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Short-term heat stress *in situ* reduces net photosynthesis and alters metabolite composition in the arctic poppy *Papaver dahlianum*

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Preface

This master's thesis has been written at the Norwegian University of Science and Technology Department of Biology, with the support of the Arctic Field Grant.

The day has come where the only thing left to write is the preface, and oddly enough, it is as hard to write as the rest of it, albeit for different reasons. To whomever who would read this, thank you for taking an interest in my work, and I hope that you find what you are seeking.

To my supervisor Richard Strimbeck and co-supervisor Jens Rohloff, thank you for the invaluable guidance, answering one to many questions and feedback during the writing process. Thank you, Richard, for making this project possible and sparking my interest in plant physiology, it means the world to me. Also, a big thank you to UNIS for allowing me to use their facilities during the field period. Although I like to think that I am of a practical nature (contrary to popular opinion), I could never have built the necessary modifications and sensor equipment, thank you Eigil and Rolf.

Vegard, I have decided to retire young. Thanks for the cheese. Catch ya's later.

Maja, thank you for being there, with all that it entails, and still standing by my side.

Abstract

Climate change is increasing the mean summer temperature in the Arctic and with that the probability of extreme climatic events including heat waves. An increase in heat waves may cause increased stress in a number of arctic plants, but to what extent, is a current research question. There was no evidence of an increase in the frequency, length or average daily maximum temperature of heat waves at Longyearbyen, Svalbard. Irrespective of future changes in temperature, present conditions could result in leaf temperatures upwards of 30 °C. I investigated the photosynthetic and metabolomic responses of *Papaver dahlianum* Nordh. to a short-term heating (17, 24 and 31 °C) event (45 min) in a field study on Svalbard. Net photosynthesis declined in single leaves with an increase in temperature along with a corresponding decrease in stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i). The reduction in net photosynthesis persisted after a return to 13 °C in the 31 °C heat treatment. Elevated temperatures caused an increase in metabolic activity, (likely) alterations in membrane composition, and an accumulation of metabolites related to or with direct antioxidant capabilities, indicating that plants experienced photoinhibition both directly after and two hours after heating.

Sammendrag

Som følge av klimaendringer vil den gjennomsnittlige sommertemperaturen i Arktis øke, noe som har blitt påvist ved Svalbard Lufthavn. Temperaturøkningen har derimot ikke medført en økning i frekvens, lengde eller gjennomsnittlige maks-temperatur for hetebølger i perioden 1976-2015. Det er uvisst hvilken effekt hetebølger har på arktiske planter, og eksisterende data indikerer at bladtemperaturen på nåværende tidspunkt kan bli rundt 30 °C under ekstreme forhold. Responsen til en kortvarig (45 min) økning av temperatur (17, 24 og 31 °C) i *Papaver dahlianum* Nordh. på Svalbard ble undersøkt for å forstå effekten av hetebølger i Arktis. Dette ble gjort gjennom måling av gassutveksling og endringer i metabolittkomposisjon hos utvalgte individer. Netto fotosyntese sank med en økning i temperatur, og denne reduksjonen vedvarte for individer behandlet med 31°C, etter at temperaturen ble senket til 13 °C. Det var en korresponderende nedgang i den stomatale ledningsevnen (g_s) og den sub-stomatale CO_2 konsentrasjonen (C_i). En økning i metabolitter med direkte eller indirekte anti-oksiderende egenskaper indikerer at enkelte planter ble fotoinhibert. Det ble også funnet bevis for endringer i membrankomposisjonen hos hos hetebehandlede planter

Selected abbreviations

A – Net CO_2 assimilation (photosynthesis)

$\mathbf{A_G}$ – Single leaf gross photosynthesis

$\Delta\mathbf{A_G}$ – The difference in single leaf gross photosynthesis from before to after heating

$\mathbf{A_N}$ – Single leaf net photosynthesis

$\mathbf{A_{Plant}}$ – Whole plant gross photosynthesis including soil respiration

$\Delta\mathbf{A_{Plant}}$ – The difference in whole plant gross photosynthesis (+soil respiration) from before to after heating

$\mathbf{C_i}$ - Sub-stomatal CO_2 concentration

$\Delta\mathbf{CO_2}$ – The difference in CO_2 concentration within the canopy assimilation chamber after a 60 second measurement

$\Delta\mathbf{CO_{2\text{-}Dark}}$ – The difference in CO_2 concentration within the canopy assimilation chamber after a 60 second measurement in natural sunlight

$\Delta\mathbf{CO_{2\text{-}Light}}$ – The difference in CO_2 concentration within the canopy assimilation chamber after a 60 second measurement in the dark

GC-MS – Gas chromatography-mass spectroscopy

$\mathbf{g_s}$ – Stomatal conductance

$\mathbf{F_v/F_m}$ – PSII maximum efficiency

Δ**Light** – The difference in light intensity experienced by treated plants with the canopy assimilation chamber from before to after heating

NPQ – Non-photochemical quenching

$\mathbf{R_{Leaf}}$ – Single leaf respiration

$\Delta\mathbf{R_{Leaf}}$ – The difference in single leaf respiration from before to after heating

$\mathbf{R_{Plant+S}}$ – Whole plant respiration including soil respiration

$\Delta\mathbf{R_{Plant+S}}$ –The difference in whole plant respiration (+soil respiration) from before to after heating

$\mathbf{Tmax_H}$ – The average of the maximum temperature recorded for each day in one heat wave, where one heat wave was defined as three or more consecutive days with a max temperature ≥10 °C

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1. Introduction

The effect of global warming is expected to be greater in the Arctic compared to other climatic regions (Collins et al., 2013), and this will cause changes in Arctic plant ecology (Chapin et al., 1995). Higher temperatures will also entail an increase in the frequency of heat waves (Collins et al., 2013, Karl et al., 1997) and it is vital to understand the responses to climatic extremes to fully explain the effects of climate change (Parmesan et al., 2000).

The projected increase in mean annual temperature for the Arctic region ranges from 2.2 ±1.7 °C (minimum greenhouse gas concentration trajectory: RCP2.6) to 8.3 ±1.9 °C (maximum greenhouse gas concentration trajectory: RCP8.5) for the 2081-2100 period compared to 1986-2005 (Collins et al., 2013). In Svalbard the mean summer temperatures have already increased by 0.33-0.55 °C per decade from 1975 to 2011 (Førland et al., 2011) and the warmest year in the composite temperature series at Svalbard Airport from 1898-2012 was 2006 (Nordli et al., 2014). However, there was no corresponding increase in the average maximum temperature from the combined meteorological data for East Greenland, Jan Mayen and Hopen (Svalbard) in the period 1951-1990 (Przybylak, 2002). There is to my knowledge no published research on the frequency of heat waves in the Arctic region, their length or average temperature, but there is both empirical and climate model evidence linking the changes in heat wave properties and frequency in temperate regions to increased greenhouse gas concentration. The length of western Europe summer heat waves has doubled since 1880 (Della-Marta et al., 2007) and the five highest temperatures measured in a heat wave in Europe have occurred after 2001 (Coumou and Rahmstorf, 2012). The climate model by Meehl and Tebaldi (2004) predicts higher temperatures, increased frequency, and longer lasting heat waves in the 21st century.

The adaptations of arctic plants (i.e. hairy leaves and low stature) that allow for a decoupling between leaf temperature and ambient temperature (Billings and Mooney, 1968) are under normal growth temperatures beneficial as photosynthesis increases with temperature up to a thermal optimum before it declines (Sage and Kubien, 2007), but can also be detrimental if the temperature increases (i.e. through a heat wave) such that photosynthesis or other biosynthetic pathways are negatively affected (heat stress) (Orsenigo et al., 2014). Previous studies show a leaf-air temperature difference up to 8 °C for several arctic plants (Mølgaard, 1989, Wilson, 1957), with 18 °C as the maximum leaf-air temperature difference recorded in *Papaver radicatum* Rottb. (Mølgaard, 1982). Scherrer and Körner (2010) also demonstrate a strong decoupling between the ambient temperature and surface temperature of arctic and alpine environments through infrared

thermometry, where an SW slope in Colesdalen, Svalbard had a surface-air temperature difference of 9.3 ±2.3 °C. Several studies in alpine environments also indicate that the actual leaf temperature may be substantially higher (up to 21 °C) than the ambient air temperature (Buchner et al., 2015, Graham et al., 2012, Salisbury and Spomer, 1964).

The photosynthetic response to experimentally created heat waves in herbs and shrubs (n = 32 species over different studies) irrespective of habitat or region, is species specific, with positive and negative responses in F_v/F_m (PSII maximum efficiency), while net photosynthetic rate either showed a neutral or negative response (Orsenigo et al., 2014). Marchand et al. (2006b) found a decrease in F_v/F_m with a 7.6 °C temperature increase (by infrared heating) over 13 days in three target species at Disko Island, West Greenland. A 9 °C temperature increase (mean ambient temperature 7.7 °C) over 8 days in four target species at Zackenberg, North-East Greenland caused increases in F_v/F_m and gross canopy photosynthesis during the simulated heat wave indicating that the raised temperatures were not above the photosynthetic thermal optimum, although the heated plants were more stressed than unheated plants after the heating period (Marchand et al., 2005). An increase in F_v/F_m was also found in an another (arctic) heatwave experiment, although there was an increase in leaf mortality with a second successive heat wave (Marchand et al., 2006a). A delayed impact of heat stress was also observed after an unusually hot summer (fifth warmest and the driest summer in 65 years) in Barrow, Alaska where gross primary productivity (GPP) was reduced the following year, and GPP was restored to normal levels after two years (Zona et al., 2014). Net photosynthesis was inhibited by leaf temperatures >38 °C in the artic-alpine species *Ranunculus glacialis* L. (Larcher et al., 1997) and a reduction in net photosynthesis were observed from 27.7 °C (Körner and Diemer, 1987).

The temperature experienced during plant growth and development is an important factor in determining the temperature range and optimum of photosynthesis, while the limiting factors of photosynthesis vary with temperature and sub-stomatal CO_2 concentration (C_i) (Sage and Kubien, 2007). The net CO_2 assimilation rate (A) is mainly under the control of three processes: (1) the capacity of ribulose-1-5-bisphosphate carboxylase (RuBisCO) to catalyze the carboxylation of ribulose bisphosphate (RuBP), (2) the capacity of the Calvin cycle and the light dependent reactions to regenerate RuBP and (3) the regeneration rate of inorganic phosphate (P_i) for photophosphorylation (Sage and Kubien, 2007). In tobacco leaves (*Nicotiana tabacum*, L. cv W38) with a C_i of 300 ppm, A is limited by the P_i concentration up to 18 °C and at temperature >18 °C by RuBisCO capacity, the decline in A >40 °C is due to the reduction in electron transport capacity (Sage and Kubien, 2007). The limitation of A by the regeneration rate of P_i in cold-adapted plants

is reduced and occurs at lower and shorter temperature ranges (Savitch et al., 2000, Strand et al., 1997). The decline in (RuBisCO limited) A above the thermal optimum may be explained by a reduction in the activation state of RuBisCO with increasing temperature, due to the heat lability of RuBisCO activase (Kurek et al., 2007, Salvucci and Crafts-Brandner, 2004). There is a contrasting view where the reduction in the activation state with an increase in temperature is a regulated response due to a decrease in the electron transport capacity. Heat-induced reduction in electron transport capacity may be (there is no clear consensus) due to an activation of cyclic electron transport (through PSI) at the expense of linear electron transport, with a resulting reduction in nicotinamide adenine dinucleotide phosphate (NADPH) concentrations (Sharkey and Schrader, 2006). At high temperatures (<40 °C) there is loss of cofactors at the OEC (oxygen evolving complex) further reducing the electron transport capacity (Enami et al., 1994, Havaux, 1996, Nash et al., 1985, Yamane et al., 1998). Recent research also indicates that moderate temperatures decelerate the repair rate of photodamaged PSII (Nishiyama and Murata, 2014).

Maximum leaf temperatures in the Arctic are likely reached under high irradiance. The combination of heat stress and photoinhibition is, therefore, important in order to fully understand the effects of heat stress on Arctic plants. Photoinhibition can be defined as "the reduction of photosynthetic capacity, independent of gross changes in pigment concentration, induced by exposure to visible light (40-700 nm)" (Powles, 1984). If the rate of absorbed light energy through photosynthetic pigments exceeds the consumption rate in the chloroplast and the rate that excess energy is dissipated through non-photochemical quenching (NPQ), then photoinhibition will accelerate (Demmig-Adams and Adams, 1992, Melis, 1999, Powles, 1984). The reduction in photosynthesis above the thermal optimum could, therefore, accelerate the extent of photoinhibition, for example through increased formation of reactive oxygen species (ROS) (Gururani et al., 2015, Melis, 1999)

Short-term heat stress affects not only photosynthesis, but also metabolite composition through an increase in the Q_{10} of enzymatic reactions and an active metabolic response that may involve protection of cellular processes, recovery after heating and/or an acclimation to increased temperatures. Kaplan et al. (2004) showed that the biggest change in metabolite composition of a plant in response to a heat shock (40 °C) happens in the first 30 minutes, with a gradual change in metabolite composition over several hours after the initial heat shock, indicating that a short-term increase in temperature can have a long-term impact on metabolite composition (Hemme et al., 2014, Kaplan et al., 2004). The main effect of increased temperatures on metabolic pathways is through secondary stresses such as oxidative damage and osmotic imbalances (Wang et al., 2003), causing changes in the level and composition of primary and secondary metabolites. The

metabolites can be stressors in themselves, while also acting as signalling molecules and/or be the components of disintegrated macromolecules.

In the present study, we investigate *in situ* (Svalbard) the effects of a short-term (45 min) heat stress on photosynthesis using whole plant and single leaf measurement of *Papaver dahlianum* Nordh. The temporal metabolite response to heat stress was also analyzed with gas chromatography-mass spectrometry (GC-MS) using field extracted leaf material in order to explore changes in biosynthetic pathways and identify novel compounds that exhibit temperature specific responses. The applied temperatures (15, 24 and 31 °C) was chosen to reflect the possible temperatures experienced by Arctic plants under present and future heat waves. The overall objective was to explore the *in situ* temperature response of *P. dahlianum* and to evaluate as to what temperatures that constitute heat stress.

2. Method

2.1 Study area

The fieldwork was conducted in and around Longyearbyen, Svalbard at the following locations: the water tower (78.2161 °N, 15.6758 °E), the south-west ridge of Vannledningsdalen (78.2121 °N, 15.6264 °E), 50-meters east (78.2220 °N, 15.6595 °E) and south-east (78.2210 °N, 15.6526 °E) of the UNIS building, in the time period 11-25 July 2015. The town of Longyearbyen is situated on the west side of Spitsbergen in Isfjorden, with an average summer temperature of 5.2 °C (1981-2010), and average precipitation of 47 mm (Førland et al., 2011)

2.2 Study species

P. dahlianum is a perennial Artic herb that is found in Svalbard, North Greenland, the Canadian Artic Archipelago (westwards to the Banks Islands). There are some records (supported genetically) of *P. dahlianum* east of Svalbard at Franz Joseph Land, Novaya Zemlya, Taimyr and northern Fennoscandia (Alsos et al., 2016). Until recently only one species of *Papaver* was thought to be present on Svalbard, but recent studies show that both *P. dahlianum* and *Papaver cornwallisense* D.Löve. are present (Solstad et al. 2014). *P. dahlianum* is found throughout the Svalbard archipelago and is the dominant species on the west side of Spitsbergen (Alsos et al., 2016). It is one of the hardier arctic species and is found on rocky sites where there is little competition from other species, such as moraine, mountain plateaus and fell fields. (Alsos et al., 2016). The individual plants are solitary, and one plant may have one or several basal rosettes that form a cushion, and several flowers. The flowers are heliotropic and will follow the sun's movement throughout the day (Kevan, 1975). Reproduction is sexual and the plants are highly polyploid (2n=70) (Alsos et al., 2016, Solstad et al., 2014).

2.3 Whole plant measurements

A modified CPY-5 canopy assimilation chamber (14.5 x 14.6 cm) connected to the CIRAS-3 portable photosynthesis system (PP Systems, Amesbury, MA, USA) was used to measure CO_2 concentration (μmol mol^{-1}), temperature (°C), light intensity (μmol m^{-2} s^{-1}) and other parameters in a closed circulatory system (no external air entering the chamber) when placed over individual *P. dahlianum* (Figure 1). The air within the chamber was circulated through the CIRAS-3, and the CO_2 concentration was measured continuously to get the net increase (respiration) or decrease (photosynthesis) of CO_2 in a 60 second measurement period. The net change in CO_2 is referred to as ΔCO_2.

The chamber was modified with a heating element at the top of the chamber and a PT100 temperature sensor (note that the temperature within the chamber was recorded with the chambers internal sensor, not the PT100 sensor). The PT100 and the heating element were connected via a PID controller (JUMO Quantrol – Compact Controller 702030) that adjusted the power to the heating element based on the temperature in the chamber and the pre-programmed target temperature, creating a feedback loop keeping the temperature relatively constant within the chamber. A small fan at the bottom of the chamber ensured mixing of the air and thereby a relatively uniform temperature distribution in the chamber.

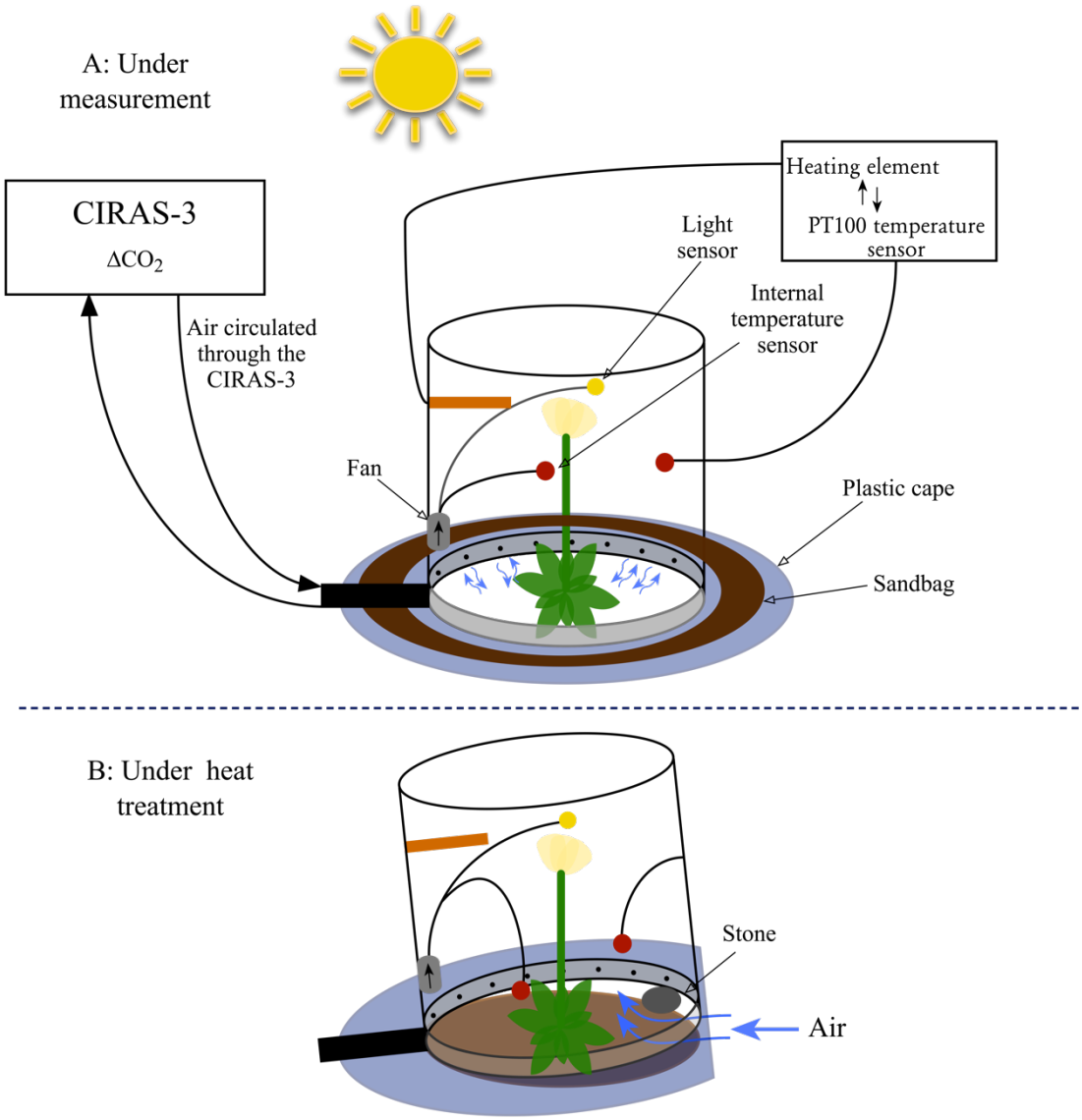

Figure 1. A modified CPY-5 canopy assimilation chamber (made of hard plastic) connected to the CIRAS-3 portable photosynthesis system was used to heat individual *Papaver dahlianum*. **Panel A** depicts the 60 second gas-exchange measurement in the light. A dark measurement was done by covering the chamber in aluminum foil. Note that the sandbag placed over the plastic cape prevents air from leaking in or out of the chamber, making it a closed system. **Panel B** shows the configuration of the chamber during the 45 min heating period in the light. The chamber was slightly tilted (2-3 cm) on one side to keep the amount CO_2 to ambient levels.

The chamber restricted both the height and width of the plants that were possible to treat and as such the individuals that were used in this experiment were chosen partially on the basis of this size limit. The substrate surrounding the plant were also an important factor. The chamber measurement is done in a closed system, and it was, therefore, important to seal the bottom part of the chamber. As a consequence, only plants surrounded by a uniform and even substrate (no big rocks, etc.) were used in the experiment. A plastic cape was taped to the bottom part of the chamber and before the measurement a tube of sand was put on this plastic cape, sealing off the chamber (Figure 1).

A 60 second measurement was done first in natural light ($\Delta CO_{2\text{-Light}}$) and then in the dark ($\Delta CO_{2\text{-Dark}}$) by covering the chamber with aluminum foil. The selected plants were then heated to either 17 (low), 24 (medium) or 31 °C (high) in an open system allowing external air to enter the chamber for 45 minutes in natural light. This was done by slightly raising one side of the chamber as the CPY-5 does not have an open flow mode (Figure 1). After the temperature, treatment another 60 second measurement was done first in natural light and then in the dark. For the majority of the treated individuals, all measurement was repeated twice (consecutively) or more (e.g. two light measurements and then two dark measurements before treatment and two light measurements and then two dark measurements after treatment). Each temperature treatment was repeated six times, for a total of 18 treated plants.

The heating system was not powerful enough to heat the air within the chamber to 31 °C in low sunlight (ca. <800 μmol m^{-2} s^{-1}, personal observation) or in high winds. Therefore, the day and time of the high temperature treatment were not randomly chosen, and the 31 °C heating treatment was only conducted when conditions allowed.

2.4 Single leaf measurements

The CIRAS-3 portable photosynthesis systems PLC3 leaf cuvette was used to manipulate the temperature and light experienced by individual *P. dahlianum* leaves and to measure/calculate the temperature of the leaf (°C), transpiration (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}), sub-stomatal CO_2 concentration (μmol mol^{-1}) and net CO_2 exchange (μmol CO_2 m^{-2} s^{-1}). The cuvette window (4.5 cm^2) was clamped over a single leaf of separate *P. dahlianum* individuals, and the experimental procedure was performed (Figure 2) where the main temperature treatment consisted of a 45-minute period of elevated temperature to either 17 (low), 24 (medium) or 31 °C (high). All measurements with the cuvette were done first in the light (800 μmol m^{-2} s^{-1}) and then in the dark. The measurements with the cuvette were in an open system and measured the

differential CO_2 and H_2O (i.e. the difference in CO_2 / H_2O of the air entering and exiting the cuvette). Several replicates of each temperature treatment were done and in some instances the stem of the leaf broke during the treatment, resulting in 5 replicates within the low temperature treatment and 6 replicates in the high and medium treatments. All treated leaves were photographed with millimeter paper as a background for later leaf area measurements.

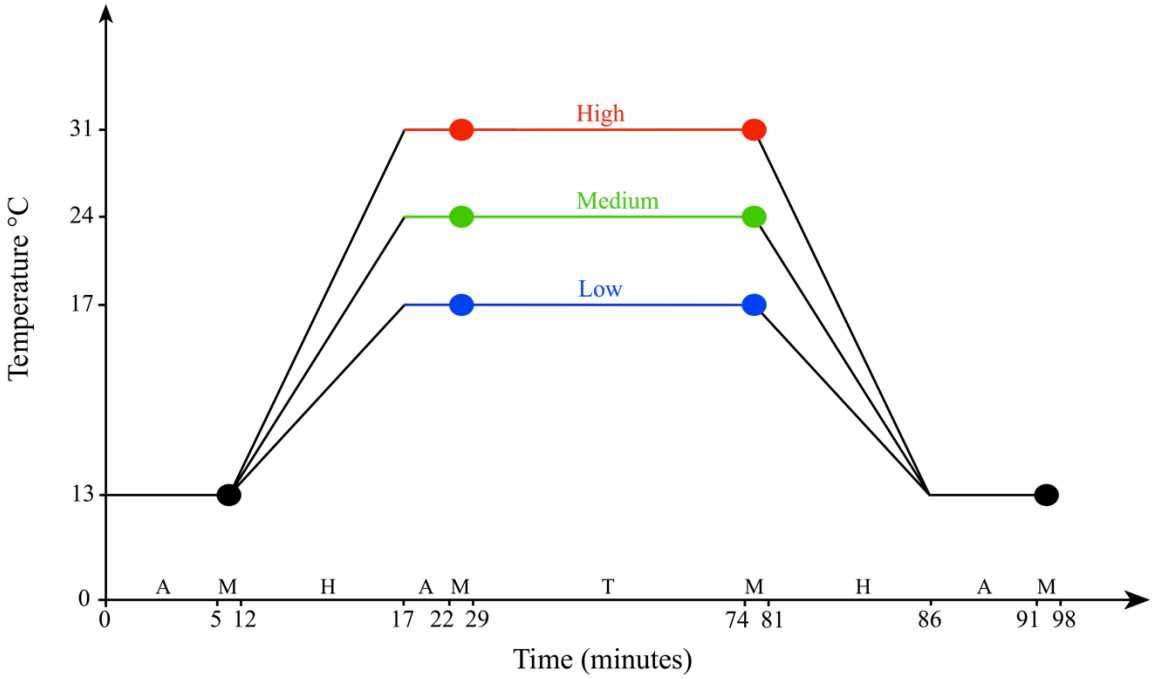

Figure 2. The experimental procedure of heating and measuring *Papaver dahlianum* leaves with the CIRAS-3 portable photosynthesis systems PLC3 leaf cuvette. A: Acclimatization (5 minutes), M: Measurement (7 minutes), H: Increase or decrease to/from the treatment temperature (5 minutes), T: Treatment (45 minutes at high, medium or low temperatures). Each measurement period (represented by a circle) consisted of one measurement in the light (2 minutes) and one in the dark (2 minutes) with 3 minutes between the light and dark measurement

2.5 Metabolite analysis and extraction

2.5.1 Field extraction

Leaf tissue samples from the chamber experiments, cuvette experiments, and untreated control plants were cut into small pieces and sampled into round-bottomed Eppendorf tubes (2 mL) containing a premixed solution of 1000 µL EtOH:H_2O (80:20) with an internal standard (ribitol, 70 µg/mL). The Eppendorf tubes were kept in a styrofoam box that contained ice mixed with salt, and salt was added over the course of the day to keep the temperature as low as possible. At the end of each day the samples were put in a -20 °C freezer. The samples were transported back to Trondheim, Norway (once the fieldwork was completed) in a thermos at -20°C, and stored at -20 °C until extraction.

Leaf tissue from plants treated with the chamber was collected immediately after the final measurements. Depending on the size of the leaves (the tissue sampled from one plant was kept as close to 100 mg as possible, using a known weight to size ratio) one or more leaves were collected. If two or more leaves were collected, then the tissue was mixed before being sampled into the Eppendorf tube. A single leaf was sampled from the plants used in the cuvette experiment before the cuvette treatment started (control sample) and the (single) treated leaf was sampled after being photographed. Additional control samples from untreated plants were taken from the water tower location on two different days in the field period.

2.5.2 Lab extraction

The leaf samples were extracted in three different batches using the same protocol and each sample was randomly assigned to its batch. The Eppendorf tubes with the leaf tissue were centrifuged for 10 minutes at 13,000 rpm, and 850 µL of aliquots were transferred to a new 1.5 mL Eppendorf tube, with 5 holes in the lid (created with a syringe). The samples were then dried overnight with a DNA110 Speed Vacuum (Thermo Electron Corporation) and the dried residue re-dissolved in 100 µl of 20 mg/ml methoxyamine hydrochloride in pyridine and derivatized at 30 °C for 90 minutes. The derivatized samples were treated with 100 µL of MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) and incubated at 37 °C for 30 minutes, before being transferred to 1.5 mL auto samples vials with glass inserts. All samples were stored at -80 °C before being analyzed by gas chromatography-mass spectroscopy (GC-MS).

Separations were performed on an Agilent 6890/5975 GC/MS (Agilent Technologies, Palo Alto, CA) equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) (Agilent Technologies, Palo Alto, CA). Sample volumes of 1 µl were injected with a split ratio of 15:1. Injection and interface temperature were set to 230°C and 250°C, respectively. The GC temperature program was held isothermally at 70°C for 5 min, ramped from 70 to 310°C at 5°C/min, and finally held at 310°C for 7 min (run time: 60 min). The MS source was adjusted to 230°C, and a mass range of m/z 70–700 was recorded (EI mode).

2.6 Statistical analysis

All gas-exchange and meteorological data were analyzed with the software R, version 3.2.3 (R Core Team, 2015). A significance level of $p < 0.05$ was used for all statistical analyses. The assumptions of the ANOVA models were checked with the Bartlett´s test (Zeileis and Hothorn, 2002), while the Breusch-Pagan test (Zeileis and Hothorn, 2002) was used to test the linear regression model assumptions, additionally all models were evaluated by visual inspection of the residual plots.

2.6.1 Heat waves

Meteorological data from Svalbard Airport was analyzed and the length (days), frequency and average daily max temperature (°C) was calculated for all heat waves in the period 1976-2015 for the months June, July, August, and September. Where one heat wave was defined as three or more consecutive days with a max temperature ≥10 °C. The average daily max temperature was calculated by summing the max temperature of each day in a heat wave divided by the number of days in that heat wave; this was defined as $Tmax_H$. A seasonal Kendall test (non-parametric) was used to investigate changes in length and $Tmax_H$ of all the heat waves in the 1976-2015 (extended summer) period, when correcting for the seasonality of each month (Hirsch and Slack, 1984, Hirsch et al., 1982, Marchetto, 2015). It was assumed that there was a positive trend within each month for the whole period. A Mann-Kendall test (non-parametric) was used to test if there was an increase in the frequency of heat waves (Mann, 1945, Marchetto, 2015).

2.6.2 Whole plant measurements

Negative and positive values were switched for all ΔCO_2 values, such that a negative ΔCO_2 represents respiration while a positive ΔCO_2 represents photosynthesis. The $\Delta CO_{2\text{-Light}}$ incorporates soil respiration and whole plant net photosynthesis while $\Delta CO_{2\text{-Dark}}$ gives whole plant respiration and soil respiration ($\Delta CO_{2\text{-Dark}}$ is henceforth referred to as $R_{Plant+S}$). Subtracting $\Delta CO_{2\text{-Dark}}$ from $\Delta CO_{2\text{-Light}}$ gives the whole plant gross photosynthesis and is defined as A_{Plant}. If there were two or more consecutive $\Delta CO_{2\text{-Light}}$ or $\Delta CO_{2\text{-Dark}}$ measurements, then the average was used. Subtracting A_{Plant} before the heat treatment from A_{Plant} after the heat treatment ($A_{Plant\text{-}After}$ - $A_{Plant\text{-}Before}$) results in the difference in A_{Plant}, defined as ΔA_{Plant}. The same calculation was done for $R_{Plant+S}$, and this was defined as $\Delta R_{Plant+S}$.

The light intensity experienced by the plant at the end of the 60 second measurement (in the light) before the treatment was subtracted from the light intensity at the end of the 60 second measurement (in the light) after the treatment, this was defined as ΔLight (in the instances where two or more measurements in the light were conducted consecutively, then the average of these measurements were used). An analysis of covariance (ANCOVA) was used to test if ΔLight had an effect on ΔA_{Plant} and if there was an interaction effect with the temperature treatments. If no interaction effect was found between the predictor variables, then the ANCOVA was fitted without an interaction effect, and non-significant predictor variables were removed from the model. An analysis of variance (ANOVA) followed by a Tukey honest significance difference (Tukey HSD) test (if the ANOVA was significant) was used to test for differences among temperature treatment means in initial A_{Plant}, initial light intensity, ΔA_{Plant}, and $\Delta R_{Plant+S}$. The difference between the

treatments in (chamber) temperature before heating was tested with a non-parametric Kruskal-Wallis test (Kruskal and Wallis, 1952, Pohlert, 2014) and the Kruskal-Wallis post hoc test after Nemenyi (Pohlert, 2014) was used to test which treatments differed from each other.

2.6.3 Single leaf measurements

Net photosynthesis (A_N), stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i) were all calculated from the raw measurements using PP Systems' software as described in the CIRAS-3 portable photosynthesis system instruction manual (pp. 118-122) and corrected for leaf area. The leaf area was calculated with Image J (version 1.49) by using the known pixel to distance on the mm paper used as a background in the pictures of the treated leaves. A_N, g_s, and C_i were averaged from all the logged values (12) within one light or dark measurement (2 min). A_N measured in the dark was the respiration of the leaf and was defined as $R_{Leaf.}$ Gross photosynthesis (A_G) for a measurement period (Figure 2) was calculated by subtracting R_{Leaf} from A_N for the same measurement period. The difference in A_G after heat treatment (defined as ΔA_G) was calculated by subtracting A_G at 13 °C before heat treatment from A_G at 13 °C after the heat treatment ($A_{G\text{-}After}$ - $A_{G\text{-}Before}$). The same calculations were done for R_{Leaf}, and this was defined as ΔR_{Leaf}.

Due to an unbalanced design, a non-parametric Kruskal-Wallis test was used to test for differences in initial A_G, initial R_{Leaf}, initial g_s, ΔA_G and ΔR_{Leaf} and initial A_N between the temperature treatments. The Kruskal-Wallis post hoc test after Nemenyi was used to test which treatments differed from each other (when the Kruskal-Wallis test showed a significant difference). There was a total of six replicates in the high and medium temperature treatment while the low temperature treatment had 5 replicates. Linear regression was used to test if the leaf temperature had an effect on A_N, g_s and C_i at the end of the 45 min treatment period (in the light) and R_{Leaf} (in the dark).

2.6.4 Metabolomics

Data alignment and processing were carried out using the MetAlign software (Rikilt, Wageningen, NL). Compound identification was achieved using MS libraries, such as NIST/EPA/NIH MassSpectralLibrary NIST05 (National Institute of Standards and Technology, Gaithersburgh, MD), the Golm Metabolome Database containing MS spectra of derivatized metabolites (Hummel et al., 2010), in combination with an *in-house* retention index library of trimethylsilylated (TMS) metabolites. The Automated Mass Spectral Deconvolution and Identification System (AMDIS; National Institute of Standards and Technology, Boulder, CO) software were used to interpret GC-MS data.

Principle component analysis (PCA) (Wold et al., 1987) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) (Trygg and Wold, 2002) were performed with SIMCA 14 (Umetrics, Umeå, Sweden) on pareto scaled and log transformed data. Unsupervised PCA was used to provide an initial visualization and inspection of trends within the dataset and OPLS-DA was used to further explore the differences in metabolite concentration between the treatment groups. The OPLS-DA models were evaluated with the R^2X, R^2Y (an estimate of the goodness-of-fit) and Q^2Y (an estimate of the predictability of the model), additionally, the ellipse defined by Hotelling´s T^2 confidence region (95 %) and the distance to the model in X-space (DModX) was used to asses the models. A second cross-validation step was performed in addition to to the 7-fold cross validation performed by SIMCA, where one-third of the samples (randomly chosen) from each group (in the model) was removed from the final model, and the new reduced dataset was used as a training set to predict the class membership of the excluded samples. This was repeated three times such that each sample was predicted once. A volcano plot was calculated with BioStatFlow version 2.7.7 (National Institute of Agronomic Research, France) and used to further explore the fold change and significance levels of the metabolites between the groups in selected OPLS-DA models.

3. Results

3.1 Heat waves

There were no significant trends for the frequency (p=0.98), $Tmax_H$ (p=0.19) or the length of the heat waves (p=0.55) in the 1976-2015 data series from Svalbard Airport (Figure 3; Table S4), when correcting for the seasonality of each month (August and July). September and June were not included in the analysis because there were less then four heat waves in these two months for the whole time period. The mean $Tmax_H$ of all heat waves between 1976-2015 was 12.29 °C.

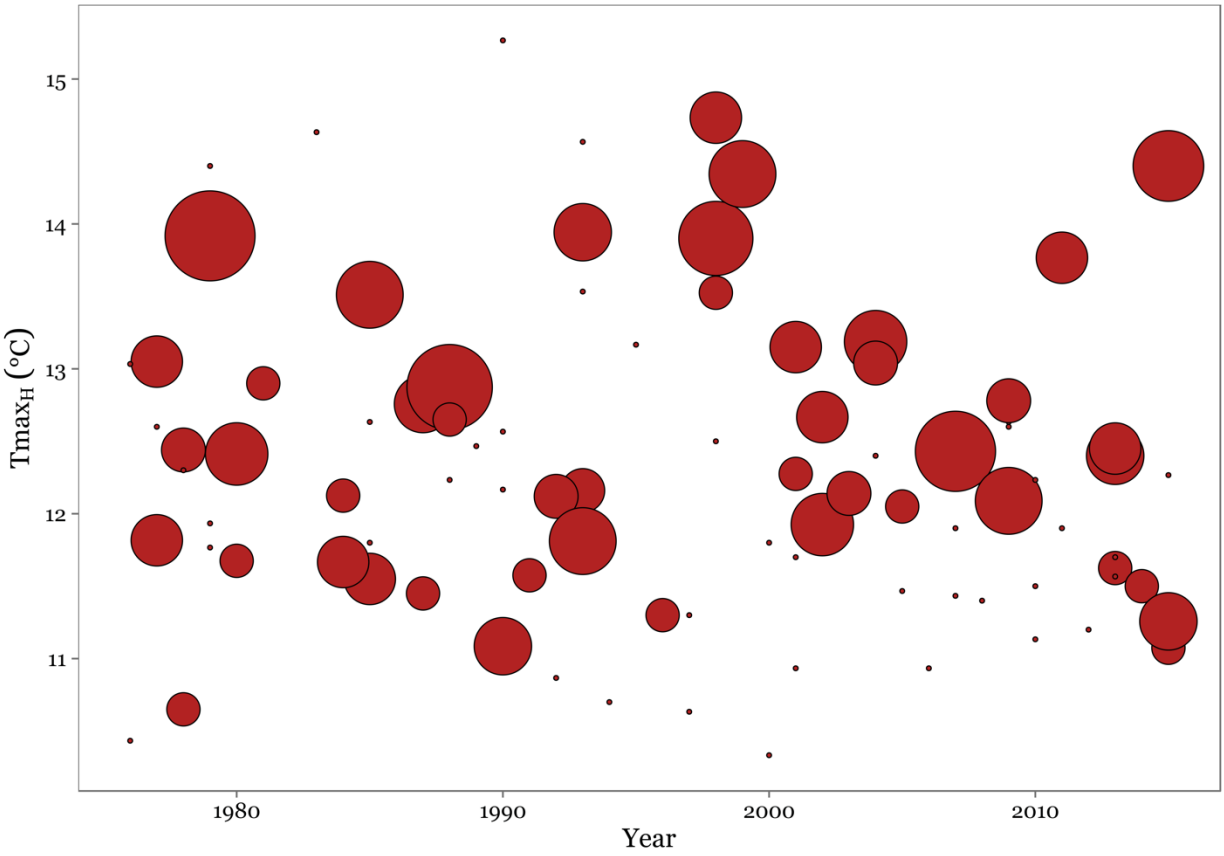

Figure 3. The average daily max temperature ($Tmax_H$) of heat waves at Svalbard Airport between 1976-2015 for the months June, July, August, and September. One heat wave is defined as three or more consecutive days with a max temperature ≥10 °C and is represented by a single circle. The size of the circle corresponds to the length of the heat wave, where the biggest circle corresponds to a 17-day heat wave, and the smallest circle(s) corresponds to a 3-day heat wave.

3.2 Whole plant gas exchange

The ANOVA showed a significant temperature effect on ΔA_{Plant} (R^2=0.43, p=0.015; Table S1). There was a varied response in ΔA_{Plant} to the medium temperature treatment, whereas the high temperature treatment showed a consistent reduction in ΔA_{Plant} (Table 1; Figure S1). There was a small increase in ΔA_{Plant} for individuals in the low temperature treatment (Table 1; Figure S1). The high and low temperature treatments both have one outlier with an opposite response to heating

(Figure S1), but there was still a significant decline in ΔA_{Plant} in the high temperature treatment compared to the low temperature treatment (p=0.017; Table S1). Soil and plant respiration has been shown to increase with temperature, but with the whole treated plants there was, surprisingly, no difference in $\Delta R_{Plant+S}$ between any treatments (R^2=0.023, p=0.84; Table S1) (see Discussion for further details).

Table 1. The average A_{Plant}, ΔA_{Plant}, $R_{Plant+S}$ and $\Delta R_{Plant+S}$ (CO_2 μmol mol^{-1}) of whole plant (*Papaver dahlianum*) measured with a modified CPY-5 canopy assimilation chamber connected to CIRAS-3 portable photosynthesis systems at three different temperature categories (high, medium and low), before and after a 45 min heat treatment, with each temperature treatment replicated six times. The given temperature (°C) and light intensity (μmol m^{-2} s^{-1}) is the average temperature/sunlight within the chamber during a 60 second closed system measurements in the light either before or after heating and are approximations of the conditions during the 45 min temperature treatment. CIRAS-3 measured the increase (respiration) or decrease (photosynthesis) of the CO_2 concentration (μmol m^{-2} s^{-1}) in the chamber, with the net change in CO_2 being the ΔCO_2. Negative values (photosynthesis) were converted to positive values, and positive values (respiration) were converted to negative values. Measurements were done both in the light ($\Delta CO_{2\text{-Light}}$) and in the dark ($\Delta CO_{2\text{-Dark}}$). The $\Delta CO_{2\text{-Light}}$ incorporates soils respiration and whole plant net photosynthesis while $\Delta CO_{2\text{-Dark}}$ ($R_{Plant+S}$) gives whole plant respiration and soil respiration. Subtracting $\Delta CO_{2\text{-Dark}}$ from $\Delta CO_{2\text{-Light}}$ gives the whole plant gross photosynthesis and the unknown soil respiration component (A_{Plant}). If there was two or more consecutive $\Delta CO_{2\text{-Light}}$ or $\Delta CO_{2\text{-Dark}}$, then the average was used. Subtracting A_{Plant} before the heat treatment from A_{Plant} after the heat treatment results in the difference in A_{Plant} (ΔA_{Plant}), the same calculation was done for $R_{Plant+S}$ ($\Delta R_{Plant+S}$), and the light intensity, giving ΔLight. Different raised letter indicates differences between treatments at $p<0.05$. The significant difference in A_{Plant} (before) is based on log transformed A_{Plant} values. However, the mean and standard error of A_{Plant} are not log transformed. Note that all standard errors were calculated group wise and are not the total standard error given by the ANOVA, see Table S1 for supplemental details and transformed values.

	High		Medium		Low	
	Before	After	Before	After	Before	After
A_{Plant}	42.01 ±9.62[a]	22.47 ±7.06	19.67± 5.97[ab]	16.97 ±2.84	9.82 ±2.81[b]	10.66 ±3.64
ΔA_{Plant}	-19.53 ±5.36[a]		- 3.19 ±4.90[ab]		0.85 ±3.10[b]	
$R_{Plant+S}$	-25.20 ±7.96	-25.77 ±6.37	-14.78 ±2.60	-17.67 ±3.45	-9.50 ±2.81	-9.99 ±3.57
$\Delta R_{Plant+S}$	0.57 ±3.07[a]		0.50 ±2.94[a]		2.89 ±3.64[a]	
Temperature (°C)	19.11 ±0.70[a]	32.89 ±0.91	14.88 ±0.56[ab]	21.88 ±0.88	15.55 ± 1.74[b]	17.01 ±1.17
Light intensity	1282.25 ± 118.30[a]	1159.33 ± 135.76	714.42 ± 172.58[b]	707.25 ± 186.74	761.50 ± 121.30[b]	711 ± 149.87
ΔLight	-122.92 ± 69.69		-7.16 ± 228.73		-50.50 ± 215.14	

The response in A_{Plant} to an increase in temperature is among other factors (see Discussion) dependent on the initial A_{Plant} level before temperature treatment. There was, as with the ΔA_{Plant} a significant difference in A_{Plant} before heat treatment between the high and low temperature treatments (p=0.013; Table S1), with a higher initial A_{Plant} in the high treatment compared to the

low temperature treatment (Table 1). A possible explanation for the variation in A_{Plant} before treatment is the non-random selection of individuals in the high temperature treatment (see Methods), as seen by the significantly higher initial light intensity in the high temperature treatment compared to the two other treatments (Table 1; Table S1; Figure S4) and the significantly higher initial (chamber) temperature in the high temperature treatment compared to the low temperature treatment ($p=0.024$; Table 1; Table S2). There was no interaction effect between temperature treatments and ΔLight ($p=0.46$) in predicting ΔA_{Plant} or main effect of ΔLight ($p=0.31$) in predicting ΔA_{Plant} (Table S1).

3.3 Single leaf measurements

The Kruskal-Wallis test showed a significant temperature effect on ΔA_G ($p=0.019$; Table S2) where all three temperature treatments caused a reduction in ΔA_G (Table 2; Figure S2), with a significantly bigger decline in ΔA_G in the high temperature treatment compared to the low temperature treatment ($p=0.015$; Table S2).

Table 2. The average A_G, ΔA_G, R_{Leaf} and ΔR_{Leaf} (CO_2 μmol m^{-2} s^{-1}) of single leaves (corrected for leaf area) on individual *Papaver dahlianum* at three different temperature categories (high, medium and low) before and after a 45-minute heat treatment measured with a CIRAS-3 portable photosynthesis systems PLC3 leaf cuvette under constant light (800 μmol m^{-2} s^{-1}). The leaf temperatures (°C) are the average leaf temperature under a 2-minute measurement period in the light at the end of the 45 min heat treatment period and are approximations of the leaf temperature during the 45 min treatment period. The high and medium heat treatments were replicated six times while the low temperature treatment was replicated five times. Net photosynthesis (A_N) measured in the dark is the respiration of the leaf and is defined as $R_{Leaf.}$ Gross photosynthesis (A_G) was calculated by subtracting R_{Leaf} from A_N. The difference in A_G from before to after the heat treatment (defined as ΔA_G) was calculated by subtracting A_G at 13 °C before heat treatment from A_G at 13 °C after the heat treatment. The same calculations were done for R_{Leaf} giving ΔR_{Leaf}. Different raised letter indicated differences between treatments at $p<0.05$. All standard errors were calculated group wise, see Table S2 for supplemental details

	High		Medium		Low	
	Before	After	Before	After	Before	After
A_G	11.17 ±1.99[a]	6.83 ±1.08	12.14 ±1.60[a]	8.31 ±1.44	15.57±1.82[a]	15.06 ±1.76
ΔA_G	-4.33 ±0.92[a]		-2.65 ±0.96[ab]		-0.33 ±0.18[b]	
R_{Leaf}	-0.94 ±0.16[a]	-0.86 ± 0.24	-0.78 ± 0.14[a]	-0.07 ±0.53	-0.79 ±0.47[a]	-1.01 ±0.32
ΔR_{Leaf}	-2.09 ±0.80[a]		-0.48 ±0.17[a]		-1.20 ±0.78[a]	
Leaf Temperature (°C)	30.20 ±0.82		23.79 ±0.42		15.87 ±0.37	

The significant decline in ΔA_G (at high temperatures), shows that the observed reduction in net photosynthesis (Figure 4) persists after the heat treatment (under constant light). The lack of any difference in ΔR_{Leaf} between the treatments (p=0.28; Table S2) indicates that the observed (persistent) reduction in A_N is (likely) due to a reduction in gross photosynthesis rather than an increase in respiration after heat treatment.

A_N, g_s, and C_i all decreased with an increase in temperature (Figure 4; Table S3). A_N decreased by -0.54 ±0.14 µmol CO_2 m^{-2} s^{-1} with a 1°C increase in leaf temperature (p=0.0051, r^2=0.51). g_s was reduced by -11.55 ± 2.59 mmol H_2O m^{-2} s^{-1} (p<0.001, r^2=0.57) and C_i decreased by -3.65 ±1.13 µmol mol^{-1} (p=0.0057, r^2=0.41) with a 1°C increase in leaf temperature.

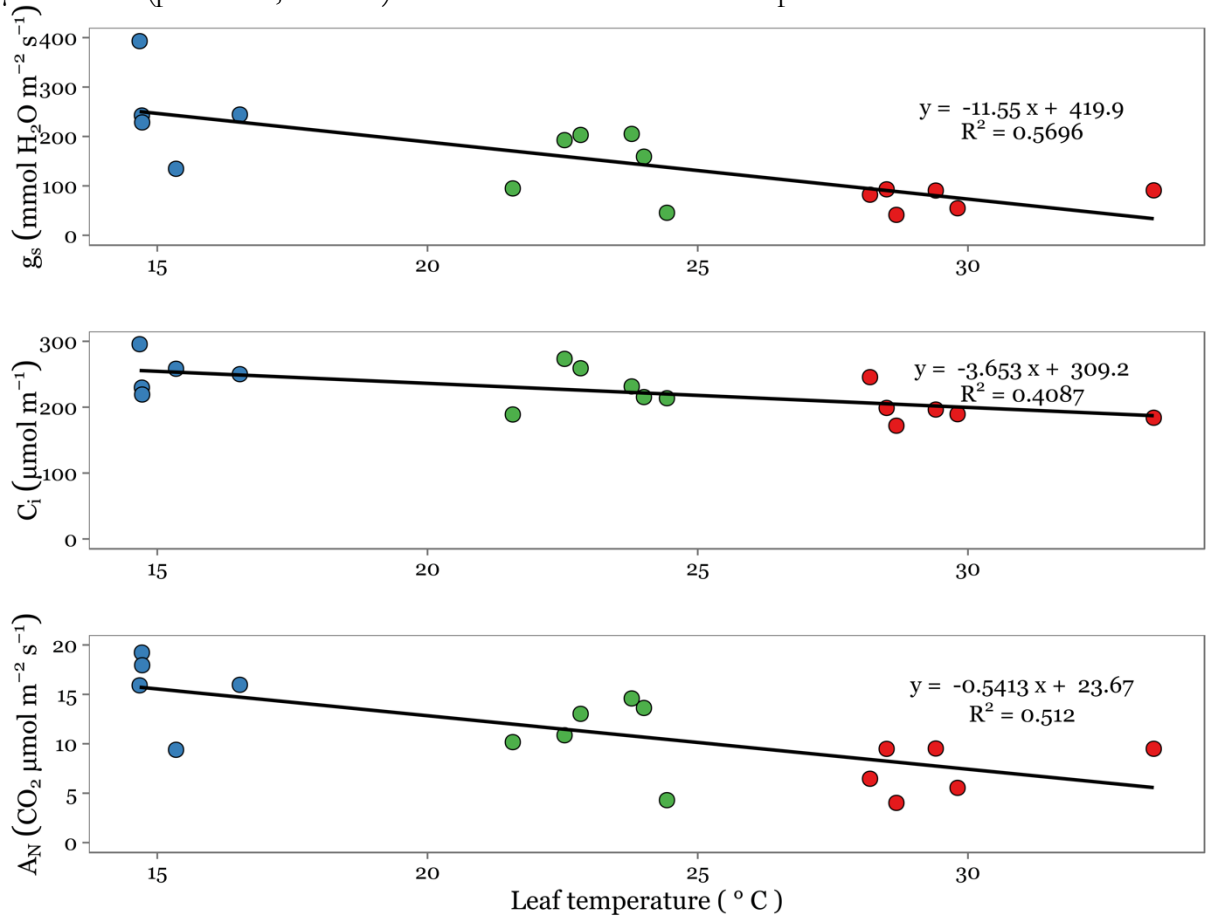

Figure 4. The effect of leaf temperature on stomatal conductance (mmol H_2O m^{-2} s^{-1}) (g_s), sub-stomatal CO_2 concentration (µmol mol^{-1}) (C_i) and net photosynthesis (CO_2 µmol m^{-2} s^{-1}) (A_N) on *Papaver dahlianum* leaves. Measurements were done on single leaves of individual plants at three different temperature categories, high (red), medium (green), low (blue) under constant light (800 µmol m^{-2} s^{-1}) at the end of a 45 min heat treatment, with a CIRAS-3 portable photosynthesis systems PLC3 leaf cuvette. One circle represents a single leaf on an individual plant and is the average of 12 logged values (leaf temperature, A_n, g_s and C_i) taken under a 2-minute measurement period in the light at the end of the heat treatment period. The regression line and its coefficients are derived from a linear model with leaf temperature as a predictor variable and the g_s, C_i and A_N as response variables.

The individual responses to the temperature treatment for A_N and g_s were mostly uniform within each treatment (Figure 5), except in the low temperature treatment for g_s. There was no difference

for either g_s (p=0.34) or A_N (p=0.11) before treatment in the light between the temperature treatments (Table S2; Figure 5), showing that the decline in net photosynthesis is not due to initial differences in A_N and/or g_s. Further supporting the temperature effect on A_N is the lack of any differences in A_G (p=0.17) and R_{Leaf} (p=0.72) before heating (Table S2).

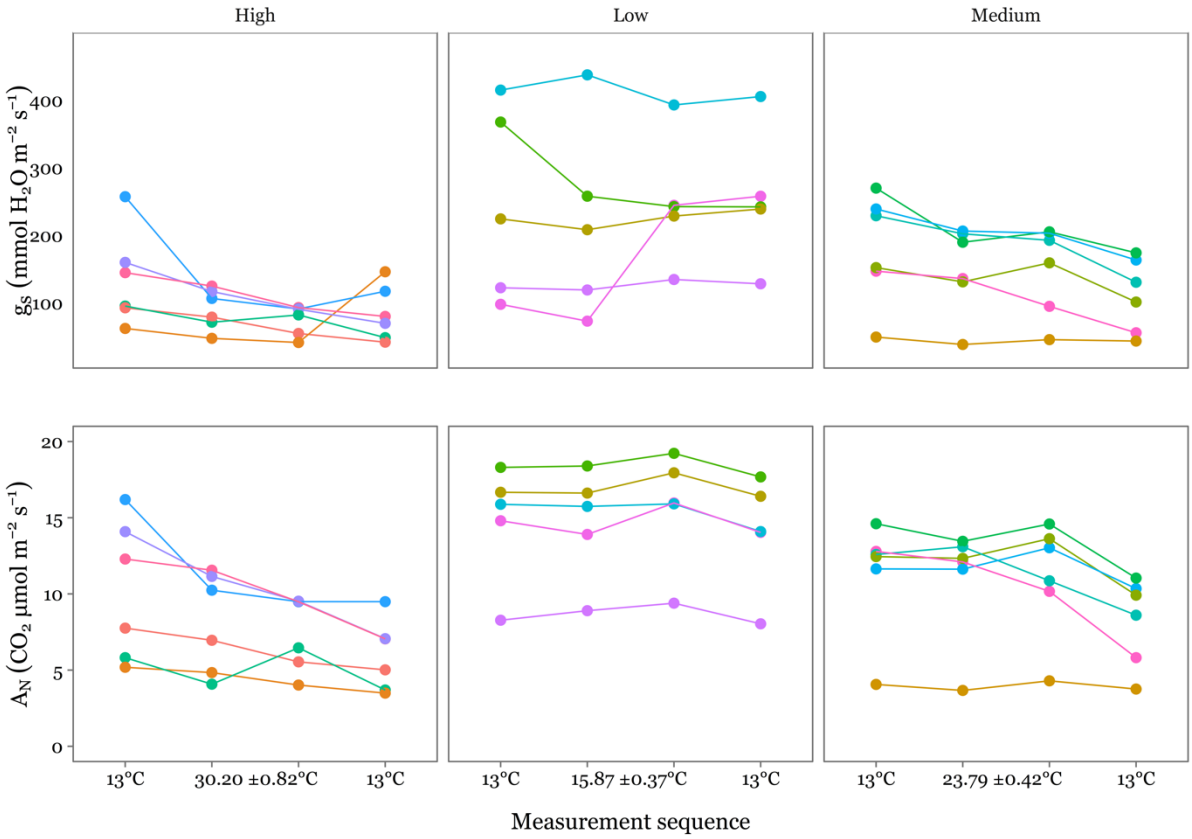

Figure 5. Stomatal conductance (mmol H_2O m^{-2} s^{-1}) (g_s) and net photosynthesis (CO_2 µmol m^{-2} s^{-1}) (A_N) of single *Papaver dahlianum* leaves under an experimental setup where g_s and A_N was measured four times (sequentially) at three different temperature categories (high, medium and low) with a CIRAS-3 portable photosynthesis systems PLC3 leaf cuvette under constant light (800 µmol m^{-2} s^{-1}). The measurement sequence was as following (from left to right): (1) Before heating at 13 °C, (2) before treatment at the treatment temperature (given in the panel), (3) at the end of a 45 min treatment period at the treatment temperature and (4) after heating at 13 °C. One circle represents a single leaf on an individual plant and is the average of 12 logged values (leaf temperature, A_N, and g_s) taken under a 2-minute measurement period in the light. Circles connected by lines are measurements of the same leaf (on individual plants), lines with the same color in the g_s and A_N panels are the same leaves/individuals.

3.4 Metabolomics

The PCA and the OPLS-DA analysis did not show any separation in metabolite concentration between temperature treatment groups (high, medium and low) for single treated leaves or leaves from whole treated plants. The samples, when combined irrespective of heat treatment, did not show any separation in the PCA analysis when comparing samples from single treated leaves and leaves from whole treated plants (both immediately and after two hours) to the control samples. However, the OPLS-DA did show a separation (Table S5) between samples from whole treated plants taken immediately after the treatment and control samples (r^2X = 0.28, r^2Y= 0.87, Q^2= 0.34;

Model name: Treated vs. Control) and between samples taken 2 h after heat treatment control samples (r^2X = 0.43, r^2Y= 0.96, Q^2= 0.47; Model name: Two-hour vs. Control).

The large discrepancy between the r^2Y and Q^2 value (>0.5) coupled with Q^2 values <0.5 in both models indicates overfitting (see Discussion) and a manual cross-validation was performed to test the true predictive capacity of the OPLS-DA models (see Method section). The Treated vs. Control OPLS-DA model had a 64.44 % correct prediction rate of the excluded samples class membership and the Two-hour vs. Control OPLS-DA model had a 66.53 % prediction rate (the average prediction rate of three training sets, Table S5). The less than optimal prediction rates of the training sets suggest that there are some differences in metabolite concentration between heated plants, but not large enough to create a reliable multivariate model. As such, a volcano plot was used to explore which metabolites that had a significant change in concentration (fold change) in the Treated vs. Control and Two-hours vs. Controls models (Figure 6), and those metabolites were viewed as important for the immediate and delayed heat shock response.

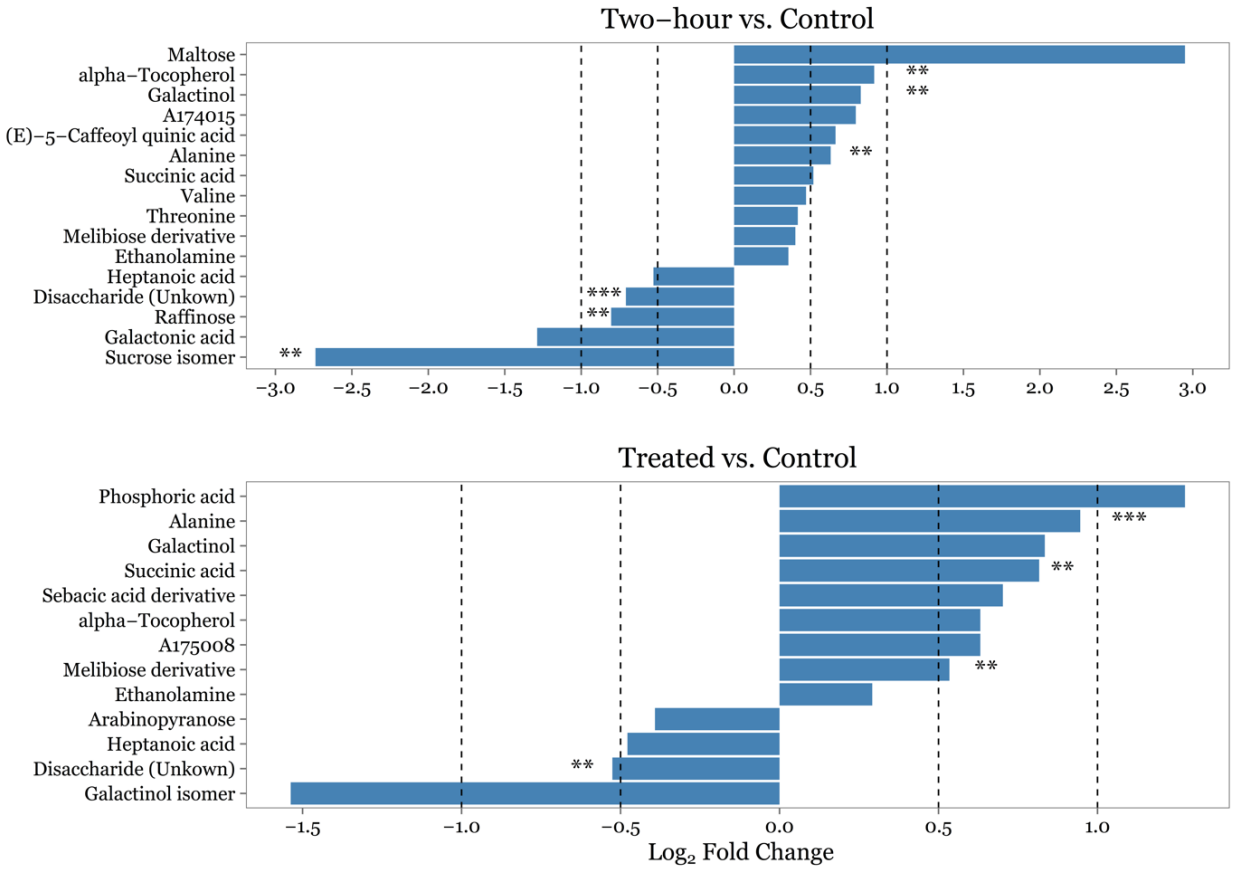

Figure 6. The $\log_2$ fold change in metabolites from heat treated *Papaver dahlianum* (irrespective treatment temperature) that differed significantly when compared to the control sample group (n=26) (calculated with a volcano-plot). The entire plant was heated with a modified CPY-5 canopy assimilation chamber connected to CIRAS-3 portable photosynthesis system under natural sunlight for 45 min to one of three different temperature categories: high (32.89 ±0.91 °C), medium (21.88 ±0.88 °C) and low (17.01 ±1.17 °C). The Treated vs. Control model consists of all control samples and all samples taken immediately from heat treated individuals (n=19), the Two-hour vs. Control model consists of samples taken 2 hours after the heat treatment (n=15) and all control samples. All metabolites not marked differed from the control samples with $p<0.05$ while **: $p<0.01$, ***: $p<0.001$.

4. Discussion

4.1 Heat waves

Following the method of De Boeck et al. (2010) and the guidelines of Klein Tank et al. (2009) in analyzing extreme temperature events, temperatures above the 90th percentile were viewed as extreme events. The 90th percentile daily max temperature of Svalbard Airport was 10.7 °C (i.e. 10% of all daily max temperatures are ≥10.7 °C), suggesting that 10 °C is a relevant cutoff point for extreme temperatures at Svalbard Airport. This study was only concerned with the short-term heat stress, and therefore, a heat wave was defined as three or more consecutive days with max temperatures ≥10 °C. The lack of an increase in heat wave frequency at Svalbard Airport is contrary to the predictions by Collins et al. (2013) given the observed increase in temperature at Svalbard Airport by Nordli et al. (2014), although the chances of detecting a significant trend from a single location is generally small (Klein Tank et al., 2009). It is also possible that the projected increases in max temperatures are negated by the proximity to the ocean and that continental arctic areas will see a much higher temperature increase and extreme climatic events (Collins et al., 2013). The filtration of the dataset could have an equalizing effect on the observed temperature trends explaining why there is no change in $Tmax_H$, e.g. if there are several heat waves close to the cutoff temperature then they will equalize the (statistical) effect of the heat waves with a high $Tmax_H$ (Hygen H.O., personal communication, 2015). A higher temperature cutoff point could, therefore, show if there was an increase in more extreme values, however with a 12 or 14 °C cutoff point, the sample sizes becomes too small to detect any trends.

4.2 Photosynthesis

The mean temperature of the field period at Svalbard Airport was 7.95 ±0.33 °C, which is nearly three degrees above the normal mean summer temperature (Førland et al., 2011), and during the field period there was a ten-day long heat wave (14-23 July 2015) with a mean temperature of 8.26 ±0.41 °C and $Tmax_H$ of 11.21 ±0.33 °C. These temperatures were coupled with moderate winds (the average wind speed of the field period was 4.79 ±0.33 m/s, with max winds at 11.2 m/s) and would likely keep the leaf temperature close to the ambient air temperature (Wilson, 1957). Despite the effect of the wind (on the leaf temperature), it is possible that there was *a priori* heat stress in the treated plants given the higher than normal temperatures.

The heat wave during the field period illustrates the relevance of the chosen treatment temperatures, with a max temperature of 11.7 °C a leaf-air temperature difference of 9 °C would

increase the leaf temperature to 20 °C and under rare circumstance up to 30 °C with a 18 °C leaf-air temperature difference, as observed by Mølgaard (1982). The maximum temperature recorded at Svalbard Airport is 21.3 °C (July 1979) and the more common leaf-air temperature difference of 8-9 °C, would result in a leaf temperature of ca. 30 °C. A 2-6 °C increase in temperature at Svalbard (Førland, 2010) could conceivably make leaf temperatures of 24-30 °C a regular occurrence.

4.2.1 Whole plant measurements

The significant reduction in ΔA_{Plant} at high temperatures compared to the low temperature treatment indicates that >30 °C temperatures negatively affect whole plant photosynthesis. While temperatures around 24 °C cause a more individual response with the treated plants split equally between a reduction or increase in ΔA_{Plant}. There was no interaction effect between ΔLight and the temperature treatments or main effect of ΔLight on ΔA_{Plant}, indicating that changes in the light intensity throughout the treatment period did not cause the observed reduction in ΔA_{Plant}. However, it cannot be concluded that temperature alone is causing the decrease in ΔA_{Plant} due to three factors: (1) The temperature and light intensity within the chamber during the 45 min heating period is unknown and the temperature would likely fluctuate due to the use of unheated air to keep the CO_2 concentration at ambient levels in the chamber, (2) the initial A_{Plant} is significantly higher in the high temperature treatment (probably caused by a significantly higher temperatures and light intensity before heating as a result of non-random selection of individuals) compared to the low temperature treatment and substantially higher than the medium temperature treatment and (3) $\Delta R_{Plant+S}$ did not increase with temperature, contrary to the respiration of treated single leaves (Figure S3) and previous studies that show a definite increase in respiration with temperature across several plant taxa (Ryan, 1991, Tjoelker et al., 2001), including arctic plant species (Wager, 1941). Additionally, ΔR_{Plant} could also be influenced by an increase in soil respiration (Lloyd and Taylor, 1994). Due to the uncertainty of the whole plant measurements, these results will be used as supporting evidence for the single leaf measurements.

4.2.2 Single leaf measurements

The decline in net photosynthesis of single leaf measurements at high and medium temperatures persisted in the high temperature treatment at the control temperature (13 °C) (after heating). There was no difference in ΔR_{Leaf} between treatments indicating that the observed (persistent) reduction in A_N is (likely) due to a reduction in gross photosynthesis, not an increase in respiration. The reduction in net photosynthesis at ca. 24 and 30 °C is expected given that these temperatures exceed the predicted thermal optimum for photosynthesis in arctic plants (Sage and Kubien, 2007) and greatly exceed the temperatures in the (Arctic) heating experiments by Marchand et al. (2006b),

Marchand et al. (2006a), Marchand et al. (2005). The small, but consistent reduction in ΔA_G after the low temperature treatment may be relevant over longer time periods as the study by Marchand et al. (2006b) indicate that sustained leaf/vegetation temperatures around 15 °C can be detrimental. The reduction in ΔA_G could be caused by the cuvette itself, for example through blocking the veins in the stem of the leaf and as a consequence reduced water flow to the leaf. If this were the cause, then it would be expected to see a large general decline in g_s for all treated individuals (irrespective temperature treatment), no such pattern was found. The temperature effect on ΔA_G is further supported by the corresponding decrease in ΔA_{Plant} with an increase in temperature.

In situ heating to 45 °C (a temperature that corresponds to the higher summer temperatures in the Alps compared to the Arctic) of four alpine plants produced a reversible (over several days) reduction in photosynthetic performance and F_v/F_m (Buchner et al., 2015). It is, therefore, possible that the reduction in net photosynthesis observed at 13 °C (after heating) would be reversed over a several hours/ days. The reduction in photosynthetic performance observed in Buchner et al. (2015) is strongly correlated with a reduction in stomatal conductance (as observed in this experiment), and Buchner et al. (2015) views this correlation as a possible cause (reduction in g_s) and effect (reduction in photosynthetic performance) scenario. If stomatal conductance were limiting net photosynthesis by reducing the diffusion of CO_2, then one would expect a corresponding relevant decrease in C_i. There is a small (significant) decrease, but not large enough to shift the limitation away from RuBisCO limited photosynthesis (Sage and Kubien, 2007). Net photosynthesis decreases with C_i under RuBisCO limited photosynthesis at constant temperatures (Long and Bernacchi, 2003, Sage and Kubien, 2007). However, if the leaf temperatures in the high and/or medium temperature treatment were above the thermal optimum for RuBisCO, then the reduction in the activation state of RuBisCO may have contributed to or been responsible for the observed decline in net photosynthesis (Cen and Sage, 2005, Sage and Kubien, 2007, Salvucci and Crafts-Brandner, 2004). This view is further supported by the findings of Farquhar and Sharkey (1982), Jones (1998), who conclude that stomatal conductance does not exert a large control over photosynthesis.

The decrease in F_V/F_m in Buchner et al. (2015) is not surprising given the >40 °C temperatures, where inhibition of PSII has been observed (Enami et al., 1994, Havaux, 1996, Nash et al., 1985, Yamane et al., 1998). The possibility that inhibition of PSII is responsible for the reduction in net photosynthesis at high temperatures (30 °C) cannot be excluded, as the hypothetical reduction in RuBisCO activation state could be due a reduction in the electron transport chain (Sage and Kubien, 2007). There are to my knowledge no studies indicating that <35 °C temperatures inhibit

PSII in itself, however, a saturation of the non-photochemical quenching capacity due to reduced carbon assimilation at 30 °C could increase photoinhibition (Demmig-Adams and Adams, 1992, Melis, 1999, Powles, 1984). This would partly explain the relatively large reduction in ΔA_{Plant} at the high temperatures (assuming the data is valid) compared to the reduction in net photosynthesis of single leaves, as the light in the single leaf measurements was at 800 μmol m^{-2} s^{-1} compared to ca. 1200 μmol m^{-2} s^{-1} (at >30 °C) during the whole plant measurements. However, preliminary F_V/F_m measurements during the two first days of the whole plant treatments (n=11) did not show any difference in F_V/F_m between treatments (data not shown).

4.3 Metabolomics

OPLS-DA is a type of discriminant analysis where the number of classes is specified *a priori*, and due to this, there is a high chance of overfitting the model to the dataset (Worley and Powers, 2013). The <0.5 Q^2 of the two models (two hours vs. control and treated vs. control) are lower than what is recommended (SIMCA 14 User Guide, p. 341) indicating overfitting and a lack of reliability (Triba et al., 2015). This was further confirmed by the prediction rate of the training datasets (60-80 %), which is too low to confirm the validity of the model, but suggests a difference in metabolite concentration between treated leaf classes (both immediately and two-hours after treatment) and the control plants, as shown by the $\log_2$ fold change.

The increased metabolic activity with temperature is evident with the doubling in phosphoric acid (P_i) immediately after heat treatment (presumably through the use of adenosine triphosphate), and the rise in succinic acid could either be a results of an up-regulation of the citrate cycle (which correlates to the increase in P_i) and / or through a (non-detected) transient increase in γ-aminobutyric acid (GABA), as GABA is ultimately converted to succinate (Bouche and Fromm, 2004, Gilliham and Tyerman, 2016). Additional evidence for the increased metabolic activity comes from the accumulation of valine, alanine, and threonine which could derive from increased amino acid production and/or enhanced heat-induced protein disintegration (Krasensky and Jonak, 2012b, Mayer et al., 1990). The accumulation of amino acids may also come from their role as compatible solutes (Yancey et al., 1982), which are thought to mediate osmotic adjustments and protect sub-cellular structures, but the present evidence of this is more correlative than causal (Hare et al., 1998).

A study by Panikulangara et al. (2004) using transgenic *A. thaliana* overexpressing a heat shock factor that targets the galactinol synthase-1 gene (GolS1) showed a substantially accumulation of raffinose while knockout GolS1 failed to accumulate galactinol and raffinose after a heat shock.

Kaplan et al. (2004) further confirmed the galactinol and raffinose relationship with a sustained increase (over 4 h) in both metabolites. In the present study, there was a sustained increase in galactinol, but an immediate decrease in raffinose. It is possible that the immediate reduction in raffinose is caused by increased metabolic activity and that there is delayed raffinose synthesis not detected within the two-hour timeframe. The accumulation of raffinose is observed in response to several types of abiotic stresses. The role of raffinose and to what extent it in itself confers increased stress tolerance is unclear (Krasensky and Jonak, 2012a), although elevated raffinose and galactinol levels are suggested to function as hydroxyl radical scavengers (Nishizawa et al., 2008). Galactinol is also a product of the conversion of D-galactose to D-myo-inositol, and myo-inositol has been linked to several stress responses (Loewus and Murthy, 2000), including oxidative stress (Smirnoff and Cumbes, 1989).

The increase in α-tocopherol and (E)-5-caffeoyl quinic acid two hours after the heat treatment and immediately after the treatment suggest an increase in antioxidant activity (possibly caused by photoinhibition). α-Tocopherol (Vitamin E) is an essential component for the prevention of photooxidative damage to membranes, both through hydroxyl scavenging activities and membrane stabilization (Fryer, 1992). Caffeoyl quinic acid is a metabolite of the phenylpropanoid pathway showing reactive oxygen species scavenging activity and involvement in biotic and abiotic stress responses (Atanasova-Penichon et al., 2016, Foyer and Noctor, 2005, Harborne and Williams, 2000, Oh et al., 2009).

High temperatures alter the properties of (plant) membranes through an acceleration of kinetic energy increasing the permeability of the membranes (Wahid et al., 2007). The increase in ethanolamine suggest changes within the membrane caused by the heat treatment as ethanolamine is important for the synthesis of phosphatidylethanolamine (PE) (Gibellini and Smith, 2010, Kennedy and Weiss, 1956), which constitutes a large part of the phospholipids in eukaryotic membranes (van Meer et al., 2008). PE is important for attaining a functional membrane architecture, and it is thought that PE causes an increase in lateral pressure and thereby introduces curvature stress in the membrane allowing for proper function of peripheral membrane proteins (Dowhan and Bogdanov, 2009). Recent research suggest that increased membrane fluidity could be counteracted by an increase in PE (Dawaliby et al., 2016) in eukaryotic cells. There was an increase in specific PE species in wheat (*Triticum aestivum* L.) leaves after exposure to heightened day and night growth temperatures, using the heat-susceptible genotype Karl 92 (Narayanan et al., 2016). Alternatively, there was no change in PE levels after exposure to 38 °C in wt *A. thalina* (Chen et al., 2006).

There was a 9-fold increase in maltose after two hours and Kaplan et al. (2004) found a corresponding (transient) increase in maltose. It is possible that the large increase in maltose is due to mobilization of the cells energy reserves caused by increased metabolic activity, as maltose is produced by the breakdown of glucans (starch) by β-amylases. Maltose can be converted to glucose in the cytosol and, subsequently, fructose and sucrose (Kotting et al., 2010). The increased metabolic activity after two hours could be a response to heat induced stress as demonstrated by the possible reduction in ΔA_{Plant} and the suggested metabolic evidence of photoinhibition/membrane alterations. Alternatively, the accumulation of carbohydrates could also be as a result of their (possible) role as compatible solutes (Hare et al., 1998).

4.4 Future directions

The effects of heat stress on *P. dahlianum* are probably reversible over a certain timeframe given the results by Buchner et al. (2015), and the biggest detrimental effects of heat waves on arctic plants are likely to be come from successive heat waves within one growing season (Marchand et al., 2006a). Future research should, therefore, focus on repeated heat stress events both within and between growing seasons. The accumulated evidence of heat wave studies indicates that an increase in the frequency and intensity of heat waves may be detrimental for Arctic plant growth and development, albeit with a highly species-specific response. On the molecular level there is a need for long-term heat stress studies to fully elucidate the metabolic response to heat waves. The species specific response to heat stress may also alter community composition and could cause an increase in the establishment of non-arctic plant species that are better adapted to higher temperatures.

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6. Appendix

Table S1. A_{Plant}, ΔA_{Plant}, $R_{Plant+S}$ and $\Delta R_{Plant+S}$ (CO_2 μmol mol^{-1}) of whole plant (*Papaver dahlianum*) was measured with a modified CPY-5 canopy assimilation chamber connected to CIRAS-3 portable photosynthesis systems at three different temperature categories (high, medium and low), before and after a 45-minute heat treatment, with each temperature treatment replicated six times. The measured increase (respiration) or decrease (photosynthesis) of the CO_2 concentration (μmol m^{-2} s^{-1}) in the chamber, with the net change in CO_2 being the ΔCO_2. Negative values (photosynthesis) was converted to positive values, and positive values (respiration) were converted to negative values. Measurements were done both in the light ($\Delta CO_{2\text{-}Light}$) and in the dark ($\Delta CO_{2\text{-}Dark}$). The $\Delta CO_{2\text{-}Light}$ incorporates soils respiration and whole plant net photosynthesis while $\Delta CO_{2\text{-}Dark}$ ($R_{Plant+S}$) gives whole plant respiration and soil respiration. Subtracting $\Delta CO_{2\text{-}Dark}$ from $\Delta CO_{2\text{-}Light}$ gives the whole plant gross photosynthesis and the unknown soil respiration component (A_{Plant}). If there was two or more consecutive $\Delta CO_{2\text{-}Light}$ or $\Delta CO_{2\text{-}Dark}$, then the average was used. Subtracting A_{Plant} before the heat treatment from A_{Plant} after the heat treatment results in the difference in A_{Plant} (ΔA_{Plant}), the same calculation was done for $R_{Plant+S}$ ($\Delta R_{Plant+S}$), and the light intensity giving, ΔLight. A Kruskal-Wallis test was used to test if there was any difference in the temperature (°C) before treatment, results are presented in Table S2. Analysis of variance (ANOVA) and Tukey honest significance difference (Tukey HSD) was used to test for differences between temperature treatment group means (high, medium and low) in A_{Plant} and light intensity (μmol m^{-2} s^{-1}) before heating, in addition to ΔA_{Plant} and $\Delta R_{Plant+S}$, with a $p<0.05$ significance level (significant differences are marked in bold). An ANCOVA was performed to test the effect of ΔLight both as a main effect and as an interaction effect with the treatment groups on ΔA_{Plant}, the R^2 in these two models are the adjusted R^2. Note that the values of A_{Plant} are log transformed and have not been back transformed. All standard errors are those from the ANOVA /ANCOA models.

Response variable	Treatment	Mean	±SE	df	F	Model p-value	R^2	Treatment contrasts	Tukey HSD p-value
A_{Plant} (Before)	High	3.57	0.34	2,15	5.48	**0.016**	0.42	**Low-High**	**0.013**
	Medium	2.01						Medium-High	0.17
	Low	2.67						Medium-Low	0.37
Light (Before)	High	1282.3	139.6	2,15	5.09	**0.020**	0.40	**Low-High**	**0.046**
	Medium	761.5						**Medium-High**	**0.029**
	Low	714.4						Medium-Low	0.97
$\Delta R_{Plant+S}$	High	0.57	3.23	2,15	0.18	0.84	0.023	NA	NA
	Medium	0.50							
	Low	2.89							
ΔA_{Plant}	High	-19.53	4.56	2,15	5.60	**0.015**	0.43	**Low-High**	**0.017**
	Medium	0.85						Medium-High	0.056
	Low	-3.19						Medium-Low	0.81
ANCOVA								ANCOVA p-value	
ΔA_{Plant}	Temperature	NA	NA	2,14	5.56	**0.027**	0.36	**0.016**	NA
	ΔLight			1,14	1.11			0.31	
ΔA_{Plant}	Temperature	NA	NA	2,12	5.51	**0.070**	0.34	**0.02**	NA
	ΔLight			1,12	1.08			0.32	
	ΔLight*Temperature			2,12	0.83			0.46	

Table S2. A_G, ΔA_G, A_N, R_{Leaf}, ΔR_{Leaf} (CO_2 μmol m^{-2} s^{-1}) and g_s (mmol H_2O m^{-2} s^{-1}) of single leaves (corrected for leaf area) on individual *Papaver dahlianum* was measured at three different temperature categories (high, medium and low) before and after a 45-minute heat treatment measured with a CIRAS-3 portable photosynthesis systems PLC3 leaf cuvette under constant light (800 μmol m^{-2} s^{-1}). The high and medium heat treatments were replicated six times, while the low temperature treatment was replicated five times. Net photosynthesis (A_N) measured in the dark is the respiration of the leaf and is defined as $R_{Leaf.}$ Gross photosynthesis (A_G) was calculated by subtracting R_{Leaf} from A_N. The difference in A_G from before to after the heat treatment (defined as ΔA_G) was calculated by subtracting A_G at 13 °C before heat treatment from A_G at 13 °C after the heat treatment. The same calculations were done for R_{Leaf} giving ΔR_{Leaf}. A Kruskal-Wallis test and Kruskal-Wallis post hoc test after Nemenyi was used to test if there was a difference between treatments in A_G, R_{Leaf}, A_N and g_s before heating, in addition to ΔA_G and ΔR_{Leaf}, with a significance level of $p<0.05$ (significant differences are marked in bold).

Response variable	Treatment	Mean	±SE	df	χ^2	Model p-value	Treatments contrasts	Post hoc test p-value
A_G (Before)	High	11.17	1.99	2	3.60	0.17	NA	NA
	Medium	12.14	1.60					
	Low	15.57	1.82					
ΔA_G	High	-4.33	0.92	2	7.89	**0.019**	**Low-High**	**0.015**
	Medium	-2.65	0.96				Medium-High	0.60
	Low	-0.33	0.18				Medium-Low	0.15
R_{Leaf} (Before)	High	-0.94	0.16	2	0.65	0.72	NA	NA
	Medium	-0.78	0.14					
	Low	-0.79	0.47					
ΔR_{Leaf}	High	-2.09	0.80	2	2.53	0.28	NA	NA
	Medium	-0.48	0.17					
	Low	-1.20	0.78					
A_N (Before)	High	10.22	1.88	2	4.50	0.11	NA	NA
	Medium	11.36	1.51					
	Low	14.79	1.73					
g_s (Before)	High	135.28	28.46	2	2.17	0.34	NA	NA
	Medium	181.01	33.09					
	Low	245.37	63.64					
Temperature (Before)*	High	19.11	0.70	2	7.06	**0.029**	**Low-High**	**0.024**
	Medium	14.88	0.56				Medium-High	0.21
	Low	15.55	1.74				Medium-Low	0.61

* Temperature (before) is the temperature of the modified CPY-5 canopy assimilation chamber, see Table S1 for further details.

Table S3. The effect of leaf temperature on stomatal conductance (mmol H_2O m^{-2} s^{-1}) (g_s), sub-stomatal CO_2 concentration (µmol mol^{-1}) (C_i), respiration (µmol CO_2 m^{-2} s^{-1}) (R_{Leaf}) and net photosynthesis (µmol CO_2 m^{-2} s^{-1}) (A_N) of *Papaver dahlianum* leaves, tested with linear regression. Measurements were done on single leaves of individual plants in three different temperature categories (°C), high (30.20 ±0.82), medium (23.79 ±0.42), low (15.87 ±0.37) with a CIRAS-3 portable photosynthesis systems PLC3 leaf cuvette under constant light (800 µmol m^{-2} s^{-1}). Each data point in the regression is the average of 12 logged values (leaf temperature, A_N, g_s, C_i, and R_{Leaf}) taken under a 2-minute measurement period in the light at the end of a 45 min heat treatment at high, medium or low temperatures. Significant models with a $p<0.05$ are marked in bold.

Response variable	Parameter	Mean	±SE	t-value	p-value	df	F-value	Model p-value	R^2
A_N	(Intercept)	23.67	3.26	7.27	<0.001	1,15	10.73	**0.0051**	0.51
	Temperature	-0.54	0.14	-3.97	0.0012				
g_s	(Intercept)	419.85	61.90	6.78	<0.001	1,15	19.85	**<0.001**	0.57
	Temperature	-11.55	2.59	-4.46	<0.001				
C_i	(Intercept)	309.22	27.085	11.42	<0.001	1,15	10.37	**0.0057**	0.41
	Temperature	-3.653	1.13	-3.22	0.0057				
R_{Leaf}	(Intercept)	1.55	1.15	1.35	0.20	1,15	10.40	**0.0057**	0.41
	Temperature	-0.16	0.049	-3.23	0.0057				

Table S4. The trends in average daily max temperature (°C) ($Tmax_H$), length and frequency of all heatwaves at Svalbard Airport in the period 1976-2015 for the months June, July, August and September was explored with a seasonal Kendall test and a Mann-Kendall test. One heat wave is defined as three or more consecutive days with a daily max temperature ≥10°C. The trends in $Tmax_H$ and length were tested with a seasonal Kendall test, while the trends in frequency was tested with a Mann-Kendall test.

Response Variable	Date	Block	SKT slope	Tau	Score	Two-sided p-value
$Tmax_H$	Year	Month	-0.017	-0.14	-129	0.19
Length	Year	Month	0	-0.054	-48	0.55
Frequency	Year	Month	NA	0.0043	3	0.98

Table S5. The r^2X, r^2Y and Q^2, components for two orthogonal projections to latent structures discriminant analysis (OPLS-DA) models and their respective training sets on metabolites from heat treated *Papaver dahlianum* leaves. Leaves where taken from plants heated with a modified CPY-5 canopy assimilation chamber connected to CIRAS-3 portable photosynthesis system under natural sunlight for 45 min to one of three different temperature categories: high (32.89 ±0.91 °C), medium (21.88 ±0.88 °C) and low (17.01 ±1.17 °C). The Treated vs. Control model consists of all control samples (n=26) and all samples taken immediately from heat treated individuals (n=19), the Two-hour vs. Control model consists of samples taken 2 hours after the heat treatment (n=15) and all control samples. Each training set consists of 2/3 of the complete dataset and was used to predict the class membership of the excluded classes, such that all samples were predicted once. The prediction rate is the percentage of the correctly predicted class membership.

Model	r^2X	r^2Y	Q^2	Components	Treated	Control	N	Prediction rate (%)
Treated vs. Control	0.28	0.87	0.34	1+2+0	19	26	45	
Training set A	0.33	0.91	0.24	2+0+0	12	18	30	60
Training set B	0.47	0.99	0.50	1+5+0	13	17	30	80
Training set C	0.14	0.49	0.033	1+0+0	13	17	30	53.33
Two-hour vs. Control	0.43	0.96	0.47	1+4+0	16	26	42	
Training set A	0.34	0.907	0.24	1+2+0	10	18	28	64.29
Training set B	0.41	0.97	0.64	1+3+0	11	17	28	64.29
Training set C	0.44	0.99	0.38	1+4+0	11	17	28	71.43

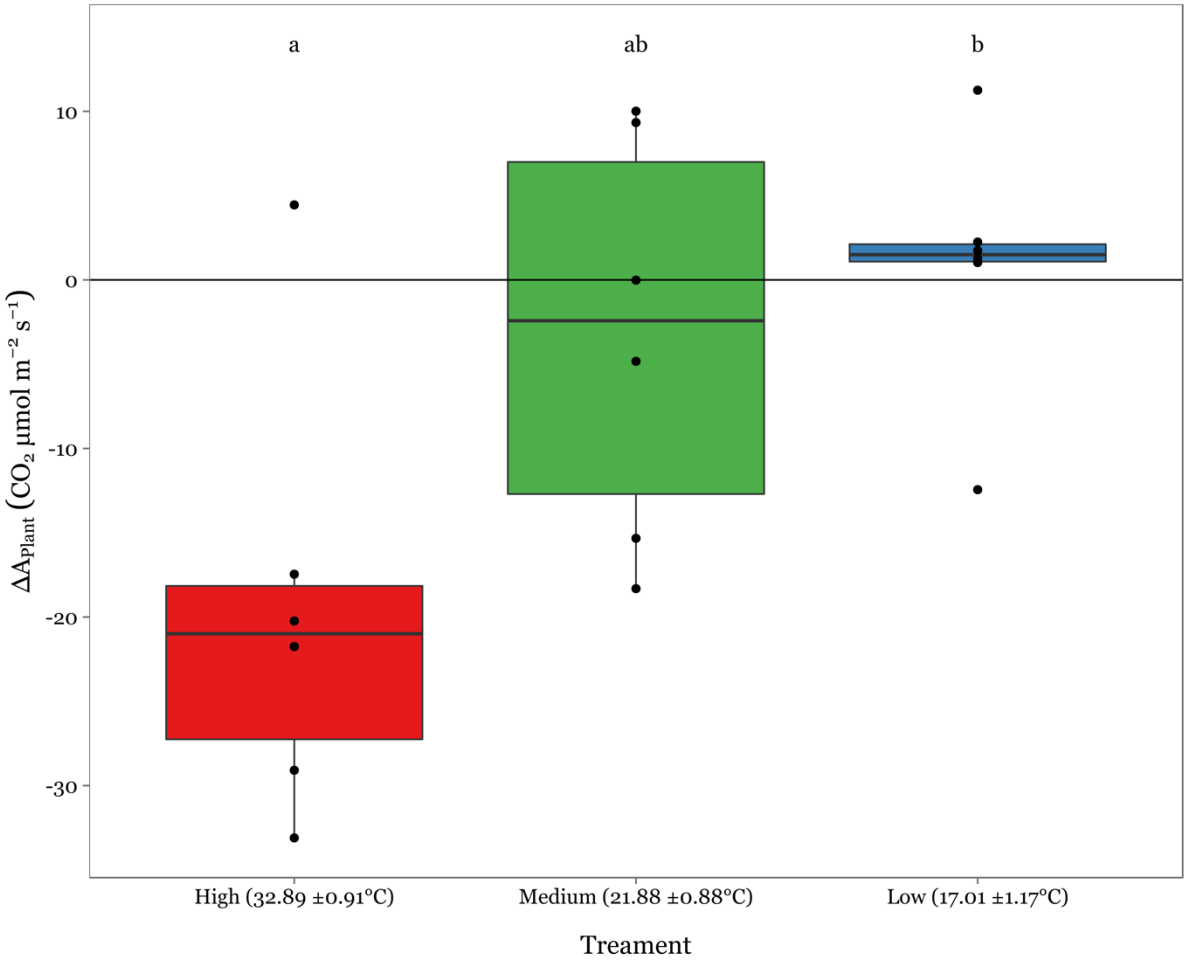

Figure S1. The ΔA_{Plant} (CO_2 µmol mol^{-1}) of the whole plant (*Papaver dahlianum*) measured with a modified CPY-5 canopy assimilation chamber connected to CIRAS-3 portable photosynthesis systems at three different temperature categories (high, medium and low), before and after a 45 min heat treatment under natural sunlight. The given treatment temperature (°C) is the temperature within the chamber during the 60-seconds closed system measurements in the light at the end of the 45 min treatment period and are approximations of the 45 min treatment temperature. Measurements were done both in the light ($\Delta CO_{2\text{-}Light}$) and in the dark ($\Delta CO_{2\text{-}Dark}$). The $\Delta CO_{2\text{-}Light}$ incorporates soil respiration and whole plant net photosynthesis while $\Delta CO_{2\text{-}Dark}$ gives whole plant respiration and soil respiration. Subtracting $\Delta CO_{2\text{-}Dark}$ from $\Delta CO_{2\text{-}Light}$ gives the whole plant gross photosynthesis and the unknown soil respiration component (A_{Plant}). If there was two or more consecutive $\Delta CO_{2\text{-}Light}$ or $\Delta CO_{2\text{-}Dark}$, then the average was used. Subtracting A_{Plant} before the heat treatment from A_{Plant} after the heat treatment results in the difference in A_{Plant} (ΔA_{Plant}).

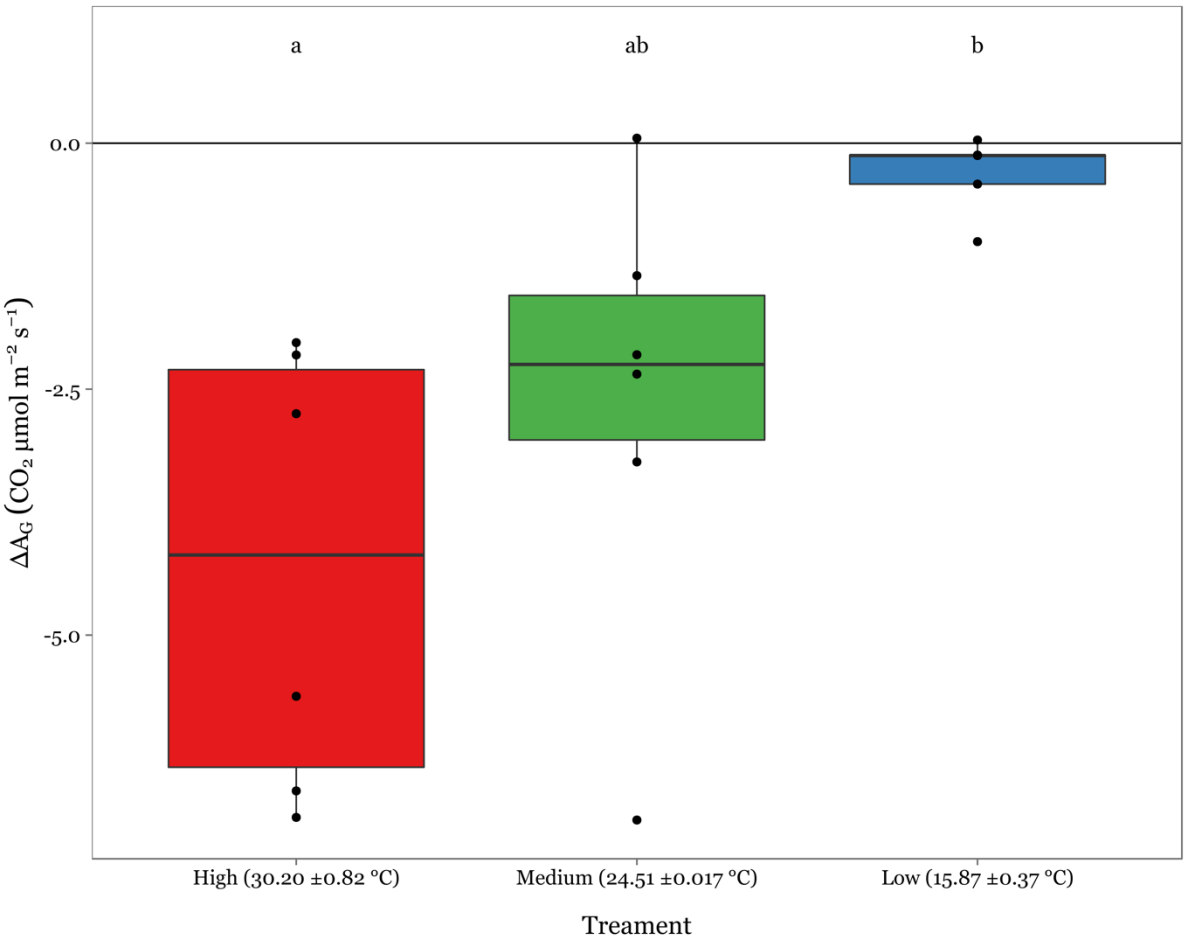

Figure S2. The ΔA_G (CO_2 µmol m^{-2} s^{-1}) of single leaves (corrected for leaf area) on individual *Papaver dahlianum* at three different temperatures categories (high, medium and low) before and after a 45-minute heat treatment measured with a CIRAS-3 portable photosynthesis systems PLC3 leaf cuvette under constant light (800 µmol m^{-2} s^{-1}). The given treatment temperatures (°C) is the average leaf temperature under a 2-minute measurement period in the light at the end of the 45-minute treatment period and are approximations of the leaf temperature during the heat treatment. Net photosynthesis (A_N) measured in the dark is the respiration of the leaf and is defined as R_{Leaf} (where one measurement is the average of 12 logged values within a two-minute period in the light and dark). Gross photosynthesis (A_G) for was calculated by subtracting R_{Leaf} from A_N, the difference in A_G from before to after the heat treatment (defined as ΔA_G) was calculated by subtracting A_G at 13 °C before heat treatment by A_G at 13 °C after the heat treatment.

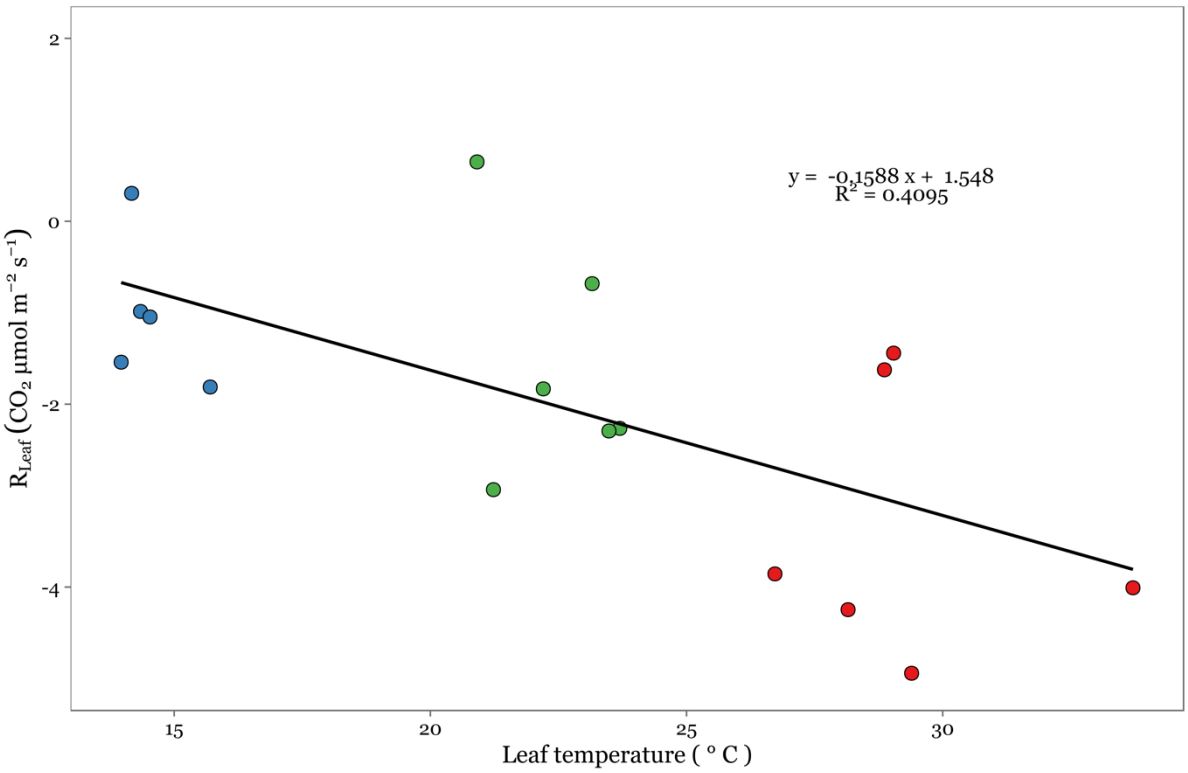

Figure S3. The effect of leaf temperature on respiration (CO_2 µmol m^{-2} s^{-1}) (R_{Leaf}) on *Papaver dahlianum* leaves. Measurements were done on single leaves of individual plants at three different temperature categories, high (red), medium (green), low (blue) with a CIRAS-3 portable photosynthesis systems PLC3 leaf cuvette under constant light (800 µmol m^{-2} s^{-1}) at the end of a 45 min heat treatment. One circle represents a single leaf on an individual plant and is the average of 12 logged values (leaf temperature and R_{Leaf}) taken under a 2-minute measurement period in the light at the end of the heat treatment period. The regression line and its coefficients are derived from a linear model with leaf temperature as a predictor variable and R_{Leaf} as a response variable.

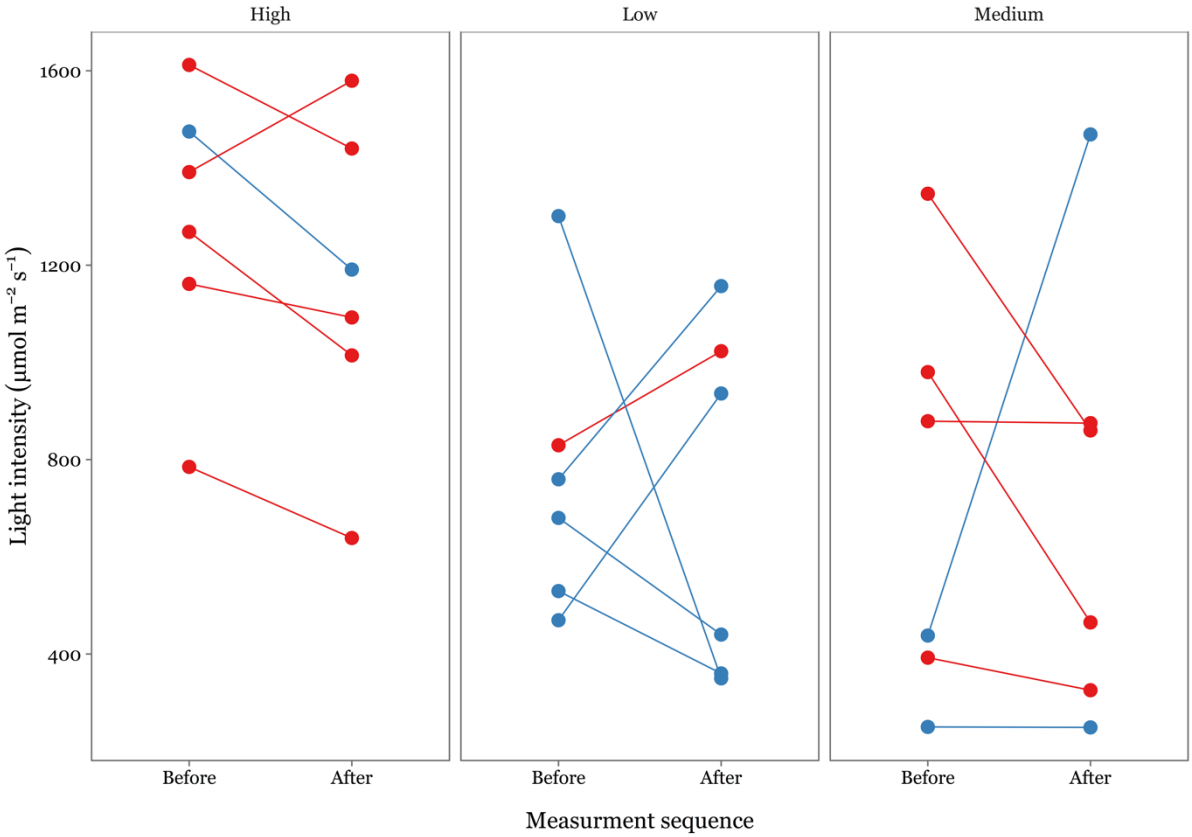

Figure S4. Light intensity (µmol m^{-2} s^{-1}) experienced by *Papaver dahlianum* under whole plant gas-exchange measurements with a modified CPY-5 canopy assimilation chamber connected to CIRAS-3 portable photosynthesis systems at three different temperature categories (high, medium and low), before and after a 45 min heat treatment, with each temperature treatment replicated six times. The light intensity is the sunlight within the chamber during a 60-seconds closed system measurement. The temperatures (°C) within the chamber (containing the plant) was 19.11 ±0.70 before and 32.89 ±0.91 after heating at high temperature, 14.88 ±0.56 before and 21.88 ±0.88 after heating at medium temperature and 15.55 ± 1.74 before and 17.01 ±1.17 after heating at low temperature. The increase (respiration) or decrease (photosynthesis) of the CO_2 concentration (µmol mol^{-1}) in the chamber was measured, with the net change in CO_2 being the ΔCO_2. Negative values (photosynthesis) was converted to positive values, and positive values (respiration) were converted to negative values. Measurements were done both in the light ($\Delta CO_{2\text{-Light}}$) and in the dark ($\Delta CO_{2\text{-Dark}}$). The $\Delta CO_{2\text{-Light}}$ incorporates soils respiration and whole plant net photosynthesis while $\Delta CO_{2\text{-Dark}}$ ($R_{Plant+S}$) gives whole plant respiration and soil respiration. Subtracting $\Delta CO_{2\text{-Dark}}$ from $\Delta CO_{2\text{-Light}}$ gives the whole plant gross photosynthesis and the unknown soil respiration component (A_{Plant}). The connected points show the change in light intensity from before to after the treatments for a single individual. A red line indicates a decrease in A_{Plant} from the before to the after treatment measurements, blue line indicates an increase in A_{Plant}.