# Influence of artificial sunlight from a microwave plasma lamp on morphology and secondary metabolism of horticultural plants

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# Abbreviations

| | |
|---|---|
| % | percent |
| λ | lambda; wavelength |
| µ | micro |
| AS | artificial sunlight |
| a.u. | arbitrary units |
| B | blue light [400-500 nm] |
| c | speed of light [299792458 m/s] |
| °C | degree Celsius |
| CDM | ceramic metal halide |
| CE | catechin equivalents |
| CRI | color rendering index |
| cry | cryptochrome |
| d | day |
| Da | dalton |
| DAD | diode array detector |
| Dim | dimension |
| DLI | daily light integral [mol/m²d] |
| dw/DW | dry weight |
| et al. | et alii |
| FAD | flavin adenine dinucleotide |
| F-C | Folin-Ciocalteu |
| FL | fluorescent lamp |
| FMN | flavin mononucleotide |
| FR | far-red light [700-800 nm] |
| FRET | Förster resonance energy transfer |
| fw/FW | fresh weight |
| G | green light [500-550 nm] |
| g | gram |
| GAE | gallic acid equivalents |
| GC | gas chromatography |
| h | hour |
| *h* | Planck's constant [$6.62607004 \cdot 10^{-34}$ Js] |
| HPLC | high-pressure liquid chromatography |
| HPS | high-pressure sodium |
| IR | infrared radiation |
| J | joule |
| k | kilo |
| L | liter |
| LOV | light oxygen voltage |
| LED | light-emitting diodes |
| lm | lumen |
| LMA | leaf mass per area [mg dw/cm²] |
| m | meter |
| m² | square meter |
| M | molar [moles per liter] |
| min | minute |
| ml | milliliter |
| mm | millimeter |
| MPL | microwave plasma lamp |
| MS | mass spectroscopy |
| NC | negative control |
| $N_A$ | Avogaphdro's constant [$6.02214076 \cdot 10^{23}$ $mol^{-1}$] |
| nm | nanometer |
| NIR | near infrared radiation |
| PAR | photosynthetic active radiation [400-700 nm] |
| PBAR | photo-biologically active radiation [280-800 nm] |
| PC | positive control |
| PCA | principal component analysis |
| phot | phototropin |
| PF | photon flux [µmol/s] |
| $P_{FR}$ | far-red light sensing phytochrome |
| $P_n$ | net photosynthesis |
| $P_R$ | red light sensing phytochrome |

| | |
|---|---|
| PFE | photon flux per electricity power [µmol/J] |
| PPFD | photosynthetic photon flux density [µmol/m²s] |
| QE | quercetin equivalents |
| $R^2$ | coefficient of determination |
| R | red light [600-700 nm] |
| RA | rosmarinic acid |
| R:FR | red to far-red ratio |
| ROS | reactive oxygen species |
| RT | room temperature |
| s | second |
| sd. | standard deviation |
| sp. | species |
| TAC | total anthocyanin content |
| TPC | total phenolic content |
| $TFC_{415\ nm}$ | total flavonoid content (measured at 415 nm) |
| $TFC_{510\ nm}$ | total flavonoid content (measured at 510 nm) |
| UV | ultraviolet |
| UV-A | ultraviolet A light [315-400 nm] |
| UV-B | ultraviolet B light [280-315 nm] |
| V | volt |
| v | volume |
| W | watt |
| Y | yellow light [550-600 nm] |

# 1. Abstract

Light is one of the most important abiotic factors for plant physiological processes. In addition to light intensity, the spectral quality of light can also influence the plant morphology and the content of secondary metabolites. In the horticultural industry, artificial light is used in to enable year-round production of herbs, ornamental plants and vegetables in winter terms. Until today, discharge lamps like high-pressure sodium (HPS) lamps, emitting predominantly orange and red light and high amounts of infrared radiation, are the most common lamp systems in greenhouses. In the last decades, light-emitting diodes (LEDs) emerged as an efficient alternative light source. LEDs have the advantage of distinct adjustments to the light spectrum. For a usage in horticultural industry LEDs are often too expensive. Furthermore, reduced plant growth can occur due to incorrectly adjusted light spectra and lower leaf temperatures caused by the lack of infrared radiation.

In a research project (LOEWE, funding no. 487/15-29) funded by the Hessen State Ministry of Higher Education, Research and Arts, Microwave plasma lamps (MPL) were tested as new light sources for horticultural industry and plant research. The electrodeless lamp systems emit light in similar properties like sun light. The aim of the study was to determine the influence of artificial sunlight of the MPL on the accumulation of secondary metabolites, plant architecture and plant physiology of three different species (coleus, basil and potted roses). The MPL was compared with other light systems such as commercial HPS lamps, LEDs or ceramic metal halide lamps (CDM). In addition to morphological parameters such as plant height, internode length or fresh and dry weight, the phenolic content of leaves grown under the respective light sources were examined.

Overall an increased far-red light content in the emission spectra of the MPL showed high influence on the plant architecture which was observed in all three plant species. Artificial sunlight from MPL induced stem elongation in coleus and basil plants, compared to the other tested light sources. In potted roses a reduced branching degree was observed under MPL light compared to HPS grown plants.

In addition to the impact of far-red light also the blue light content of the emission spectra was found to be a strong influencing factor for plant physiological processes. A positive correlation between blue light content and leaf thickness was determined in coleus cultivated under MPL, LED, HPS and CDM lamps. Low blue light content in HPS emission spectra resulted in shade-adapted leaves with low photosynthetic capacity and susceptibility to high

irradiances. Blue light was assumed to increase phenolic metabolites in basil and rose leaves. Furthermore, the different light treatments resulted in an alteration of the composition of essential oils of basil.

Experiments with coleus plants demonstrated that besides light color also the infrared radiation, had an influence on secondary metabolites by causing different leaf temperatures. Coleus plants grown with MPL showed the lowest content of phenolic compounds such as rosmarinic acid per dry weight. Infrared radiation resulted in a faster plant development indicated by increased biomass production and higher leaf formation rate as observed in coleus and basil plants.

The results obtained in this study show that the influence of leaf temperature should always be considered when comparing different lamp systems. Especially when LEDs are compared to discharge lamps an overestimation of light color can be a consequence since also infrared radiation influences the content of phenolic compounds and plant growth.

# 2. Summary

Plants need to adapt to changing environmental conditions. In addition to abiotic conditions such as temperature, humidity, nutritional resources and light intensity the spectral light quality also has an influence on morphology and phytochemical constitutions of plants. In nature, the quality of light changes during the day, through the weather or through shading by neighboring plants. Plants implement morphological and physiological adaptations to distinct light colors, which are signals for an altered light environment.

Far-red light (700-800 nm) results in an increased elongation growth in the term of shade avoidance, while blue light (400-500 nm) may trigger the accumulation of secondary metabolites in order to protect plant cells from high irradiances and harmful ultraviolet (UV) radiation.

For around 160 years, plants are exposed to an artificial light environment through plant lighting. Different lamp systems emerged enabling the plant cultivation in northern hemispheres where in winter periods days are too short and light intensities are too low. Through the use of artificial light, constant plant quality can be guaranteed all year round.

Until today, discharge lamps like high-pressure sodium (HPS) lamps, emitting predominantly orange and red light and high amounts of infrared radiation, are the most common lamp systems in greenhouses. In the last decades, light-emitting diodes (LEDs) emerged as an efficient alternative light source. LEDs have the advantage of distinct adjustments to the light spectrum. Due to the lack of emission of infrared radiation, they are suitable for multilayered approaches and small climate chambers. For a usage in horticultural industry LEDs are often too expensive. Furthermore, reduced plant growth can occur due to incorrectly adjusted light spectra and lower leaf temperatures caused by the lack of infrared radiation.

In a research project (LOEWE, funding no. 487/15-29) funded by the Hessen State Ministry of Higher Education, Research and Arts, Microwave plasma lamps (MPL) were tested as new light sources for horticultural industry and plant research. The electrodeless lamp systems emit light in similar properties like sun light. The influence of artificial sunlight of the MPL on the accumulation of secondary metabolites, plant architecture and plant physiology was examined. The results are divided into four chapters and were carried out with three different plant species:

| | |
|---|---|
| Chapter 5 & 6 | Coleus *(Plectranthus scutellarioides)* |
| Chapter 7 | Sweet basil (*Ocimum basilicum*) |
| Chapter 8 | Potted rose (*Rosa hybrida*) |

**Summary: Chapter 5**

In chapter 5, the influence of the artificial sunlight of the MPL on the morphology and the content of secondary metabolites of coleus was examined. The MPL was compared with other lamp systems such as commercial HPS, LEDs and ceramic metal halide lamps (CDM). The experiments were carried out in a climate room in order to evaluate the influence of the light sources in the absence of global radiation. Coleus are used as ornamental plants, but are also of pharmaceutical relevance due to the high content of phenolic ingredients. The blue light content of the four lamp systems is different and it was suspected that this could influence the content of secondary metabolites.

The lamp systems differ not only in the spectral quality of light, but also in the emission of infrared radiation. Especially when LEDs are compared with discharge lamps, leaf temperature can be altered since LEDs are not emitting infrared radiation which might lead to an overestimation of the impact of light color. It was suspected that an altered leaf temperature can also lead to a change in the content of secondary ingredients. The aim was therefore to investigate both aspects: The influence of spectral light quality and the influence of leaf temperature on the plant physiological processes of coleus.

The phenolic content was quantified using colorimetric methods and HPLC analysis. In addition to morphological parameters such as plant height, internode length, fresh and dry weight, leaf histological analysis were carried out. The leaf temperature under the respective lamp systems was measured with a thermal imaging camera.

The elongation growth was significantly higher in plants grown under the light from the MPL and the LED treatment, due to an increased content of far-red light. A higher proportion of the infrared radiation from the MPL and HPS lamps led to higher leaf temperatures and faster plant growth.

In addition, the content of phenolic ingredients such as rosmarinic acid in leaves that were developed under the light of CDM and LED was significantly higher. It was assumed that the reason for a higher absolute content of phenol components was not the blue light

component, but a lower leaf temperature. However, it was determined that the blue light content of the emission spectra correlated positively with the leaf thickness, leading to a higher accumulation of phenolic constitutions per leaf area.

**Summary: Chapter 6**

Chapter 6 shows additional light experiments with coleus. In previous investigations, it was found that leaves which were cultivated with a higher proportion of blue light had a thicker leaf thickness. In this chapter the effects of an altered leaf morphology on the photosynthetic capacity as well as the adaptation to high irradiance were examined.

Via gas exchange measurements, light saturation curves of coleus plants, which were cultivated with three different light qualities. were recorded. In addition, light stress experiments were carried out under direct sunlight with plants that had previously been cultivated under MPL, HPS, CDM and LED light in the absence of global irradiance.

In addition, the influence of the MPL in the greenhouse was examined. The experiments were carried out with different varieties of coleus to determine differences in the cultivar. Commercial HPS lamps were used as a reference light source.

The experiments showed that plants that were produced with a higher proportion of blue light had a higher photosynthetic capacity and were less susceptible to higher irradiances. This was due to the increased leaf thickness under a blue light enriched environment

The influence of the increased far-red light on the elongation growth under the sun-like light of the MPL could also be observed in the presence of global radiation and was detected in all cultivars of coleus. In regard to the content of secondary ingredients, cultivar differences were found.

**Summary: Chapter 7**

Chapter 7 displays how the light from the MPL changes the content of secondary ingredients in sweet basil. Basil is one of the most popular herbs in Europe. The typical taste and smell is caused by essential oils. However, basil also contains methyl eugenol, which is suspected to be carcinogenic. The aim was to investigate whether the content of methyl eugenol can be reduced by using different lamp systems, while increasing the internal and external plant quality. In addition to the sun-like light of the microwave plasma lamps, MPL were used, which emit a blue / green weighted light spectrum. Commercial HPS lamps were used as a reference. The essential oils were quantified by GC-MS. Colorimetric methods were used to

quantify non-volatile phenolic compounds. In addition to the analysis of secondary metabolites, morphological parameters were measured.
The elongation growth was higher under the sun-like light of the MPL compared to the other light sources due to the increased content of far-red light. A higher content of phenolic ingredients was measured in basil leaves that were grown under the sun-like light of the MPL. The content of essential oils was also changed under the respective light sources. Leaves grown under the light of the HPS showed lowest amount of linalool. The methyl eugenol content was the lowest under the sun-like light of the MPL.

**Summary: Chapter 8**

In chapter 8, experiments with potted roses were carried out to test if the MPL would be suitable for the horticultural industry. The influence of the sun-like light from the MPL was compared to commercial HPS lamps. Experiments were conducted in greenhouse and in a climatic room in the absence of global radiation. Potted roses are of great economic importance especially in the winter months with an increased demand due to the large number of public holidays. In addition to morphological parameters such as the degree of branching, number of flowers and plant height, the chlorophyll and flavonol content in the leaves was measured using a chlorophyll-phenol meter.
Potted roses, which were grown under the sun-like light, showed an earlier flowering, but displayed a lower degree of branching and lower number of flowers compared to plants grown with the light of the HPS lamps. This was due to the increased proportion of far-red light in the sun-like light spectrum of the MPL. The results were observed in the greenhouse and in the climatic room. Leaves of pot roses showed a higher flavonoid content and a lower chlorophyll content due to the increased blue light content in the light spectrum of the MPL.

The results obtained in this thesis show that the influence of leaf temperature should always be taken into account when different lamp systems are compared. Especially in the comparison of LEDs and discharge lamps, the spectral influence can be overestimated if the influence of the lamp systems on the leaf temperature is not taken into account. However, blue light and far-red light have a strong influence on plant physiological processes. Blue light leads to sun-adapted leaves with a high photosynthetic capacity and la lower susceptibility against high irradiances. Due to the increased leaf thickness, the content of secondary ingredients per leaf area is indirectly influenced by blue light. However, blue light

can also directly influence the content of phenolic ingredients and essential oils. Far-red light was the strongest factor influencing plant morphology.

Due to negative effects on plant morphological parameters such as increased elongation growth or a reduced degree of branching and moreover due to the low light efficiency, the MPL are rather unsuitable for commercial usage in horticulture. Nevertheless, the artificial sunlight from the MPL could be an alternative source of light for plant research if environmental close light conditions are to be simulated. LEDs are more suitable for future culture systems due to their higher efficiency and flexibility.

# 3. Zusammenfassung

Pflanzen müssen sich an veränderte Umweltbedingungen anpassen. Neben abiotischen Faktoren wie Temperatur, Wasser- und Nährstoffressourcen, Luftfeuchtigkeit sowie Lichtintensität hat auch die spektrale Lichtqualität einen Einfluss auf die Pflanzenmorphologie und den Gehalt von pflanzlichen sekundären Inhaltsstoffen. In der Natur verändert sich die spektrale Lichtqualität in Abhängigkeit des Tagesverlaufs, der Wetterlage oder durch Beschattung von benachbarten Pflanzen. Pflanzen nehmen unterschiedliche Lichtfarben als Signale für eine veränderte Lichtumgebung war. Infolgedessen reagieren Pflanzen mit morphologischen und physiologischen Veränderungen.

Zum Beispiel führt dunkelrotes Licht (700-800 nm) zu einem erhöhten Streckungswachstum im Prozess der Schattenvermeidungsreaktion, während blaues Licht (400-500 nm) zu einer erhöhten Akkumulation von Sekundärmetaboliten führen kann, um Pflanzenzellen vor hohen Strahlungsintensitäten sowie vor schädlichem ultravioletten Licht zu schützen.

Seit etwa 160 Jahren nehmen Pflanzen durch den Einsatz von Pflanzenbeleuchtung auch ein künstliches Lichtumfeld wahr. In den nördlichen Hemisphären ermöglichen verschiedene Lampensysteme eine Kultivierung von Nutz- und Zierpflanzen sowie Kräutern auch im Winter, wenn die Tage zu kurz und die Lichtintensitäten zu niedrig werden. Durch den Einsatz von künstlichem Licht kann somit das ganze Jahr über eine gleichbleibende Pflanzenqualität gewährleistet werden.

Entladungslampen wie Natriumdampf-Hochdrucklampen (HPS), die vorwiegend oranges und rotes Licht sowie Infrarotstrahlung emittieren, sind bis heute die meist eingesetzten Lampensysteme in Gewächshäusern. In den letzten Jahrzehnten haben sich Licht-Emittierende-Dioden (LEDs) zu einer effizienten, alternativen Lichtquelle entwickelt. LEDs haben den Vorteil, dass das Lichtspektrum gezielt angepasst werden kann. Aufgrund der fehlenden Emission von Infrarotstrahlung eignen sie sich für mehrlagige Kultursysteme sowie für die Beleuchtung in Klimakammern. Für die Gartenbauindustrie sind die Anschaffungskosten der LEDs jedoch häufig zu hoch. Außerdem kann aufgrund von suboptimalen Lichtqualitäten und fehlender Wärmestrahlung ein vermindertes Pflanzenwachstum auftreten.

In einem vom Hessischen Ministerium für Wissenschaft und Kunst geförderten Forschungsprojekt (LOEWE, Nr. 487/15-29) wurden Mikrowellen-Plasmalampen (MPL)

als neue Lichtquelle für den Gartenbau sowie für die Pflanzenforschung getestet. Die elektrodenlosen Lampensysteme emittieren ein kontinuierliches, sonnenähnliches Licht.
In dieser Arbeit wurde der Einfluss des künstlichen Sonnenlichts der MPL auf die Akkumulation von pflanzlichen sekundären Inhaltsstoffen sowie der Einfluss auf die Pflanzenmorphologie untersucht. Die Untersuchungen wurden mit drei verschiedene Pflanzenspezies durchgeführt und die Ergebnisse sind in vier Kapitel eingeteilt:

| | |
|---|---|
| Kapitel 5 & 6 | Buntnessel *(Plectranthus scutellarioides)* |
| Kapitel 7 | Basilikum (*Ocimum basilicum*) |
| Kapitel 8 | Topfrose (*Rosa hybrida*) |

**Zusammenfassung: Kapitel 5**

In Kapitel 5 wurde der Einfluss des sonnenähnlichen Lichts der MPL auf die Morphologie und den Gehalt an sekundären Inhaltsstoffe von Buntnesseln untersucht. Dabei wurde die MPL mit anderen Lichtsystemen wie handelsüblichen HPS, LEDs und Keramik-Metallhalogendampflampen (CDM) verglichen. Die Experimente wurden in einem Klimaraum durchgeführt, um den Einfluss der Lichtquellen ohne Globalstrahlung bewerten zu können. Buntnesseln werden in der Regel als Zierpflanzen genutzt, haben jedoch aufgrund des hohen Gehalts an phenolischen Inhaltsstoffen auch eine pharmazeutische Relevanz. Der Blaulichtgehalt der vier Lampensysteme ist unterschiedlich und es wurde vermutet, dass dies den Gehalt an sekundären Inhaltsstoffen beeinflussen könnte. Die verwendeten Lampensysteme unterschieden sich jedoch nicht nur in der spektralen Lichtqualität, sondern auch in der Emission von infraroter Strahlung.
Insbesondere, wenn LEDs, die keine Wärmestrahlung emittieren, mit Entladungslampen verglichen werden, kann der Einfluss der Lichtfarbe überbewertete werden. Es wurde vermutet, dass auch eine Veränderung der Blatttemperatur zu einer Veränderung des Gehalts an sekundären Inhaltsstoffen führen kann. Ziel war es daher beide Aspekte zu untersuchen: Den Einfluss der spektralen Lichtqualität sowie den Einfluss der Blatttemperatur auf die pflanzenphysiologischen Prozesse von Buntnesseln.
Der Gehalt phenolischer Inhaltsstoffen wurden mit kolorimetrischen Methoden sowie per HPLC quantifiziert. Neben morphologischen Parametern wie Pflanzenhöhe, Internodienlänge, Frisch- und Trockengewicht wurden auch blatthistologische

Untersuchungen durchgeführt. Die Blatttemperatur unter den jeweiligen Lampensystemen wurden mit einer Wärmebildkamera gemessen.
Das Streckungswachstum war bei Pflanzen die unter dem sonnenähnlichem Licht der MPL sowie der LED gewachsen waren, aufgrund des höheren Gehalts an dunkelrotem Licht, signifikant höher, aufgrund des höheren Gehalts an dunkelrotem Licht. Ein höherer Anteil der infraroten Strahlung der MPL und HPS Lampen führte zu höheren Blatttemperaturen und einem schnelleren Pflanzenwachstum.
Andererseits war der Gehalt phenolischer Inhaltsstoffen wie Rosmarinsäure in Blättern, die unter dem Licht der CDM und LED gewachsen waren deutlich höher. Es wurde vermutet, dass nicht der Blaulichtanteil, sondern eine niedrigere Blatttemperatur der Grund für einen höheren absoluten Gehalt an phenolischen Inhaltsstoffen war. Es zeigte sich jedoch, dass der Blaulicht-Anteil der Emission-Spektren positiv mit der Blattdicke korrelierte, sodass sich unter einem erhöhten Blaulichtanteil mehr phenolische Inhaltstoffe pro Blattfläche akkumulieren.

**Zusammenfassung: Kapitel 6**

In Kapitel 6 sind weitere Lichtexperimente mit Buntnesseln dargestellt. In vorherigen Untersuchungen zeigte sich, dass Blätter, die mit höheren Blaulichtanteilen kultiviert wurden, eine höhere Blattdicke aufwiesen. In diesem Kapitel wurden die Auswirkungen einer veränderten Blattmorphologie auf die photosynthetische Kapazität sowie die Adaptation gegenüber höheren Bestrahlungsstärken untersucht.
Mithilfe von Gaswechselmessungen wurden Lichtsättigungskurven von Buntnessel-Pflanzen aufgenommen, die mit drei unterschiedlichen Lichtqualitäten kultiviert wurden. Zudem wurden Lichtstressexperimente unter direktem Sonnenlicht mit Pflanzen durchgeführt, die vorher unter MPL-, HPS-, CDM- und LED-Licht ohne Globalstrahlung kultiviert wurden.
Des Weiteren wurde der Einfluss der MPL im Gewächshaus untersucht. Die Experimente wurden mit unterschiedlichen Buntnessel-Arten durchgeführt, um mögliche Sortenunterschiede festzustellen. Kommerzielle HPS-Lampen wurden als Referenz verwendet.
In den Experimenten zeigte sich, dass Pflanzen, die mit einem höheren Blaulichtanteil produziert wurden, eine höhere photosynthetische Kapazität aufwiesen und weniger anfällig

gegenüber höheren Bestrahlungsstärken waren. Dies lässt sich auf die erhöhte Blattdicke zurückführen.

Der Einfluss des erhöhten Anteils von dunkelrotem Licht auf das Streckungswachstum unter dem sonnenähnlichen Licht der MPL konnte auch in Anwesenheit von Globalstrahlung erfasst werden und wurde bei allen Buntnesselsorten beobachtet. In Hinblick auf den Gehalt von sekundären Inhaltsstoffen wurden jedoch sortenspezifische Unterschiede festgestellt.

**Zusammenfassung: Kapitel 7**

Wie das sonnenähnliche Licht der MPL den Gehalt sekundärer Inhaltstoffen in Basilikum verändert, ist in Kapitel 7 dargestellt. Basilikum ist eine der meistgefragten Topfkräuterarten in Europa. Der typische Geschmack und Geruch werden durch ätherische Öle verursacht. Jedoch enthält Basilikum auch Methyl-Eugenol, das im Verdacht steht krebserregend zu sein. Ziel dieser Versuchsreihe war es, zu untersuchen, ob der Gehalt von Methyl-Eugenol durch den Einsatz von verschieden Lampensystemen gesenkt werden kann, bei einer gleichzeitigen Erhöhung der inneren und äußeren Pflanzenqualität. Dabei wurden neben dem sonnenähnlichen Licht der Mikrowellen-Plasmalampen auch MPL verwendet, die ein blau/grün-gewichtetes Lichtspektrum emittieren. Kommerzielle HPS Lampen wurden als Referenz verwendet. Die ätherischen Öle wurden per GC-MS quantifiziert. Kolorimetrische Methoden wurden zur Quantifizierung von nicht flüchtigen Inhaltsstoffen verwendet. Neben inhaltsstofflichen Untersuchungen wurde morphologische Parameter gemessen.

Das Streckungswachstum war unter dem sonnenähnlichen Licht der MPL im Vergleich zu den anderen Lichtquellen aufgrund des höheren Dunkelrotlicht-Anteils höher. In Basilikumblättern, die unter dem sonnenähnlichen Licht der MPL gewachsen waren, wurde ein höherer Gehalt phenolischer Inhaltsstoffen gemessen. Auch der Gehalt ätherischen Ölen wurde unter den jeweiligen Lichtquellen verändert. Blätter, die unter dem Licht der HPS gewachsen waren, zeigten einen geringeren Gehalt an Linalool. Der Methyl-Eugenol Gehalt war unter dem sonnenähnlichen Licht der MPL am Niedrigsten.

**Zusammenfassung: Kapitel 8**

In Kapitel 8 wurden Experimente mit Topfrosen durchgeführt, um die Eignung der MPL für den Einsatz in der Gartenbauindustrie zu testen. Der Einfluss des sonnenähnlichen Lichts der MPL wurde mit kommerziellen HPS-Lampen verglichen. Experimente wurden mit und ohne Globalstrahlung durchgeführt. Topfrosen stellen insbesondere in den Wintermonaten

aufgrund der Vielzahl an Feiertagen eine große wirtschaftliche Bedeutung dar. Neben morphologischen Parametern wie Verzweigungsgrad, Blütenanzahl und Pflanzenhöhe wurde der Chlorophyll- und Flavonol-Gehalt in den Blättern mit einem Chlorophyll-Phenol-Meter gemessen.
Topfrosen, die unter dem sonnenähnlichen Licht gewachsen waren, blühten früher, zeigten jedoch im Vergleich zu Pflanzen, die mit dem Licht der HPS-Lampen kultiviert wurden, einen geringeren Verzweigungsgrad sowie eine geringere Anzahl an Blüten. Dies war dem erhöhten Dunkelrotlicht-Anteil im sonnenähnlichen Lichtspektrum der MPL zuzuschreiben. Die Ergebnisse wurden sowohl im Gewächshaus als auch im Klimakammer-Versuch beobachtet. Blätter von Topfrosen zeigten unter dem Licht der MPL einen höheren Gehalt an Flavanoiden und einen geringeren Chlorophyll-Gehalt aufgrund des erhöhten Blaulichtgehaltes.

Die in dieser Arbeit gewonnen Ergebnisse zeigen, dass beim Vergleich verschiedener Lampensysteme der Einfluss der Blatttemperatur immer mitberücksichtig werden sollte. Insbesondere beim Vergleich von LED-Systemen und Entladungslampen kann es zu einer Überschätzung des spektralen Einflusses kommen, wenn der Einfluss der Lampensysteme auf die Blatttemperatur nicht beachtet wird. Blaues Licht sowie dunkelrotes Licht haben einen starken Einfluss auf die Pflanzenmorphologie und den Gehalt an sekundären Inhaltsstoffen. Blaues Licht führt zu sonnen-adaptierten Blättern, die eine hohe photosynthetische Kapazität aufweisen und weniger anfällig gegenüber hohen Bestrahlungsstärken sind. Aufgrund der höheren Blattdicke wird der Gehalt an sekundären Inhaltsstoffen per Blattfläche indirekt durch blaues Licht beeinflusst. Blaues Licht kann jedoch auch den Gehalt von phenolischen Inhaltsstoffen sowie ätherischen Ölen direkt beeinflussen. Dunkelrotes Licht zeigte sich als stärkster Einflussfaktor auf die Pflanzenmorphologie.
Aufgrund negativer Begleiterscheinungen wie ein erhöhtes Streckungswachstum oder ein verminderter Verzweigungsgrad sowie aufgrund der geringen Lichteffizienz, sind die MPL für den kommerziellen Einsatz im Gartenbau eher ungeeignet. Dennoch könnte das künstliche Sonnenlicht von MPL eine alternative Lichtquelle für die Pflanzenforschung sein, wenn umweltnahe Lichtbedingungen simuliert werden sollen. Für zukünftige Kultursysteme sind LEDs aufgrund der höheren Effizienz sowie Flexibilität besser geeignet.

# 4. Introduction

All life on earth is directly or indirectly dependent on solar radiation. The sun enables adequate climate, regulates the circadian clock of many species and is the most important resource for photoautotroph organisms using the solar irradiance for photosynthesis.

Electromagnetic irradiance with an intensity of about 1000 W/m² from short ultraviolet (UV) radiation till long infrared radiation reaches the earth's surface. The human eye can perceive an irradiance from 380-780 nm called "visible light". The plant kingdom and other photoautotroph organism are also sensing light intensity and spectral quality of light for the adaptation to changing environmental conditions.

The spectral light quality regulates morphological and physiological reactions and can lead to accumulation of secondary metabolites acting as protectors against high irradiance and UV light. Since the development of artificial light, higher plants are exposed to an unnatural light environment. Supplemental light allows the production of horticultural crops in winter periods. With the recent increase of the usage of light-emitting diodes (LEDs), distinct light colors can be adjusted to regulate inner and outer plant quality.

The morphological and physiological responses of plants to different spectral light qualities from artificial light sources are the major topic of this thesis. The following chapters give an overview of how plants perceive light and how they react to spectral quality of light.

## 4.1 Photosynthetic active radiation

Plants need light to grow. It is only a certain part of the light spectrum that is responsible for the fundamental process of photosynthesis. The photosynthetic active radiation (PAR) is defined from 400-700 nm (Mc Cree, 1972). The absorption of PAR is enabled by chlorophyll which is located in light harvesting complexes and transfers the absorbed light to the photosynthetic reaction center. The absorption maxima of chlorophyll a and b are in the blue and red range of the light spectrum. Accessory pigments like carotenoids, absorb blue and green light from about 400-530 nm and increase the light harvesting (Fig. 4.1) (Johnson, 2016).

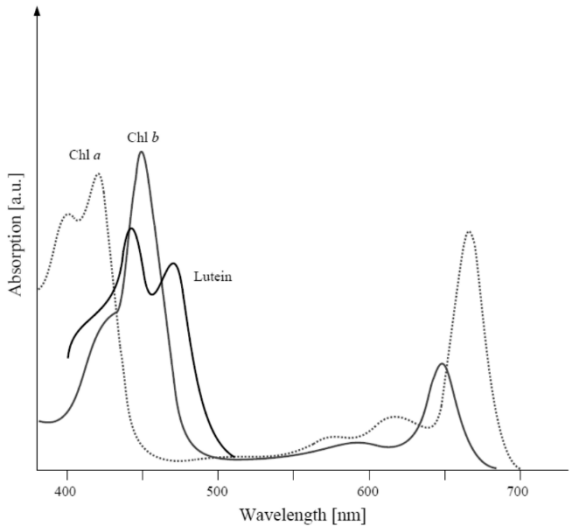


Fig. 4.1: Relative absorption of chlorophyll a, chlorophyll b and lutein modified from Heldt and Piechulla (2015, 5. Edition, p. 48).

Light with a shorter wavelength in the range of UV cannot be used for photosynthesis because it is too high in energy and can lead to cell damage and degradation of proteins and nucleic acids. On the far side light above approximately 700 nm contains insufficient amounts of energy to trigger the photosynthetic process.

Light intensity of PAR is measured in photosynthetic photon flux density (PPFD) expressed as µmol/m²s (Mõttus et al., 2015). Photosynthesis is a quantum based process; regardless of how much energy a photon contains, a photosynthetic reaction can occur, in a range of about 400-700 nm. With the absorption of a photon by a chlorophyll molecule, an electron may jump from the ground state ($S_0$) in excited states ($S_1$, $S_2$ or $S_n$) depending on the energy content of the photon (Johnson, 2016). An energy level diagram of chlorophyll excitation is shown in Fig. 4.2. Blue light (400-500 nm) shows an about 1.5 higher energy content compared to red light (600-700 nm) (see further details about light energy content in discussion chapter 9) leading to an occupation at the higher excited state $S_n$. The excess energy of blue light is released into the environment in form of heat by molecular vibration in a process called internal conversion to get in a lower $S_1$ state. Red light (660 nm) directly lead to excited state $S_1$.

From $S_1$ state several processes can occur; according to Heldt and Piechulla (2015, 5. Edition, pp. 51 ):

- the energy can be released as heat by internal conversion to return in $S_0$.
- a photon with longer wavelength compared to the absorbed photon can be emitted in the process of fluorescence.
- energy can be transferred by Förster resonance energy transfer (FRET), occurring in light harvesting complexes (Şener et al., 2011)
- the energy can be used to drive photosynthetic processes.

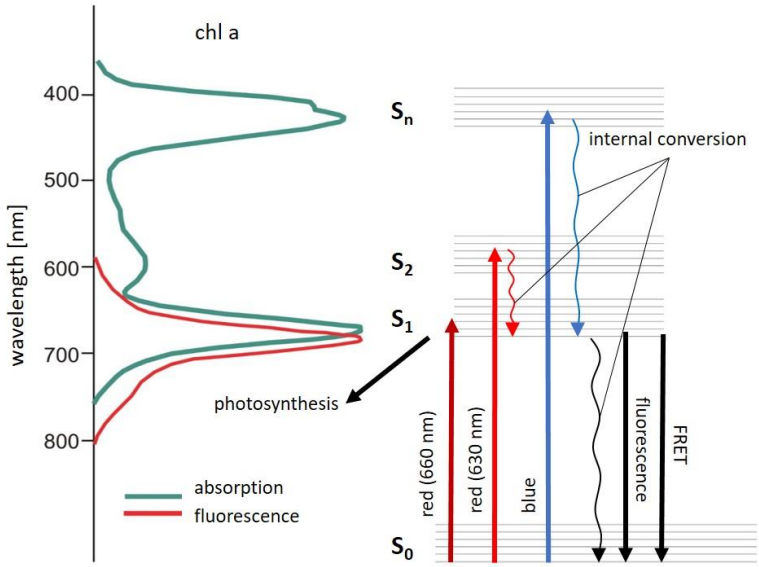


Fig. 4.2: Excitation diagram of chlorophyll. From ground state $S_0$ an excitation to higher energetic state ($S_1$, $S_2$ or $S_n$) can occur depending on the energy content of light color. The excess energy from $S_n$ or $S_2$ is released as heat by the process of internal conversion. From $S_1$ the energy can be emitted to drive photosynthetic process, Förster resonance energy transfer (FRET), can be emitted as fluorescence or as heat by internal conversion. (Diagram was modified from Claudia Büchel).

Nevertheless, light absorption and transfer is not synonymous with photosynthetic efficiency. In addition to blue and red light also green light is used for photosynthesis. This was demonstrated by a comprehensive study by Mc Cree (1972), underlining how photosynthetic efficiency dependents on wavelength. Mc Cree measured the spectral quantum yield of leaves from 22 plant species of crop plants cultivated in growth chambers or fields, over the wavelength from 350 to 750 nm. The quantum yield was defined as the rate of photosynthesis per unite rate of absorbed quanta and was calculated from the action spectrum, the energy per quantum and the spectral absorbance of the leaf. The measurements were carried out in assimilation chambers with leaf sections. The different light colors were

obtained by using a monochromator, fitted with a grating and a xenon arc light source. The resulting “MC Cree curve” displays an average quantum yield of the 22 plant species and is still used to estimate the photosynthetic efficiency dependence of wavelengths (Fig. 4.3). Plants showed highest quantum yield in red light with peaks at 620 and 670 nm. Quantum yield of blue light was about 70% with a peak at about 440 nm. The reduced quantum yield of blue light can be explained by the absorption of blue light by other plant pigments which are not involved in the process of photosynthesis. Carotenoids involved in light protection absorb blue light and release the absorbed energy in form of heat into the environment (Young, 1991). Also flavonoids and phenolic acids are leading to reduced quantum efficiency by protecting the plant cells of the absorption of UV, blue and green light (Edreva, 2005).

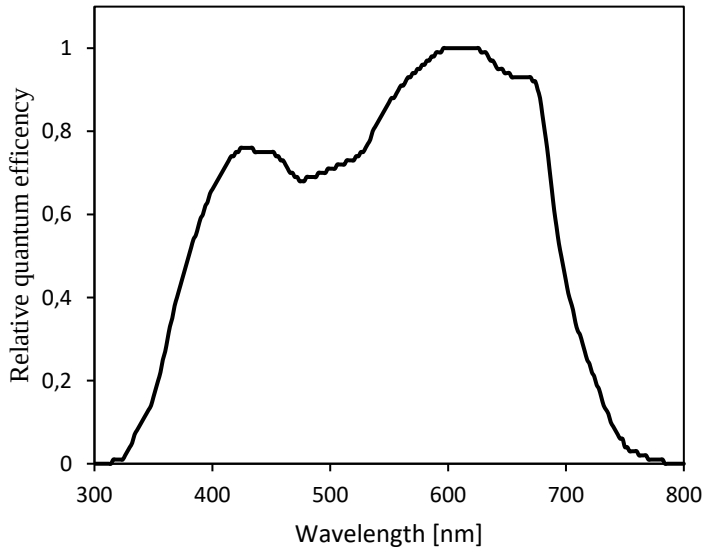


Fig. 4.3: Relative quantum efficiency of plants by McCree (1972) (own graphic).

## 4.2 Spectral quality of light is changing in the environment

Higher plants are sessile organisms and need to adapt to their environment. In addition to temperature, relative humidity or nutritional resources, light is also a variable abiotic factor. Light quality and intensity are changing during the day due to sunrise and sunset, weather conditions and shading by other plants. The light spectrum at daylight, sunset and under basil canopy in September 2019 is shown in Fig. 4.4.

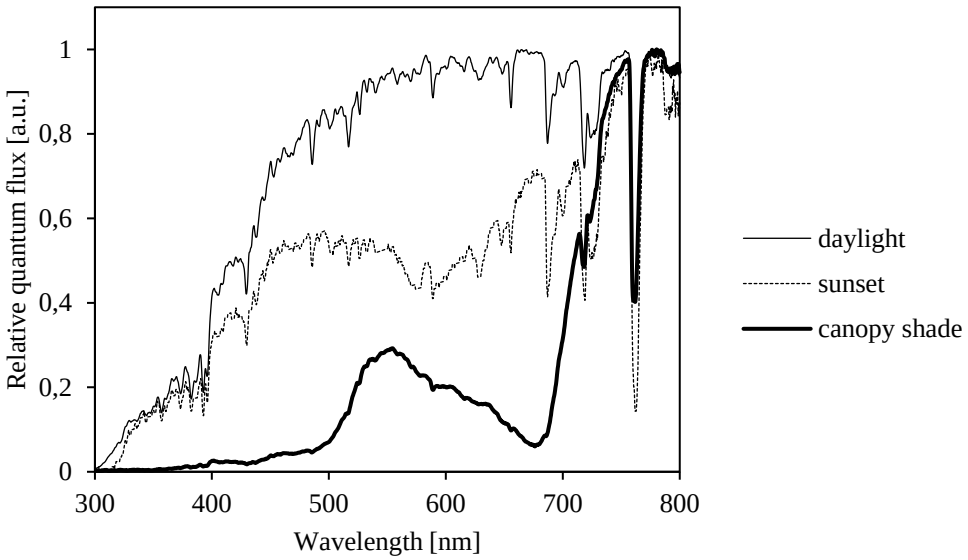


Fig. 4.4: Relative light spectra of daylight, sunset and light spectrum under a basil canopy. Measured on September 20th, 2019, Wülfrath, Germany (51° 16' 49.022" N 7° 2' 5.859" E). For better visualization, spectra were normalized at 780 nm. Light intensity (PPFD) was as follows: daylight: 1230 µmol/m²s; sunset: 7 µmol/m²s; basil canopy: 94 µmol/m²s. Daylight and light under basil canopy were measured on the apex of the sun's motion (1 pm, cloudless). Light spectrum of sunset was recorded at 7 pm.

Cloudless daylight is defined, when the light is emitted from sun in an angle of more than 10° above the horizon with a relatively constant spectrum of global radiation (Smith, 1982). Clouds are decreasing the light intensity on the earth's surface but do not change the spectrum of light drastically. However, in overcast situations clouds act as diffusion filters and increase the proportion of blue light (Smith, 1982). When the sun is below an angle of 10° at sunset or sunrise, the spectrum of the sun is changing noticeably. At twilight the longer wavelengths are increased and the proportions in an range about 550-620 nm are decreased (Fig. 4.4). The most diverse spectrum plants naturally perceive occurs when plants are shaded. Under the plant canopy the light intensity is decreased but also the spectrum of light is altered. Leaves in the plant canopy absorb high proportions of blue and red light which is used for the photosynthetic process. Other wavelengths which are not used for photosynthesis are transmitted through the leaves leading to an increased proportion of green, far-red and near infrared light under the plant canopy (Smith, 1982) resulting in an decreased R:FR (red: far-red) ratio as described in the following chapter. The proportions of light colors and R:FR ratios from daylight, sunset and shade are shown in Tab. 4.1.

Tab. 4.1: Spectral properties of daylight, sunset and from light spectrum under a basil canopy measured on September 20th, 2019, Wülfrath, Germany (51° 16' 49.022" N 7° 2' 5.859" E) (Fig. 4.4). Values are the percentage of photo-biologically active radiation (PBAR, 280–800 nm). R:FR ratio was calculated according to Smith (1982).

| Light Source | [nm] | UV-B 280-315 | UV-A 315-400 | blue 400-500 | green 500-550 | yellow 550-600 | red 600-700 | far-red 700-800 | R:FR ratio |
|---|---|---|---|---|---|---|---|---|---|
| daylight | % | 0.14 | 4.1 | 18.3 | 12.5 | 13.2 | 26.6 | 25.2 | 1.13 |
| sunset | % | 0.08 | 5.0 | 19.2 | 11.0 | 9.8 | 23.8 | 31.1 | 1.03 |
| shade | % | 0.07 | 0.6 | 3.1 | 8.2 | 10.2 | 11.8 | 66.0 | 0.13 |

In horticulture industry spectral light quality can also artificially be changed by greenhouse covers, films or supplemental lighting. The main interest of this work lies in the reaction of plants to artificial light.

## 4.3 Plant ability to adapt to spectral light quality

To enable efficient plant growth, plants need to adjust their morphological and physiological processes to the changing light conditions as previously mentioned.

Besides the photosynthetic active radiation, plants are able to sense an extended area of light from UV to near infrared radiation (NIR) which can be defined as photo-biologically active radiation (280- 800 nm, PBAR).

In higher plants five different groups of photoreceptors exist, absorbing in different regions of the light spectrum and controlling specific morphological and physiological processes: UVR8, zeitlupe, cryptochromes, phototropins and phytochromes are the major photoreceptors and covering an absorption area of about 280-800 nm. An overview of the absorption spectra, chromophore structure and plant responses mediated by the five photoreceptor groups are illustrated in Fig. 4.5.

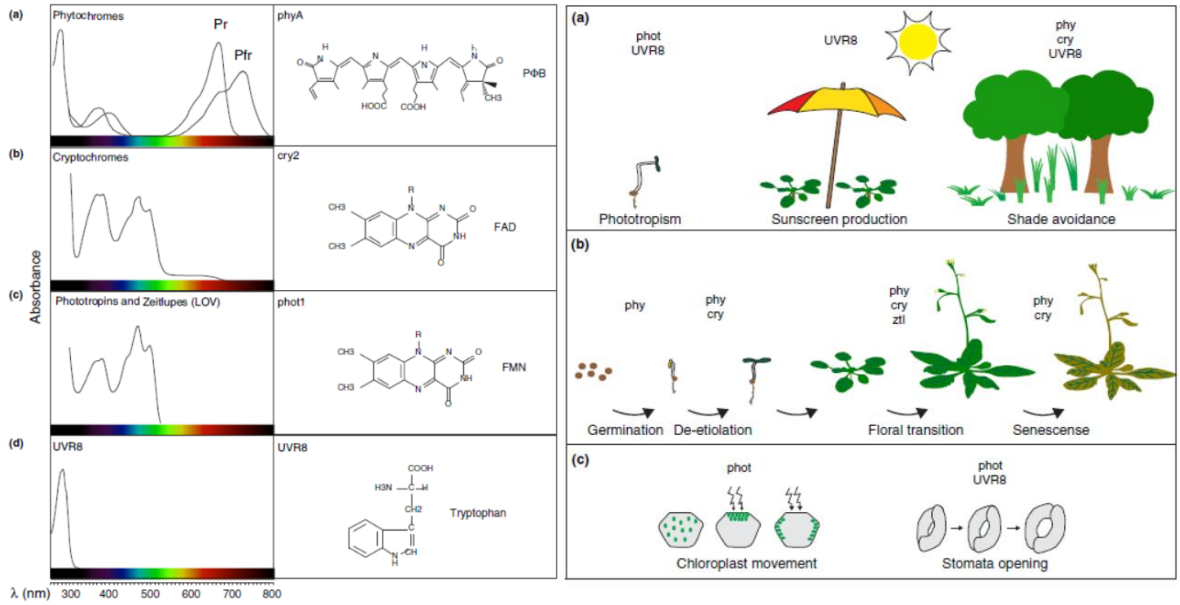


Fig. 4.5: Overview of light absorption spectrum and chromophore structure of plant receptors and their related morphological and physiological responses in *Arabidopsis thaliana*. Modified from Galvão and Fankhauser (2015).

### *4.3.1 Phytochromes*

Phytochromes are sense red and far-red light and are mainly responsible for the shade avoidance response (Casal, 2013).

As described above, plants shaded by neighboring plants receive a far-red and green light enriched environment, which is transmitted through the leaf canopy. Phytochromes are appearing in two photo-convertible forms: $P_{fr}$ (far-red light sensing phytochrome) has an absorption maximum at about 730 nm. The absorption maxima of $P_r$ (red light sensing phytochrome) is at about 660 nm (Fig. 4.5). When red light is absorbed by $P_r$, it changes into $P_{fr}$, which is considered to be the physiologically active form of the phytochrome. Phytochromes are dimeric chromopeptides with monomers of 120-130 kDa with linear tetrapyrrol acting as a chromophore (Casal, 2013).

In a far-red light enriched environment, the inactive $P_r$ dominates and leads to a signal transduction resulting in stem elongation to avoid the shading by other plants. In shade avoidance response auxin and gibberellin are the major plant hormones inducing the elongation growth under far-red light enriched light environment (Casal, 2013). In red light enriched light environment $P_{fr}$ leads to the inhibition of phytochrome interaction factors (PIFs) acting as transcriptional regulator (Galvão and Fankhauser, 2015) to suppress elongation growth.

In *Arabidopsis* five different phytochromes (phyA–E) exist regulating different physiological processes (Galvão and Fankhauser, 2015). Phytochromes are involved in flowering, germination, de-etiolation and senescence (Chen et al., 2014; Sakuraba et al., 2014; Galvão and Fankhauser, 2015). They are also absorbing light in shorter wavelength and are also involved in blue light signaling (Sullivan et al., 2016).

The red:far-red ratio ($R{:}\,FR = \frac{P_r\,(655-665\,nm)}{P_{fr}\,(725-735\,nm)}$) is a quantitative indicator of the light environment, which is correlated with the elongation growth (Smith, 1982). A low R:FR ratio is associated with increased elongation, thinner leaves and reduced branching, whereby higher R:FR ratios can lead to the opposite. Cloudless day light has a R:FR ratio of about 1.15 (53°N) and is relatively constant at changing weather conditions or different day times (Holmes and Smith, 1977). However, R:FR ratio is remarkably changed by latitude and furthermore a dependency of air moisture was observed (Lee and Downum, 1991). When plants are shaded by neighboring plants, the R:FR ratio under the leaf canopy is decreased (Tab. 4.1) depending on plant species and canopy density (Smith, 1982).

### *4.3.2 Phototropins*

Phototropins are blue light and UV-A light receptors and are responsible for directed plant growth towards the light source in a process which is called phototropism (Christie, 2007). The blue light is sensed by two flavin mononucleotide (FMN) chromophores of two light oxygen voltage (LOV1 and LOV2) domains (Galvão and Fankhauser, 2015). In *Arabidopsis* two different phototropins exist (phot1 and phot2) (Briggs and Christie, 2002). Phototropins are responsible for the control of stomata opening under blue light (Kinoshita et al., 2001). Furthermore phot1 and phot2 regulate chloroplast accumulation under low light conditions, whereby in high light intensities chloroplast light avoidance is mediated by phot2 (Wada, 2013).

### *4.3.3 Cryptochromes*

Cryptochromes (cry1, cry2 and cry3 in *Arabidopsis*) are another group of blue light and UV-A light photoreceptors. Cryptochromes have been found in bacteria, fungi and animals. They consists of a flavin adenine dinucleotide (FAD) as a chromophore (Galvão and Fankhauser, 2015).

Cryptochromes were first described for mediating hypocotyl growth inhibition under blue light in *Arabidopsis* (Ahmad and Cashmore, 1993). Cry2 was also found to promote flowering in *Arabidopsis* (El-Din El-Assal et al., 2003). Furthermore in soybean it was demonstrated that cryptochromes regulate leaf senescence (Meng et al., 2013).

### *4.3.4 UVR8*

UV-B light is sensed by UVR8 receptors, which are involved in the accumulation of UV-B absorbing secondary metabolites such as flavonoids and phenolic acids that counteract cell damage from harmful UV radiation (Kliebenstein et al., 2002; Favory et al., 2009). UV-B is sensed by a specific tryptophan acting as a chromophore and leads to monomerization and activation of UVR8 homodimers (Rizzini et al., 2011; Tilbrook et al., 2013). It has been shown that UVR8 receptor is involved in the regulation of stomata closure in Arabidopsis (Tossi et al., 2014). Furthermore, UVR8 is supposed to be involved in chloroplast differentiation and hypocotyl growth inhibition (Favory et al., 2009; Wargent et al., 2009).

### *4.3.5 Zeitlupe*

As phototropins, zeitlupe receptors bind a FMN chromophore absorbing blue and UV-A light (Galvão and Fankhauser, 2015). Members of the zeitlupe family are associated with the circadian clock and regulate flowering (Christie et al., 2015).

## 4.4 Artificial light for horticultural industry

In northern hemisphere during winter periods, light intensity is too low and days are too short to enable a production of vegetables, ornamental plants or herbs. The daily light integral (DLI) is the sum of PPFD per day (mol/m²d) and is an indicator for the light requirement for crops. The DLI and day length in Geisenheim during year 2018 and some DLI which are usually used for production of horticultural crops are shown in Fig. 4.6.

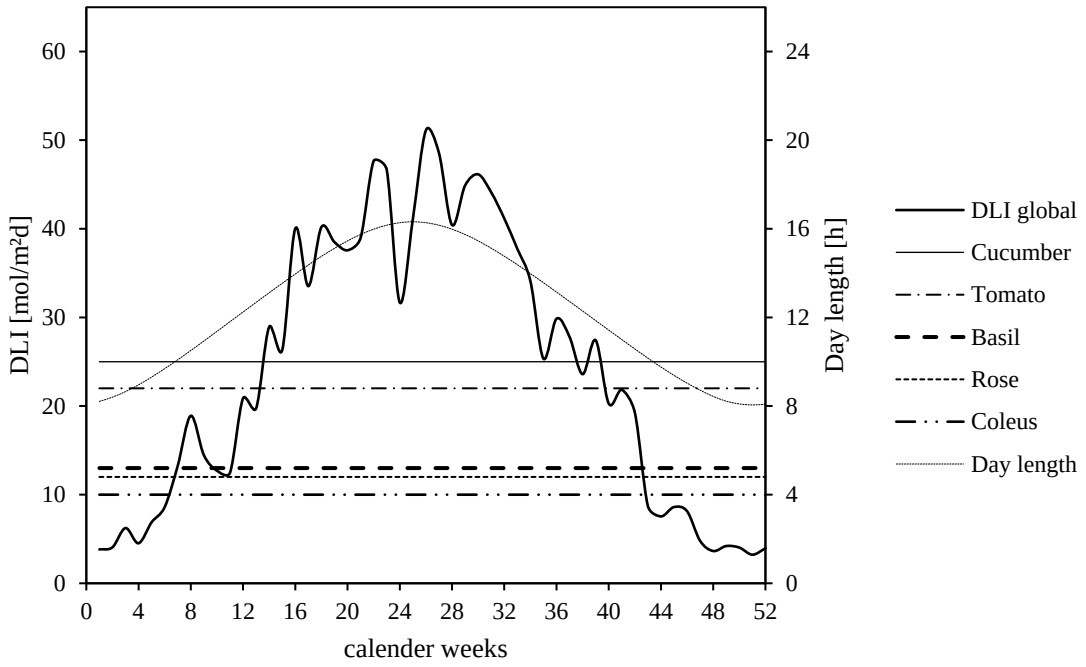


Fig. 4.6: Daily light integral [mol/m²d] and day length [h] in Geisenheim during the year 2018 and recommended DLI for some horticultural relevant crops (Garland et al., 2010; Moe et al., 2006; Mortensen, 2004; Paradiso et al., 2011).

Potted roses, coleus and basil, which were used as model plants for this thesis, are usually produced with a DLI of about 10-12 mol/m²d with a photoperiod of 16-20 h (Mortensen, 2004; Moe et al., 2006; Garland et al., 2010; Paradiso et al., 2011). From November to February (calendar weeks 44-7) the DLI is below their recommended light requirements (Fig. 4.6). Furthermore, the DLI inside the greenhouse can be reduced by greenhouse cover materials up to 60% (own measurements). Artificial light increases the DLI by increasing the light intensity and lengthening the day, resulting in a sufficient amount of light that allows a year-round production of horticultural products. Especially for crops like cucumber and tomatoes with recommended DLI up to 30 mol/m²d (Mortensen, 2004; Moe et al., 2006) artificial lighting is obligatory in winter period. In northern countries the lighting period is also extended due to lower light intensity and shorter days.

For around 160 years, electric light is used to enable plant growth in winter period (Wheeler, 2008). Different lamps were developed differing in light emission spectra and electrical efficiencies (see further details about light efficiencies in chapter 9).

In the end of the 19$^{th}$ century, one of the first lamp types used as assimilation light were carbon arc lamps, which emit a broad, blueish light spectrum (Siemens, 1880) but were also hazardous due to emission of UV radiation (Wheeler, 2008). The lamps were replaced by

incandescent filament lamps, followed by the first low-pressure discharge lamps in the beginning of the 19th century (Wheeler, 2008). In the 1960th, high-pressure sodium (HPS) lamps became the most common greenhouse lamp due to high electrical efficiencies and a long operating life. The spectrum is dominated by the emission of yellow and orange light with a low blue light content of about 5% (Terfa et al., 2013). Due to their low acquisition costs they are still the most popular lamp systems for supplemental lighting in greenhouses (Ouzounis et al., 2018). In addition to HPS lamps also fluorescent and metal halide lamps emitting white broad light are used for horticultural industry (Wheeler, 2008). In the recent years LEDs have emerged as an efficient alternative light source. With LEDs the spectra of light can be adapted to optimal plant growth or to stimulate distinct plant physiological responses as described below. LEDs are supposed to be more efficient in light emission compared to discharge lamps, since LEDs are not emitting infrared-radiation. Therefore, LEDs are suitable for multilayer applications called "Vertical Farming" (see further details about vertical farming in chapter 10).

Microwave plasma lamps (MPL) represent another light technology. They were invented in the early 90s by Fusion Systems Corporation and further developed by Fusion lighting in an NASA supported project (MacLennan et al., 1994). An overview of today's common lighting technologies used as assimilation light is shown in Fig. 4.7.

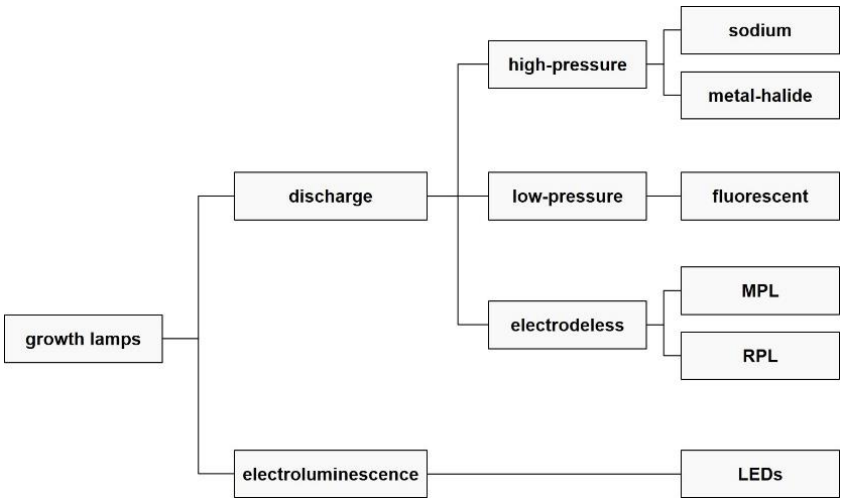


Fig. 4.7: Schematic overview of today's lighting technologies used as assimilation light. MPL= microwave plasma lamp; RPL = radio wave plasma lamp; LEDs = light-emitting diodes (according to Michael Kloss, personal communication).

In this research project the MPL were further optimized and tested for horticultural proposes in cooperation with Plasma International GmbH, Aurion Anlangen Technik GmbH and Landesbetrieb Landwirtschaft Hessen.

The project was supported by Hessen State Ministry of Higher Education, Research and Arts (LOEWE, funding no. 487/15-29) and has been coordinated by Hessen Agentur GmbH. The electrodeless MPL (Fig. 4.8) consists of a rotating light bulb supplemented with sulfur or antimony and filled with the gas argon. The light emission is induced by microwaves generated by magnetrons. These lamp types are able to emit a continuous light spectrum with nearly the same spectral properties as sunlight and are the major research objective in this thesis.

Fig. 4.8: Picture of MPL systems used in this thesis. Hessen Agentur GmbH by Jan Michael Hosan

## 4.5 Influence of the spectral light quality on morphology and secondary metabolites

Since the latest development of LEDs, light spectra can be adjusted and researches about the affection of plants due to light quality appeared numerous as recently reviewed by Bantis et al., (2018). However, the responses are often depending on plant species, cultivar or experimental conditions. Results which were produced in growth chambers are often not transferable to greenhouses in the presence of global radiation. Furthermore, different light intensities, lighting periods and different spectral properties from the light sources, make comparisons of results difficult. Thus, general statements can only be made superficially.

Tab. 4.2 summarizes some main findings to the question how secondary metabolites and plant architecture is changed by different light treatments in greenhouses and climate chambers. In general, indoor investigations are common with lettuce as one of the most popular model plants for light experiments due to their horticultural relevance and the easy cultivation procedure.

Light color, especially in growth chambers, has a distinct impact on morphological appearance and metabolic constitutions of many plant species. Since the emission of artificial light by LEDs was initially limited to red and later blue light, research was focused mainly on the effects of these light colors. Furthermore, blue and red light were assumed to be most efficient light colors for photosynthesis (Fig. 4.3) and biomass production. However, also other light colors are affecting plant architecture, flowering and metabolic constitutions of plants.

### *4.5.1 Blue light effects*

In general, it is widely accepted that blue (B) light (400-500 nm) leads to in decreased elongation growth. B light from LEDs resulted in compact plant growth as demonstrated with lettuce, poinsettia, potted roses, tomatoes and chrysanthemums (Li and Kubota, 2009; Islam et al., 2012; Ouzounis et al., 2014; Bergstrand et al., 2016). However, not only the proportion but also the absolute amount of blue light is involved in elongation inhibition as shown in radish, soybean and wheat (Cope and Bugbee, 2013). B light is also responsible for the formation of “sun-like” leaves indicated by an increased leaf mass per area (LMA), smaller leaf size or increased amount of stomata as observed in light experiments using LEDs with lettuce, roses, tomatoes and campanulas (Li and Kubota, 2009; Abidi et al., 2013; Terfa et al., 2013; Ouzounis et al., 2014; Bergstrand et al., 2016).

B light is also supposed to accumulate secondary metabolites (e.g. flavonoids and phenolic acids) which are acting as plant protectors by absorbing harmful UV-B radiation. It was shown that genes involved in the biosynthesis of flavonoids are directly induced by blue light and UV radiation (Kubasek et al., 1992). A higher content of phenolic constitutions was determined in lettuce, basil, roses, chrysanthemums, campanulas and arugula grown with LEDs including higher blue light content compared to discharge lamps (Stutte et al., 2009; Ouzounis et al., 2014; Bantis et al., 2016; Taulavuori et al., 2018). Likewise anthocyanin production was increased by B light from LEDs in leaves of lettuce (Stutte et al., 2009) or rose petals (Terfa et al., 2013).

### *4.5.2 Red light effects*

Red (R) light (600-700 nm) is supposed to be the most efficient light source to drive photosynthesis (Mc Cree, 1972). However monochromatic R light is leading to increased plant height as observed in tomato plants, chili plants and chrysanthemums (Gangadhar et al., 2012; Xiaoying, 2012; Ouzounis et al., 2014). The absence of blue light can also lead to morphologically abnormalities like leaf curling as observed in roses leaves (Ouzounis et al., 2014). This phenomenon is called the "red light syndrome" and can result in lower photosynthetic performance as demonstrated in cucumber plants (Trouwborst et al., 2016). R light can led to a decreased content of chlorophyll (Xiaoying, 2012; Trouwborst et al., 2016). Furthermore R light can leads to an increased total phenolic content (TPC) in lettuce and increased phenolic compounds in green basil microgreens (Li and Kubota, 2009; Shiga et al., 2009; Lobiuc et al., 2017).

### *4.5.3 Far-red light effects*

A higher amount of far-red (FR) light (700-800 nm) is causing an increased stem or leaf elongation in the process of shade avoidances mediated by phytochromes as previously described and as demonstrated in lettuce and petunia (Li and Kubota, 2009; Stutte et al., 2009; Lee et al., 2016; Park et al., 2016). Furthermore FR light results in an earlier flowering and lower ornamental value in petunia (Park et al., 2016). Nevertheless, FR light can also be beneficial for plant growth by increasing biomass production (Li and Kubota, 2009; Lee et al., 2016). A higher biomass production of cucumber plants by increased FR was attributed to a higher leaf unfolding rate and increased light interception (Hogewoning et al., 2010a).
Regarding the content of secondary metabolites, FR light was shown to decrease phenolic constitutions in lettuce (Li and Kubota, 2009; Stutte et al., 2009). Contradictory, FR light led to increased total phenolic content, higher antioxidant capacity, increased chlorogenic and caffeic acids in red leaf lettuce (Lee et al., 2016). A decreased chlorophyll content was observed in an FR light enriched light environment in lettuce and petunia (Li and Kubota, 2009; Lee et al., 2016; Park et al., 2016).

### *4.5.4 Green light effects*

Results with the focus on the influence of green (G) light (500-550 nm) on plant architecture and metabolic constitutions are less clear. As previously mentioned, G light and FR light is

transmitted through the leaf canopy (Fig. 4.4). Therefore, also G light is supposed to be a signal to induce shade avoidance responses leading to an increased elongation growth and is assumed to reverse the effects mediated by blue and red light (Folta and Maruhnich, 2007; Zhang et al., 2011). However, beneficial effects of G light on plant physiology were also observed. In addition to R and B LEDs green fluorescent lamps increased biomass and growth rate in lettuce plants grown in a growth chamber (Kim et al., 2004). At higher light intensities under white light it was determined that G light drives photosynthesis more efficient compared to blue or red light, due to the deeper penetration in the leaves (Terashima et al., 2009). Monochromatic G light also resulted in full developed plants (Johkan et al., 2012; Xiaoying, 2012).

Nevertheless, the beneficial impact depends on the distinct wavelength of G light. While monochromatic G light with a wavelength of 510 nm and 524 nm at higher light intensities resulted in normal plant growth, G light with 532 nm displayed succulent growth as demonstrated in red leaf lettuce (Johkan et al., 2012). Total phenolic content and anthocyanin content was increased by G light with a wavelength of 505 nm, while vitamin c and tocopherol accumulated under G light with 535 nm at highest quantities as observed in baby leaf lettuce (Samuolienė et al., 2012).

### *4.5.5 Interactions of light colors*

It is possible to cultivate plants with single colors of light, however, in general monochromatic light can results in abnormal and/or reduced plant growth (Ouzounis et al., 2014; Trouwborst et al., 2016). The combination of R and B is more effective compared to the individual light colors as demonstrated in cherry, tomato, campanulas, roses, chrysanthemums and cucumber (Gangadhar et al., 2012; Xiaoying, 2012; Ouzounis et al., 2014; Trouwborst et al., 2016). Nevertheless, a full spectrum including G light can affect plant growth positively compared to RB light treatment (Kim et al., 2004; Gangadhar et al., 2012; Xiaoying, 2012; Lin et al., 2013). In light experiments using different light spectra plant reactions are not always attributed to single light colors. These responses seem to be an interplay of several light qualities, mediated by different photoreceptors.

*4.5.6 Influence of infrared radiation on plant growth*

In light experiments using different lamp systems the impact of infrared (IR) radiation on plant growth is often not evaluated.

In an energy balance model calculated by Nelson and Bugbee (2015), it was determined that non-water stressed leaves under LED light displayed about 1.3 °C lower leaf temperatures compared to leaves under HPS light at 25 °C (air temperature). Nelson and Bugbee (2015) assumed that near IR (NIR, 800-2500 nm) radiation of HPS lamps is not resulting in an increase of leaf temperature because NIR is only slightly absorbed by the leaves. Rather long wave IR is causing an increased leaf temperature. Leaf absorption and emission spectra of HPS, LED and sunlight in a range of about 400-2500 nm are shown in Fig. 4.9.

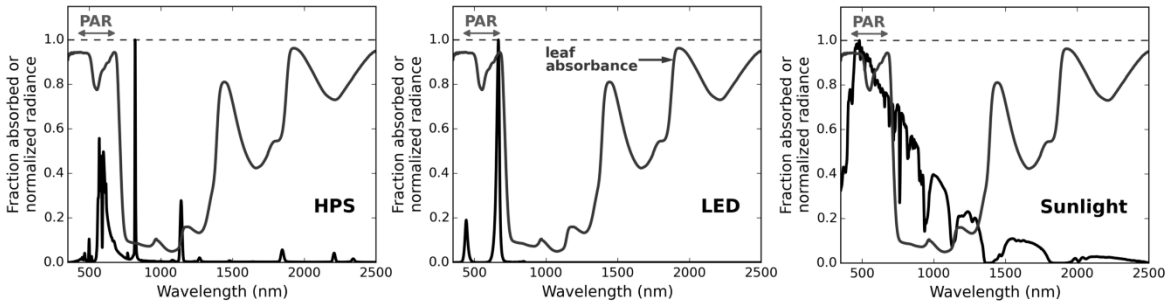


Fig. 4.9: Leaf absorbance and emission spectra of HPS, LED and sunlight. Modified from Nelson and Bugbee (2015).

However, the impact on leaf temperature of HPS fixtures is increased by lower cultivation temperatures (Nelson and Bugbee, 2015).

Due to a lack of IR, lower leaf temperatures of about 0.9-1.5 °C were measured when LEDs were used compared to a cultivation with HPS lamps as demonstrated with tomatoes, roses and poinsettia (Islam et al., 2012; Terfa et al., 2013; Bergstrand et al., 2016). Särkkä et al. (2017) measured up to 4 °C lower leaf temperatures of cucumber plants under LED light in comparison to HPS light. Lower leaf temperatures caused by LEDs are in general associated with decreased growth performance resulting in lower biomass and reduced yield (Hernández and Kubota, 2015; Bergstrand et al., 2016; Särkkä et al., 2017). Therefore, the impact of IR on plant physiological processes should not be neglected.

Tab. 4.2: Overview how spectral light quality is affecting plant morphology and accumulation of secondary metabolites of different horticultural plants. Light intensity (PPFD) is shown of supplemental light from respective lamp type. ↑ = increased; ↓ = reduced in comparison to the other light treatments.

Abbreviations: $A_{max}$, maximal photosynthesis rate; AS, artificial solar; B, blue light; DW, dry weight; FR, far-red light; FT, fluorescent lamp; FW, fresh weight; G, green light; GC, growth chamber; GH, greenhouse; HPS, high-pressure sodium; LEDs, light-emitting diodes; LMA, leaf mass per area; MPL, microwave plasma lamp; MH, metal halide; O, orange light; $P_{net}$, net photosynthesis rate; R, red light; TPC, total phenolic content; UV, ultra violet light; V, violet light; W, white light.

| Species and cultivar | Environment | Lamp type | PPFD [µmol/m²s] | h/d | Effects on morphology and physiology | Effects on secondary metabolites | Reference |
|---|---|---|---|---|---|---|---|
| **green and red leaf basil** | GC | different LEDs vs. W FL | 200 ± 20 | 14 | LED: ↑ bio mass<br>Fluorescent lamp: ↑ leaf area | LED treatment with highest R:FR ratio, high blue light content and 1% UV resulted in highest phenolic content | (Bantis et al., 2016) |
| **basil** | GC | R, B and W FL | 100 | 16 | | R and W FL: ↑ antioxidant potential, ↑ rosmarinic acid<br>W FL:↑ TPC | (Shiga et al., 2009) |
| **green and red leaf basil** | GC | W, 2R:B, 1R:1B and 1R:2B LEDs | 120 | 12 | 1R:2B:↑ FW in green cultivar | No differences in chlorophyll content<br>1R:2B: ↑ rosmarinic acid, ↑ caffeic acid in red cultivar<br>2R:1B: ↑ phenolic compounds in green cultivar | (Lobiuc et al., 2017) |
| **basil, arugula, bloody dock** | GH | HPS vs. HPS supplemented with B (450 nm) or BV (420/440 nm) LEDs | 300 | 24 | **basil**<br>BV: ↓ biomass and number of leaves<br>**arugula**<br>B: ↑ biomass<br>**bloody dock**<br>B, BV: ↓ stem elongation, ↓ biomass;<br>HPS: ↑ number of leaves | **basil**<br>B, BV: ↑ phenolic acids<br>**arugula**<br>B, BV ↑ flavonoids<br>**bloody dock**<br>no significant difference in phytochemicals | (Taulavuori et al., 2018) |
| **chili pepper** ('Cheonyang') | GC | LEDs (R, B and RB) vs. W FL | 70 | 16 | R: ↑ plant height<br>RB: ↓ plant height, ↑ FW ↑ DW, ↑ fruit yield | B: ↑ capsaicinoid content, ↑ chlorophyll content | (Gangadhar et al., 2012) |
| **cucumber** ('Hoffmann's Giganta') | GC | "Artificial solar spectrum (AS)" from MPL vs. FT and HPS | 100±5 | 16 | AS: ↑ leaf area, ↑ hypocotyl length, ↑ petiole length, ↑ number of leaves, ↑ increased plant height, ↑ total DW<br>FT: ↑ $A_{max}$; ↑ LMA | | (Hogewoning et al., 2010a) |
| **baby leaf lettuce** | GH | HPS vs. HPS supplemented with B (455/470 nm) and G (505/530 nm) LEDs | 170 (HPS)+30 (LED) | 16 | | vitamin C and tocopherol contents was increased in following order: 535 > 505 > 455 > 470 nm<br>TPC: 505 > 535 = 470 > 455 nm<br>antioxidant potential: 535 = 470 > 505 > 455 nm<br>total anthocyanins: 505 > 455 > 470 > 535 nm. | (Samuolienė et al., 2012) |
| **lettuce** ('Hoffmann's Giganta') | GC | RB LEDs, RB LEDs with G FL (RBG), G FL and W FL | 150 | 18 | RBG: ↑ leaf area, ↑ shoot DW, ↑ FW, ↑ growth rate<br>G FL: ↓ leaf area, ↓ LMA, ↓ Pn | | (Kim et al., 2004) |
| **lettuce** ('capitata') | GC | RB and RBW LEDs vs. FL | 210 | 16 | RBW and FL: ↑ shoot FW, ↑ root FW, ↑ DW<br>RBW: ↑ soluble sugar content | No significant differences in chlorophyll content | (Lin et al., 2013) |
| **red leaf lettuce** ('Outredgeous) | GC | LEDs (RGB, RB, R, RFR) vs. FL | 300 | 18 | LEDs: ↑ biomass<br>FR: ↑ leaf elongation | RB and RGB: ↑ anthocyanin; ↑ antioxidant potential<br>FR: ↓ anthocyanin content, ↓ antioxidant potential | (Stutte et al., 2009) |

Tab. 4.2 continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **baby leaf lettuce** ('Red Cross') | GC | W FL supplemented with UV-A, B, G, R and FR LEDs | 300 (UV-A: 18; B: 130; G: 130; R: 130; FR: 160) | 16 | B: ↓ stem length, ↓ leaf length<br>UV-A: ↓ stem length<br>FR: ↑ FW, ↑ DW, ↑ stem length, ↑ leaf length, ↑ leaf width<br>G and R: no significant effect | UV-A and B: ↑ anthocyanin content<br>B: ↑ carotenoids<br>R: ↑ TPC<br>FR: ↓ anthocyanin ↑ carotenoid, ↓ chlorophyll content<br>G: no significant effect | (Li and Kubota, 2009) |
| **red leaf lettuce** ('Sunmang') | GC | R and B LEDs with supplemental FR vs. FL | 130 | 12 | lower R:FR: ↑ shoot FW, ↑ root FW, ↑ DW<br>FR: ↑ leaf area | FR: ↑ TPC, ↑ antioxidant capacity, ↑ phenolic acids<br>FR: ↓ chlorophyll content | (Lee et al., 2016) |
| **red leaf lettuce** | GC | G LEDs (510, 524, 532 nm), vs. FL | 100, 200, 300 | 24 | 510 and 524 nm: at 300 µmol/m²s normal appearance<br>510 nm: ↑ leaf number, ↑ FW, ↑ DW at 300 µmol/ m²s<br>510, 524, 532: ↑ leaf area | FL: ↑ anthocyanin content<br>At high light intensities with 510 nm: anthocyanin produced was observed | (Johkan et al., 2012) |
| **petunia** ('Madness Rose') | GC | combinations of FL, MH, HL, HPS and LEDs | 25 | 16 | low R:FR: ↑ elongation growth<br>low R:FR: earlier flowering, thinner stems, ↓ branching<br>↓ ornamental value | higher R:FR: ↑chlorophyll content | (Park et al., 2016) |
| **poinsettia** ('Christmas Spirit', 'Christmas Eve' and 'Advent Red') | GH/GC | LEDs with 20% B and 80% R vs. HPS | 100±20 | 10 | LED: ↓ plant height, ↓ DW, ↓ leaf temperature | | (Islam et al., 2012) |
| **radish** ('Cherry Belle'), **soybean** ('Hoyt') and **wheat** ('Perigee') | GC | W LEDs with 11%, 19% and 28% B, | 200, 500 | 16 | B: ↓ plant height; no effect on DW | | (Cope and Bugbee, 2013) |
| **potted rose** ('Toril') | GH/GC | LED (RB) vs. HPS lamps | 100 | 20 | no difference in total DW and flowering<br>HPS: ↑ leaf area, ↑ plant height<br>LEDs: ↑ leaf biomass, ↑ photosynthetic capacity, ↑ LMA, ↑ soluble sugars, sun-type leaf anatomy with increased amount of stomata | LED: ↑ chlorophyll content, ↑ anthocyanin content | (Terfa et al., 2013) |
| **potted rose** ('Radrazz', 'Old Blush') | GC | W vs. B FL | 110 | 16 | B: ↓Pn, ↑ stomatal conductance,↑ LMA in one cultivar | B: ↑ chla/b ratio | (Abidi et al., 2013) |
| **potted rose** ('Scarlet'), **chrysanthemum** ('Coral Charm') and **campanula** ('BluOne') | GH | B and R LEDs (40%B+60%R; 20%B+80%R and 100%R) vs. W FL | 200 | 16 | **roses and chrysanthemums**<br>B: ↓ plant height<br>W FL: ↓ biomass<br>100% R: (leaf curling in rose)<br>**campanulas**<br>B: ↓ leaf area | B:↑ flavonoids; ↑ phenolic acids | (Ouzounis et al., 2014) |
| **cherry tomato** ('cerasiforme') | GC | LEDs (R, B, O, G, RB, and RBG) vs. W MH | 320 | 12 | W MH: ↑ leaf area<br>R: ↑ plant height<br>B: ↑ FW, ↑ DW<br>RB: ↑ LMA<br>RB and RBG: ↑ photosynthetic capacity, ↓ plant height | O and R: ↓ total chlorophyll and carotenoid content | (Xiaoying, 2012) |

## 4.6 Aim and content of the thesis

In experiments investigating the influence of spectral light quality, white light is often used as a control condition. But the quality of white light, simulating natural conditions depends on the used type of light technology. The development of continuous sun-like light by LEDs is generally not possibly because for each wavelength different LED chips would have to be used. Light from white LEDs is emitted by blue coated LEDs shifting blue light in longer wavelength. The emission spectrum is however not very close to the spectrum of natural sun light. The usage of FT or metal halide lamps as control is common. The emission spectrum is more balanced, but not as continuous as sunlight. Furthermore, when the impact of LEDs is compared to discharge lamps, the impact of infrared radiation is often not considered which might lead to an overestimation of the influence of single light colors on morphology and plant physiology.

In this thesis the focus lies on a microwave plasma lamp, which was tested for the influence of spectral light quality on plant architecture and secondary metabolites of horticultural relevant plants. The MPL is able to emit continuous sun-like light and might be an alternative light source for controlled conditions where natural conditions are required. Furthermore, the aspect of horticultural usability of the MPL was examined. The influence of the MPL was compared to other light systems also with regard to their impact on leaf temperature. The investigations are divided in four chapters with three different plant species used as model plants:

| | |
|---|---|
| Chapter 5 & 6 | Coleus *(Plectranthus scutellarioides)* |
| Chapter 7 | Sweet basil (*Ocimum basilicum*) |
| Chapter 8 | Potted rose (*Rosa hybrida*) |

In chapter 5 the influence of the MPL emitting artificial sunlight on morphology and secondary metabolites of coleus was examined in comparison to commercial HPS, ceramic metal halide lamps and LEDs. Coleus are ornamental plants which were also used for pharmaceutical applications due to richness of phenolic compounds. The blue light content was altered in the emission spectra of the four different lamp systems. As previously mentioned, blue light has a high impact on the accumulation of secondary metabolites. However, the used lamp systems also differ in the emission of infrared radiation. Especially

when LEDs are compared with discharge lamps, leaf temperature can be altered since LEDs are not emit infrared radiation which might lead to an overestimation of the impact of blue light.
The aim of this study was to investigate both; the influence of the spectral quality and the influence of the leaf temperature on plant physiological processes. Phenolic compounds of leaves from coleus were quantified by colorimetric methods and HPLC analysis. In addition to morphological parameters like fresh weight and internode length, investigations on leaf morphology were also carried out.

In chapter 6 additional results are displayed concerning examinations of photosynthetic capacity of coleus plants grown with different lamp types. Furthermore, light stress experiments under direct sunlight were conducted with plants precultivated with different light sources in climate chamber. In this chapter further experiments with several cultivars of coleus are presented to estimate the impact of cultivar dependency. Finally, greenhouse experiments are shown to evaluate the impact of the MPL light source in the presence of global irradiance.

Chapter 7 deals with the subject, how artificial light alters the chemical constitutions of sweet basil. Basil is one of the most demanding herbal plants in middle Europe. The descent taste and fragrance are caused by essential oils. Nevertheless, basil plants also contain methyl eugenol, a phenylpropanoid which is supposed to be carcinogenic. The main question of this study was, if the content of methyl eugenol can be reduced by spectral light quality with a simultaneous increase of plant quality parameters.
In addition to artificial light from MPL and HPS lamps an alternative light bulb from MPL was tested emitting blue and green weighted light spectra.
Essential oil composition was quantified by GC-MS. Colorimetric methods were used to estimate how non-volatile phenolic compounds are influenced by different light sources. Morphological estimations were performed to excess the impact of different spectral light quality on plant architecture and biomass production.

In chapter 8 the usage of MPL was evaluated focusing on horticultural aspects with potted roses as model plants. The production of potted roses increases in winter terms due to Mother's and Valentine's day. To enable high quality products, artificial light is required. In this study the influence of MPL with an artificial light spectrum was tested as an alternative to HPS lighting, which is still the predominated light source for horticultural industry. The experiments were conducted in greenhouse and climate chambers. Plant quality parameters like branching degree and numbers of flowers were examined.

*Parts of this thesis have been published or are accepted for publication in peer-reviewed journals:*

Chapter 5: Dörr OS, Zimmermann BF, Kögler S, Mibus H (2019): **Influence of leaf temperature and blue light on the accumulation of rosmarinic acid and other phenolic compounds in *Plectranthus scutellarioides* (L.)**. *Environmental and Experimental Botany:103830. doi: 10.1016/j.envexpbot.2019.103830.*

Chapter 7: Dörr OS, Brezina S, Rauhut D, Mibus H (2020): **Plant architecture and phytochemical composition of basil (*Ocimum basilicum* L.) under the influence of light from microwave plasma and high-pressure sodium lamps**. *Journal of Photochemistry & Photobiology, B: Biology:111678. doi: 10.1016/j.jphotobiol.2019.111678.*

Chapter 8: Dörr OS, Mibus H: **Investigation on morphology and physiology of potted roses grown with light from microwave plasma and high-pressure sodium lamps.** *Accepted for publication in European Journal of Horticultural Science*

# 5. Influence of leaf temperature and blue light on the accumulation of rosmarinic acid and other phenolic compounds in *Plectranthus scutellarioides* (L.)

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**What are the contributions of the doctoral candidate and his co-authors?**

**(1) Concept and design**
OSD: 80%
HM: 20%

**(2) Conducting tests and experiments**
OSD: 70% (establishment of colorimetric methods, HPLC-DAD, sample processing, collection of morphological data, analysis of leaf histology)
BFZ: 20% (HPLC-MS analysis)
SK: 10% (implementation of colorimetric methods)

**(3) Compilation of data sets and figures**
OSD: 80% (compilation of HPLC-MS/HPLC-DAD, morphological, leaf histological and colorimetrical data, data visualization)
BFZ: 20% (compilation of HPLC-MS data)

**(4) Analysis and interpretation of data**
OSD: 80% (statistical analysis, analysis of HPLC-MS/HPLC-DAD morphological, leaf histological and colorimetrical data, interpretation of data)
BFZ: 20% (analysis of HPLC-MS data)

**(5) Drafting of manuscript**
OSD: 85%
BFZ: 5%
HM: 5%
SK: 5%

## 5.1 Abstract

In this study, the influence of different lamp types on physiology and secondary metabolites of *Plectranthus scutellarioides* (*Solenostemon scutellarioides, Coleus blumei*) was examined. The following four lamp systems were tested: a new microwave plasma lamp (MPL) emitting artificial sunlight, a commercial high-pressure sodium lamp (HPS), a ceramic metal halide lamp (CDM), and light-emitting diodes (LEDs). The lamps differed not only in the spectral properties of the emitted light but also in the emission of infrared radiation. The aim of this study was to investigate both the influence of the spectral quality and the influence of the leaf temperature from four different lamp systems on morphology and secondary metabolites in *P. scutellarioides*. Total phenolic compounds were quantified with colorimetric methods. For detailed description of the phenolic compounds, qualitative and quantitative analysis of selected phenolic compounds were performed by HPLC.

Stem elongation was increased in plants grown with MPL and LED light, which was attributed to the higher amount of far-red light in the emission spectra. On the other hand, higher infrared radiation from MPL and HPS lamps led to increased leaf temperatures compared to plants grown with LED or CDM light, resulting in faster plant development indicated by greater leaf pair formation. In addition to rosmarinic acid (RA), which is typical for members of the family *Lamiaceae*, luteolin and apigenin glycosides were detected by mass spectrometry. The results demonstrated that *P. scutellarioides* leaves contained three acylated cyanidin diglycosides, one of them containing just a coumaroyl residue, the other two containing one and two additional malonyl residues, whereby malonylated compounds appeared in higher quantities. Leaves grown with LED and CDM light contained the highest amount of RA and flavone glycosides per dry weight, which was attributed to a lower leaf temperature compared to leaves developed under MPL or HPS light. Further, an increased blue light content in the emission spectra led to thicker leaves and consequently to a higher accumulation of secondary metabolites per leaf area.

**Key words:** artificial sunlight, *Coleus blumei*, HPLC-MS, leaf morphology, LED, microwave plasma lamp, rosmarinic acid, phenolic compounds, *Plectranthus scutellarioides*

**Abbreviations**

(a.u.), arbitrary units; CDM, metal halide lamp; Dim, dimension; DW, dry weight; FW, fresh weight; FR, far-red light; HPS, high-pressure sodium; LEDs, light-emitting diodes; LMA, leaf mass per area; MPL, microwave plasma lamp; R:FR, red to far-red ratio; RA, rosmarinic acid; sd., standard deviation; TAC, total anthocyanin content; $TFC_{415\,nm}$, total flavonoid content; $TFC_{510\,nm}$, total flavonoid content; TPC, total phenolic content; PBAR, photo-biologically active radiation; PCA, principal component analysis.

## 5.2 Introduction

Due to the increasing use of light-emitting diodes (LEDs) in assimilation lighting, studies about the influence of the spectral light quality on plant morphology and secondary metabolites have appeared numerously. LEDs enable the study of morphological and phytochemical responses to distinct wavelengths, as recently reviewed (Bantis et al., 2018). High-pressure sodium lamps emit only a low amount of blue light; therefore, many studies have focused on the influence of blue light, which can be easily supplemented with LEDs. A higher blue light content is supposed to increase the amount of phenolic substances in leaves of several plant species, such as basil, lettuce, rose, campanulas and chrysanthemums (Son and Oh, 2013; Ouzounis et al., 2014; Bantis et al., 2016). Blue light directly increased the transcriptional activity of genes involved in the flavonoid biosynthetic pathway in *Arabidopsis thaliana* seedlings (Kubasek et al., 1992). An increased absolute blue light can reduce stem elongation as well, leading to compact plants with a higher degree of branching (Cope and Bugbee, 2013). Furthermore, the induction of sun-adapted leaves is controlled by blue light (Buschmann et al., 1978).

LEDs are an energy efficient light source due to the lack of infrared radiation and are therefore suitable for the multilayered production of young plants. LEDs are often used as assimilation light in climate chamber experiments. However, especially for research under environmentally closed conditions, the production of continuous sun-like light with LEDs, including UV and infrared light, is only possible with great effort because for each wavelength, single diodes have to be used (Fujiwara and Yano, 2011). Additionally, white light from blue coated LEDs do not emit light in the same proportions as natural sunlight.

Furthermore, in many approaches, when the influence of spectral quality is examined, gas discharge lamps, such as fluorescent lamps or high-pressure sodium lamps, are often used

as control conditions (Johkan et al., 2010; Son and Oh, 2013; Bantis et al., 2016). In addition to the spectral quality, the lamp systems also differ in the emission of infrared radiation. If the influence of infrared radiation is neglected, an overestimation of the influence of different light colors might occur.

In this study, we tested four different light systems: a new microwave plasma lamp (MPL) emitting artificial sunlight, a ceramic metal halide lamp (CDM), a LED and a commercial high-pressure sodium lamp (HPS). We studied both the influence of spectral quality and infrared radiation of these lamp systems on morphological parameters and secondary metabolites.

*Plectranthus scutellarioides* (syn.: *Solenostemon scutellarioides, Coleus blumei*) was used as a model plant that can change leaf coloration depending on the light environment (Nguyen and Cin, 2009). It is a member of the family of *Lamiaceae* and is used as an ornamental plant. A detailed understanding of its secondary compounds exists because *P. scutellarioides* is used as a medicinal plant for gastrointestinal problems (Andrade-Cetto, 2009). Leaf extracts from *P. scutellarioides* have antimicrobial and antioxidant properties that probably result from its high content of rosmarinic acid (RA) (Bulgakov et al., 2012; Bauer et al., 2015). RA, which is widespread in the family of *Lamiaceae* (Shekarchi et al., 2012), is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. Due to antioxidant and antimicrobial properties, RA can increase the plant defense against pathogens (Corral-Lugo et al., 2016). It was shown that RA acts as a protective agent in roots against pathogens in the soil (Baetz and Martinoia, 2014).

*P. scutellarioides* is supposed to contain a high amount of flavonoids measured by a polyphenol meter (Sytar et al., 2015). Using thin layer chromatography, cyanidin-3,5-diglucoside acylated with p-coumaric acid, dihydrokaempferol and apigenin were detected (Lamprecht et al., 1975; Lamprecht and Powell, 1977). A detailed analysis of phenolic compounds of the genus *Plectranthus* was performed by Grayer et al. (2010). However, the assignment of *P. scutellarioides* to these examined species is not clear. Therefore, qualitative UHPLC-MS analysis was performed with a focus on anthocyanins and phenolic acids. Selected phenolic compounds were further quantified per HPLC-DAD from plants grown under four different light treatments.

The aim of this study was to examine, whether phenolic ingredients were altered in *P. scutellarioides* leaves depending on the blue light content of the four light treatments. Due to the differences between the four lamp systems regarding the emission of infrared radiation

leaf temperature was measured. Furthermore, leaf histological analysis was performed, and the content of phytochemicals was determined in relation to dry weight and leaf area to investigate the impact of the lamp systems on both reference values.

## 5.3 Materials and methods

### *5.3.1 Plant material and general growth conditions*

Cuttings from *P. scutellarioides* mother plants ('Golden Dreams', Kientzler, Gensingen, Germany) were rooted in a common substrate (LAT-Terra Standard P, pH: 5.9, N: 120 mg/L, $P_2O_5$: 120 mg/L, $K_2O$: 170 mg/L, Mg: 120mg/L, HAWITA GRUPPE GmbH, Vechta, Germany) in 12 cm pots in a greenhouse in July 2018 at the University Geisenheim for two weeks at approximately 60% rel. humidity and 21 °C day/night temperature. Afterwards, plants with a height of about 10 cm and two leaf pairs were cultivated in a climate chamber for five weeks with different light conditions generated from four different lamp systems as described below. Plants were placed at a density of approximately 20 pots per m². The day/night temperature was 21±1 °C; rel. humidity was 60±10%.

### *5.3.2 Light treatments*

The climate chamber was divided into four compartments by opaque material to separate the four light conditions with a target light intensity of 100 µmol/m²s (Photosynthetic Photon Flux Density, 400–700 nm) for 20 h. Microwave plasma lamps emitting artificial sunlight (1300 W, Plasma International, Mühlheim, Germany), commercial high-pressure sodium lamps (600 W, DH Licht, Wülfrath, Germany), light-emitting diodes emitting predominately blue and red light but also a high amount of far-red light (300 W, LEDCon, Rheine, Germany) and ceramic metal halide lamps (315 W, DH Licht, Wülfrath, Germany) were used. The light spectra from the MPL, HPS, LED and CDM systems were recorded and analyzed using a Jaz UV–Vis Spectrometer with Spectra Suite 6.2 software (Ocean Optics, Ostfildern, Germany). Normalized emission spectra are shown in Fig. 5.1. Spectra were categorized as follows: UV-B (280–315 nm), UV-A (315–400 nm), blue (400–500 nm), green (500–550 nm), yellow (550–600 nm), red (600–700 nm) and far-red (700–800 nm). The proportions of light for each section are expressed as % photo-biologically active radiation (PBAR, 280–800 nm) and are shown in Tab. 5.1. The red to far-red (R:FR) ratio was calculated with 100 nm wavebands.

Light from the MPL showed similar emission spectra compared to sunlight recorded at noon in May 2018 in Geisenheim (49° 59′ 11.161″ N 7° 58′ 0.099″ E), with a blue light content of approximately 23% PBAR. The CDM also showed a full spectrum of light with a slightly lower blue light content (18% PBAR) and more green light. The lowest blue light content was emitted from HPS light (5% PBAR), and the highest proportion of blue light was emitted from LEDs (35% PBAR). Light intensity was measured for each plant at a height of 10 cm above the pot edge with a quantum sensor (LI-190R, LI-COR, Lincoln, Nebraska, USA). The average light intensity per treatment was as follows: MPL: 102±8 µmol/m²s, HPS: 101±16 µmol/m²s, CDM: 100±13 µmol/m²s and LED 102±22 µmol/m²s. Differences in the average light intensity were not significant ($p<0.05$).

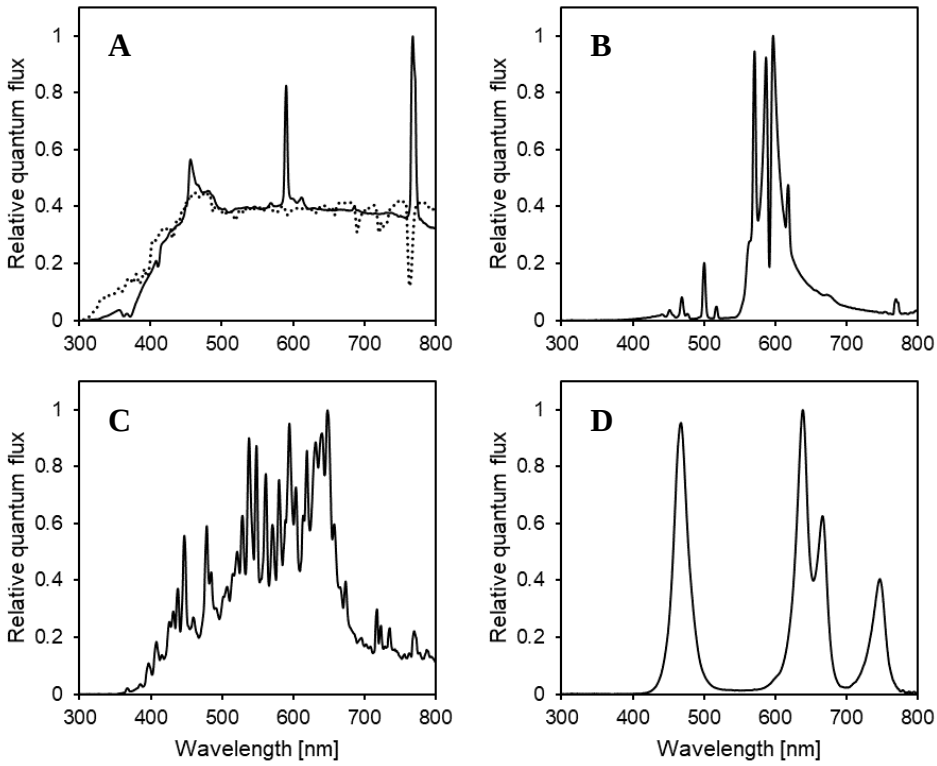


Fig. 5.1: Relative emission spectra of the four lamp systems. A: MPL (solid line) vs. sunlight[1] (dotted line). B: HPS. C: CDM and D: LED. Spectra of different lamp types were measured at 100 µmol/m²s and were normalized for better visualization.

[1]Sunlight was measured on a cloudless day (May 2018, 49° 59′ 11.161″ N 7° 58′ 0.099″ E, 90 m above sea level).

Tab. 5.1: Spectral properties of MPL, HPS, CDM and LED lamps. Values are the percentage of PBAR (280–800 nm).

| Light Sources | [nm] | UV-B 280–315 | UV-A 315–400 | blue 400–500 | green 500–550 | yellow 550–600 | red 600–700 | far-red 700–800 | R:FR ratio |
|---|---|---|---|---|---|---|---|---|---|
| MPL | % | - | 2.3 | 22.9 | 12.1 | 13.7 | 24.3 | 25.1 | 1.0 |
| HPS | % | - | 0.2 | 4.5 | 2.1 | 48.8 | 38.0 | 7.0 | 5.4 |
| CDM | % | - | 1.0 | 17.7 | 17.0 | 19.0 | 35.1 | 10.7 | 3.3 |
| LED | % | - | - | 34.8 | 1.5 | 1.5 | 47.3 | 14.8 | 3.2 |

### *5.3.3 Measurement of leaf temperature*

Due to the emission of different amounts of infrared radiation of the four light systems, leaf temperature was measured under the respective light source with a thermal imaging camera (H2640, Emissivity: 0.96, InfReC, Nippon Avionics, Tokyo, Japan). From each treatment, eight images of *P. scutellarioides* plants were recorded one week before morphological evaluation and analyzed by thermal imaging camera software (InfReC Analyzer NS9500, 2.7A, Nippon Avionics, Tokyo, Japan), whereby distance and angle were kept constant. The average leaf temperature was measured at two leaf pairs in the upper part of the plants.

### *5.3.4 Morphological parameters*

After a cultivation period of five weeks under the respective light source, 30 plants per treatment were evaluated for morphological parameters. Plant height was measured from the pot level up to the vegetation point. The number of fully developed leaf pairs was counted. Internode length was calculated from plant height divided by the number of leaf pairs. Stems and leaves were weighed individually to calculate the percentage of the stem weight among the total fresh weight (FW). Plant material was dried at 60 °C for at least three days and dry weight (DW) was determined.

### *5.3.5 Histological analysis*

Histological analysis of eight fully developed leaves from the top of the plants of each treatment was carried out according to Langkamp et al. (2015). After fixation and infiltration, leaf cross sections were embedded in paraffin and stained with methylene blue. Microscope images were taken using a Leica ICC50 camera controlled with microscope

software LAS ES 3.0.0 (Leica, Wetzlar, Germany). From each leaf cross section, leaf thickness was measured at three different positions.

### *5.3.6 Sample processing and determination of leaf mass per area (LMA)*

For determination of secondary metabolites, eight samples per treatment were analyzed. Each sample contained two fully developed leaf pairs from the top of the plants. The leaf area from each leaf was determined with a leaf area meter (LI-3100C, Li-COR, Lincoln, Nebraska, USA). After determining the leaf area, the plant material was immediately frozen at -20 °C and then freeze-dried for three days. DW of each leaf was determined with an analytical balance. LMA (mg dw/cm²) was calculated for each sample to determine the phytochemical contents in terms of g dw and cm² leaf area.

### *5.3.7 Colorimetric determination of phytochemicals*

For colorimetric determination of phytochemicals, approximately 10 mg of freeze-dried ground plant material were weighed and extracted with 2 mL 100% methanol for two days at RT (21 °C) in the dark under constant shaking. Total phenolic content (TPC), total flavonoid content (TFC) and total anthocyanin content (TAC) were measured by colorimetric methods optimized for a microplate reader as described below. Round-base 96-well microplates were used (Sarstedt, Nümbrecht, Germany). Absorbance was measured with an Infinite M200 microplate reader controlled with Magellan 7.2 Software (TECAN, Männedorf, Switzerland). For TFC, two different methods were used, which were assumed to be more selective for flavanols and luteolin ($TFC_{415\ nm}$) and for rutin, luteolin and catechine ($TFC_{510\ nm}$) (Pękal and Pyrzynska, 2014). Three technical replicates were measured for each sample. For each process, calibration curves were applied using respective reference substances as mentioned below at concentrations of 12.5, 25, 50, 100, and 250 µg/ml.

#### *Total phenolic content (TPC)*

The total phenolic content (TPC) was measured with a Folin-Ciocalteu (F-C) method first described by Singelton and Rossi (1965) and optimized for the microplate reader (Ainsworth and Gillespie, 2007). 200 µL 10% (v/v) F-C reagent (Sigma Aldrich, St. Louis, Missouri, USA) diluted in water were added to 100 µL of plant extract, and the sample was mixed

thoroughly. Afterwards, 800 µL of 700 mM aqueous $Na_2CO_3$ were added, and the samples were incubated for 15 min. Samples were centrifuged at 15,800 g for 1 min. Then, 100 µL of the supernatant were transferred to a microplate, and absorption was measured at 765 nm. TPC is expressed as gallic acid equivalents (GAE).

*Total flavonoid content at 415 nm ($TFC_{415\ nm}$) selective for flavonols and luteolin*

The flavonoid content selective for flavonols and luteolin was measured according to Ahmadi et al. (2013) and optimized for the microplate reader as follows: 100 µL of the plant extract were mixed with 300 µL methanol followed by 20 µL of a 10% (w/v) aqueous $AlCl_3$ solution (Sigma Aldrich, St. Louis, Missouri, USA) and 20 µL aqueous 1 M sodium acetate. Then, 560 µL water were added to obtain a final volume of 1000 µL. After incubation for 30 min, 100 µL of solution were transferred to a microplate, and the absorbance was measured at 415 nm. $TFC_{415\ nm}$ is expressed as quercetin equivalents (QE).

*Total flavonoid content at 510 nm ($TFC_{510\ nm}$) selective for rutin, luteolin and catechine*

The total flavonoid content selective for rutin, luteolin and catechine was measured according to Eghdami and Sadeghi (2010):100 µL of the leaf extract were mixed with 300 µL water followed by 30 µL 5% (w/v) aqueous $NaNO_2$. After 5 min, 30 µL 10% (w/v) aqueous $AlCl_3$ were added. After further 5 min, the reaction mixture was treated with 200 µL 1 mM aqueous NaOH. Then, 340 µL water were added to a final volume of 1000 µL. 100 µL of solution were transferred to a microplate, and the absorbance was measured at 510 nm. $TFC_{510\ nm}$ is expressed as catechin equivalents (CE).

*Total anthocyanin content (TAC)*

A total of 200 µL of leaf extract were diluted with 800 µL methanol, and 100 µL of this solution were transferred to a microplate. Afterwards, 10 µL of 10% (v/v) HCl solution (37%, w/v) diluted in methanol were added to each well, and the absorbance at 530 nm and 657 nm was measured. TAC was calculated according to Mancinelli (1989):

$$Antho = A_{530\ nm} - 0.25A_{657\ nm}$$

TAC is expressed as cyanidin-3,5-O-diglucoside equivalents.

### *5.3.8 Sample extraction for HPLC analysis*

For qualitative and quantitative HPLC analysis, plant powder was extracted with methanol+water+formic acid (80+19+1, v+v+v) in an ultrasonic bath two times for 20 min at RT in a concentration of 25 mg dw/ml.

### *5.3.9 Qualitative analysis of phenolic compounds by UHPLC-MS*

For qualitative analysis of phenolic compounds, an Acquity UPLC system (Waters, Milford, MA, USA) consisting of a binary pump (BSM), an autosampler (SM-FTN) cooled at 10 °C, a column oven (CM) set at 40 °C, a diode array detector (PDA) scanning from 190 to 500 nm and a tandem quadrupole mass spectrometer (Xevo TQ-S Micro) were used.

Phenolic compounds were separated using a Cortecs UPLC Shield RP18 column (150 mm x 2.1 mm; 1.6 µm particle size) from Waters. Eluent A was acetonitrile/0.1% formic acid, and eluent B was water/0.1% formic acid. The gradient program was as follows: 0 min, 90% B; 15 min, 60% B; 15.5 min, 0% B; 16.5 min, 0% B; 17.5 min, 90% B; 18.5 min, 90% B. The flow rate was 0.4 mL/min. The mass spectrometer was tuned using a solution of rutin and quercetin. The following parameters were used for negative and positive ionization, respectively: capillary voltage, –2.5 kV/2.4 kV; cone voltage 5 V/–10 V; source temperature 150 °C; desolvation temperature 600 °C; cone gas (nitrogen) flow 50 L/h; desolvation gas (nitrogen) flow, 1000 L/h. The collision energies varied from 20 to 45 eV. First, compounds known from the literature were searched using selected ion monitoring and selected reaction monitoring. Further compounds (especially those that gave major signals in the UV chromatogram) were detected using single MS scanning, followed by fragment ion scans. The resulting mass spectra were used to postulate structural assignments. Chromatograms of the qualitative UHPLC analysis are shown in Fig. S5.8.

### *5.3.10 Quantitative analysis of selected phenolic compounds by HPLC-DAD*

Selected phenolic compounds were quantified by high-performance liquid chromatography with diode array detection using a LaChrom Elite HPLC System (Hitachi High Technologies America, Inc., Schaumburg, Illinois, USA) connected with a PDA (L-2130, Hitachi High Technologies America Inc., Schaumburg, Illinois, USA). The plant extracts were manually loaded by a Hamilton syringe on a 20 µL sample loop. Phenolic compounds were separated using a RP ReproSil-Pur 120 CIB-4Q column (150 mm x 2 mm, 3 µm particle size) and a

matching guard column from Dr. Maisch (Ammerbuch, Germany). Phenolic compounds were separated at a flow rate of 200 µL/min with a mixture of eluent A containing 2% (v/v) acetic acid and solvent B containing acetonitrile/water/acetic acid (10:10:1) at RT with a linear gradient according to the following program: 0 min, 10% B; 5 min, 55% B; 20 min, 90% B; 24 min, 10% B. Chromatograms were recorded at 330 nm for rosmarinic acid and flavone derivatives (Fig. S5.9). Absorbance spectra were recorded for individual phenolic compounds, and absorbance maxima were determined. The peak area of the main phenolic compounds was calculated using EZCHrom software (Agilent Technologies, Inc., Santa Clara, California, USA). Rosmarinic acid (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) was used as an authentic standard, and a calibration curve was carried out to determine the absolute rosmarinic acid content. Other phenolic compounds were relatively quantified and are expressed as arbitrary units (a.u.) based on peak area.

### *5.3.11 Data analysis*

Statistical analysis was performed using R (R 3.4.3). Data were analyzed for normality with the Shapiro-Wilk test. Levene's test was used to assess the equality of variance. If the conditions were fulfilled, one-way analysis of variance (ANOVA) ($p \leq 0.05$) was performed with Tukey's range test as a post hoc analysis. If the data were not normally distributed and/or heterogeneous, the nonparametric Kruskal-Wallis test ($p \leq 0.05$) was used. Repetitions were based on individual plants. The experiment was repeated independently. A box plot was created with the R package ggplot2.

Multivariate analysis was performed by using R package FactoMineR for a principal component analysis (PCA) and the package factoextra for visualization based on ggplot2. Quantitative data of secondary metabolites and leaf mass per area were used for PCA. For individuals plot, the ellipse.level was set to 0.68. Average leaf temperature and proportion of blue light were used as supplementary quantitative variables.

## 5.4. Results

### *5.4.1 Leaf temperature*

The effect of infrared radiation from different lamp systems on leaf temperature are shown in thermal images (Fig. 5.2). Upper leaves of *P. scutellarioides* plants that were directly exposed to the respective light source showed slightly higher leaf temperatures compared to

shaded leaves. The average leaf temperature was significantly higher in plants under MPL and HPS lamps compared to plants under CDM and LED (Tab. 5.2). Leaves under LED light showed the lowest leaf temperature, and no distinct difference in upper leaves and shaded leaves was observed due to the missing amount of IR in LED irradiance (Fig. 5.2).

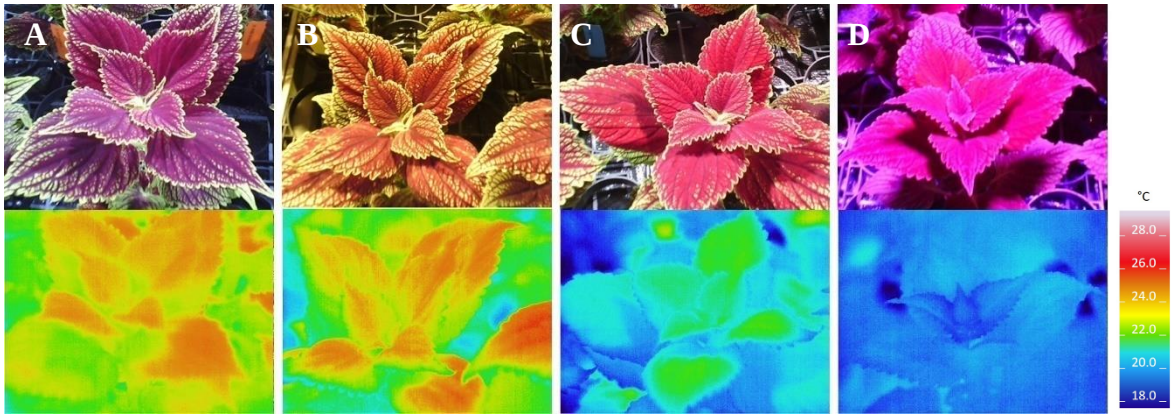


Fig. 5.2: Thermal images from *P. scutellarioides* plants under MPL (A), HPS (B), CDM (C) or LED (D) light grown for four weeks under the respective light source. The lower four images show the thermal images; the upper four images show the respective picture of the plant under the artificial light source.

Tab. 5.2: Average leaf temperature measured with a thermal imaging camera. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=8 plants.

| Leaf temperature | MPL | HPS | CDM | LED |
|---|---|---|---|---|
| [°C] | 23.6±0.3$^{a}$ | 23.0±0.8$^{a}$ | 21.7±0.6$^{b}$ | 20.3±0.4$^{c}$ |

### *5.4.2 Morphological parameters*

Plants grown under artificial sunlight from MPL and LED lamps showed the highest elongation growth, as indicated by increased plant height and internode length, compared to plants grown under HPS and CDM light (Fig. 5.3, Tab. 5.3). The internode length was the highest in plants grown with LEDs. The most compact plants were noted when CDM lamps were used as assimilation light. The average number of leaf pairs was significantly higher when plants were grown with light from MPL (7.6±0.6) and HPS lamps (7.4±0.7) in comparison to plants grown with CDM (6.9±0.7) and LED light (6.8±0.5). Artificial sunlight from the MPL led to an increase of about 13-20% in FW and about 20-25% in DW compared to the other light treatments. Plants grown with LED showed the lowest amount of FW;

nevertheless, total DW was higher compared to plants grown with HPS lamps. However, the difference in DW was not significant.

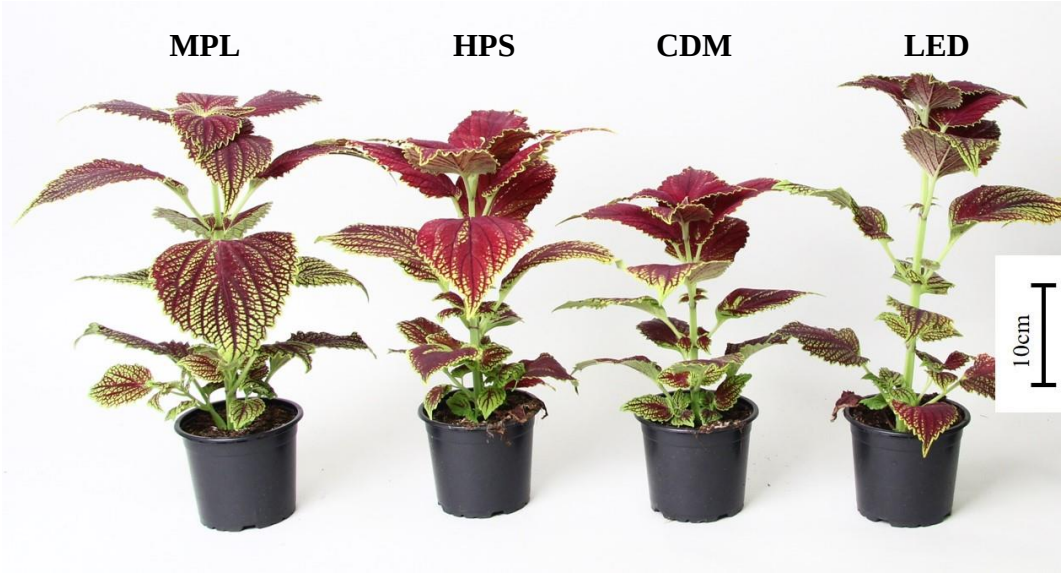


Fig. 5.3: Representative *P. scutellarioides* 'Golden Dreams' grown for five weeks in the climate chamber with MPL, HPS, CDM or LED light for 20 h with 100 µmol/m²s per day.

Tab. 5.3: Morphological parameters of *P. scutellarioides* plants grown for five weeks under the four different lamp systems: MPL, HPS, CDM and LED in a climate chamber. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=30 plants.

| Morphological parameters | MPL | HPS | CDM | LED |
|---|---|---|---|---|
| Height [cm] | 30.1±2.4$^{a}$ | 26.7±3.3$^{b}$ | 22.5±2.9$^{c}$ | 29.2±3.2$^{a}$ |
| Number of leaf pairs | 7.6±0.6$^{a}$ | 7.4±0.7$^{a}$ | 6.9±0.7$^{b}$ | 6.8±0.5$^{b}$ |
| Internode length [cm] | 4.0±0.4$^{b}$ | 3.6±0.5$^{c}$ | 3.3±0.4$^{d}$ | 4.3±0.5$^{a}$ |
| Total FW [g] | 50.3±6.8$^{a}$ | 43.9±5.4$^{b}$ | 41.9±7.8$^{bc}$ | 40.1±7.7$^{c}$ |
| Total DW [g] | 5.9±0.8$^{a}$ | 4.4±0.7$^{b}$ | 4.5±0.8$^{b}$ | 4.7±0.9$^{b}$ |
| Stem FW [%] | 31.7±2.3$^{b}$ | 26.8±2.9$^{c}$ | 26.2±2.9$^{c}$ | 36.6±3.2$^{a}$ |

The leaf mass per area was the highest in leaves grown with the LED, followed by the MPL and CDM. Leaves grown under the HPS developed the lowest amount of LMA (Tab. 5.4). Leaves that developed under the LED lamps also felt most robust. Leaf thickness measured from leaf cross sections was the highest in leaves grown with LEDs, and the results are consistent with LMA (Fig. 5.4, Tab. 5.4). The HPS leaves were much thinner compared to leaves grown with the other light sources, and the palisade parenchyma was less pronounced. A correlation between the leaf thickness and the proportion of blue light from the respective light source was found ($R^2$=0.89).

Tab. 5.4: The average leaf mass per area and leaf thickness from *P. scutellarioides* plants grown under the four different lamp systems: MPL, HPS, CDM and LED for five weeks in a climate chamber. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=8 leaves.

| Leaf histology | MPL | HPS | CDM | LED |
|---|---|---|---|---|
| LMA [mg dw/ cm²] | 3.9±0.2$^{b}$ | 2.7±0.2$^{c}$ | 3.7±0.3$^{b}$ | 4.8±0.4$^{a}$ |
| Leaf thickness [µm] | 194.2±15.4$^{b}$ | 135.5±17.2$^{c}$ | 192.5±14.7$^{b}$ | 216.1±21.1$^{a}$ |

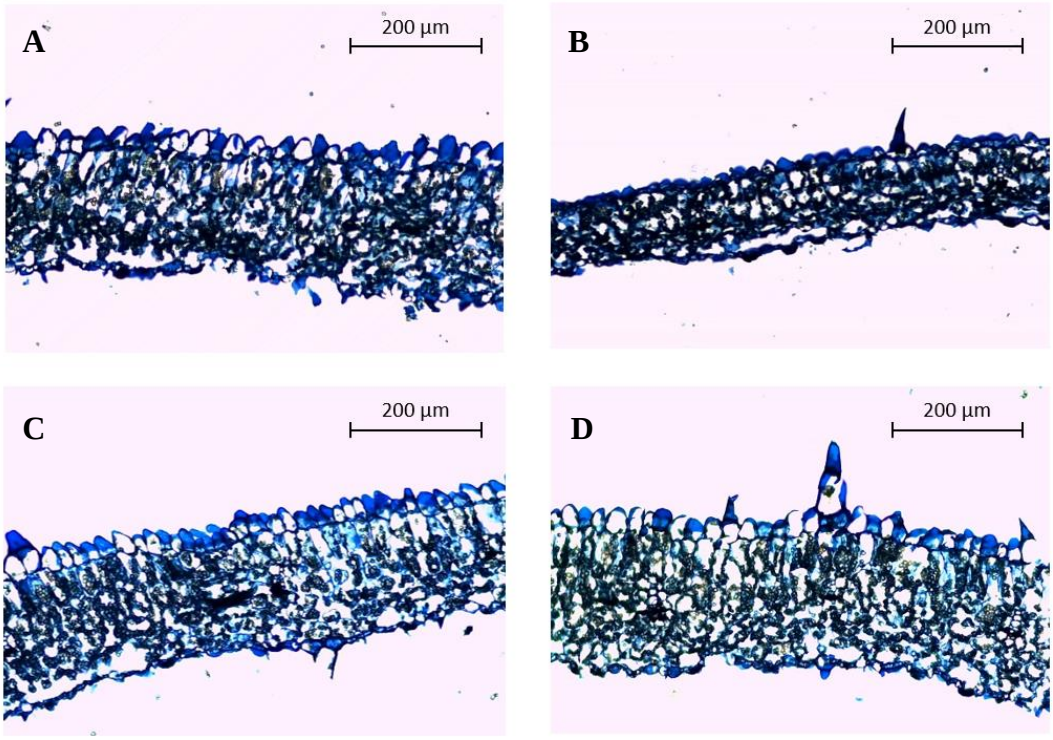


Fig. 5.4: Leaf cross section of *P. scutellarioides* plants grown under MPL (A), HPS (B), CDM (C) and LED (D) light.

### *5.4.3 Total phenolic, total flavonoid and total anthocyanin contents*

Because LMA was determined per sample, the content of phytochemicals was calculated in relation to g dw and cm² leaf area. The total flavonoid content measured with $TFC_{510\ nm}$ correlated with the total phenolic content ($R^2$=0.94); therefore, only the TPC results are shown. Total phenolic content per g dw was significantly higher in leaves grown under LED and CDM lamps compared to MPL and HPS leaf samples (Tab. 5.5). In relation to g dw, no significant difference in TPC between MPL and HPS leaves was found. Though, in relation to the leaf area, MPL leaves accumulated more phenolic compounds due to the higher leaf thickness in comparison to HPS grown leaves. Because leaf mass per area was highest in leaves from the LED treatment, the TPC per cm² was also the highest for leaves in this

treatment. Nevertheless, total anthocyanin content per g dw was highest under CDM light, whereby leaves from MPL displayed the lowest TAC per g dw. In relation to leaf area, no significant difference in total anthocyanin content per cm² was obtained under MPL or HPS light. TAC per cm² was significantly higher under CDM and LED light. The total flavonoid content measured with $TFC_{415\ nm}$ was highest in CDM leaves per g dw. The higher leaf mass per area in plants grown under LED light led to the significantly higher $TFC_{415\ nm}$ in relation to the leaf area (Tab. 5.5).

Tab. 5.5: Phenolic compounds measured by colorimetric methods in *P. scutellarioides* leaves of plants grown with light from MPL, HPS, CDM and LED lamps. The content of phenolic compounds is expressed as mg per g dw or µg per cm² leaf area. TPC= total phenolic content, expressed as gallic acid equivalents; TAC= total anthocyanin content expressed as cyanidin-3,5-O-diglucoside equivalents; $TFC_{415\ nm}$= total flavonoid content, expressed as quercetin equivalents. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=8.

| Phenolic compounds | | MPL | HPS | CDM | LED |
|---|---|---|---|---|---|
| TPC | [mg/g dw] | $52.4\pm4.2^{b}$ | $50.7\pm6.7^{b}$ | $66.8\pm3.8^{a}$ | $63.1\pm5.2^{a}$ |
| | [µg/cm²] | $181.1\pm15.6^{c}$ | $127.2\pm18.9^{d}$ | $225.9\pm22.7^{b}$ | $263.3\pm29.0^{a}$ |
| TAC | [mg/g dw] | $15.0\pm2.5^{c}$ | $18.3\pm3.8^{b}$ | $21.9\pm1.9^{a}$ | $16.2\pm2.0^{bc}$ |
| | [µg/cm²] | $52.1\pm9.5^{b}$ | $46.2\pm11.0^{b}$ | $73.9\pm8.0^{a}$ | $67.7\pm9.8^{a}$ |
| $TFC_{415\ nm}$ | [mg/g dw] | $13.1\pm0.9^{c}$ | $12.2\pm0.6^{d}$ | $16.2\pm0.4^{a}$ | $15.2\pm1.0^{b}$ |
| | [µg/cm²] | $45.3\pm3.6^{c}$ | $30.6\pm1.9^{d}$ | $54.9\pm3.7^{b}$ | $63.3\pm4.7^{a}$ |

### *5.4.4 Qualitative analysis of phenolic compounds*

A typical UHPLC chromatogram is shown in Fig. S5.8. Phenolic compounds were identified according to their *m/z* of molecular ions, their fragments in $MS^2$, elution order and absorption maxima as previously published (Zimmermann et al., 2011). Analytical data are shown in Tab. S5.7, Tab. S5.8. Rosmarinic acid (RA) ($Peak_{360\ nm}$ No. F3, *m/z* 359) was found to be the main phenolic compound in the examined *P. scutellarioides* cultivar (Tab. S5.7, Fig. S5.8C). The identity of RA was additionally confirmed by comparison with an authentic standard. *P. scutellarioides* further contains luteolin and apigenin derivatives: luteolin as such ($Peak_{360\ nm}$ No. F8, *m/z* 285), luteolin and apigenin glucuronides ($Peak_{360\ nm}$ No. F2 and F5, *m/z* 461 and 455), a luteolin hexoside ($Peak_{360\ nm}$ No. F1, *m/z* 447) and luteolin and apigenin containing an unidentified residue (abbreviated as X) of 218 Da ($Peak_{360\ nm}$ No. F6 and F7, *m/z* 503 and 487). Salvianolic acid b, an ester of two RAs, eluted next to RA ($Peak_{360\ nm}$ No.

F4, *m/z* 717). Two substances with *m/z* 313 and identical fragments were found ($Peak_{360\ nm}$ No. F9 and F10) (Tab. S5.7) but could not be identified. Apigenin and an apigenin hexoside were also detected but not further considered due to their low concentrations.

Analytical data of the peaks detected at 500 nm are shown in Tab. S5.8. Three cyanidin glycosides were found in *P. scutellarioides* leaves: cyanidin bound to a coumaroyl and two hexosyl moieties ($Peak_{500\ nm}$ No. A2, *m/z* 757), cyanidin bound to a coumaroyl, two hexosyl moieties and a malonoyl moiety ($Peak_{500\ nm}$ No. A4, *m/z* 843) and cyanidin bound to a coumaroyl, two hexosyl moieties and two malonoyl moieties ($Peak_{500\ nm}$ No. A5, *m/z* 493). A substance with *m/z* 533 could not be fragmented and thus could not be identified ($Peak_{500\ nm}$ No. A1).

### *5.4.5 Quantitative HPLC analysis of* rosmarinic acid and flavone derivatives

The main phenolic compounds were quantified from the same plant material that was also used for colorimetric analysis. Among the phenolic compounds, only RA was absolutely quantified because it appeared in highest quantities and was our main interest due to relevance as a pharmaceutical ingredient. Other substances appeared in lower amounts and were quantified in relative amounts and are expressed as arbitrary units based on their peak area. The absolute RA content in leaves grown with the four different light systems is shown in Fig. 5.5, whereby Fig. 5.5A displays the RA content in relation to g dw and Fig. 5.5B shows the RA content in relation to leaf area.

Plants that grown with artificial sunlight from MPL or HPS showed a significantly lower RA content per g dw compared to LED and CDM leaf samples. Due to the higher LMA in leaves developed under MPL light and LEDs, the RA content per $cm^2$ is significantly increased compared to leaves grown under HPS and CDM light. The content of rosmarinic acid showed a high correlation with TPC ($R^2$=0.90, Fig. 5.6) and $TFC_{510\ nm}$ ($R^2$=0.93, not shown) as measured by colorimetric methods. The relative amounts of flavone derivatives expressed as a.u. per g dw or a.u. per $cm^2$ leaf area are shown in Tab. 5.6

Overall, leaves grown with LED showed the highest content of flavone derivatives in relation to dw as well as leaf area. Apigenin-X per g dw was, however, significantly higher in plants grown under CDM light. The lowest amount of flavone derivatives was found in leaves from HPS plants.

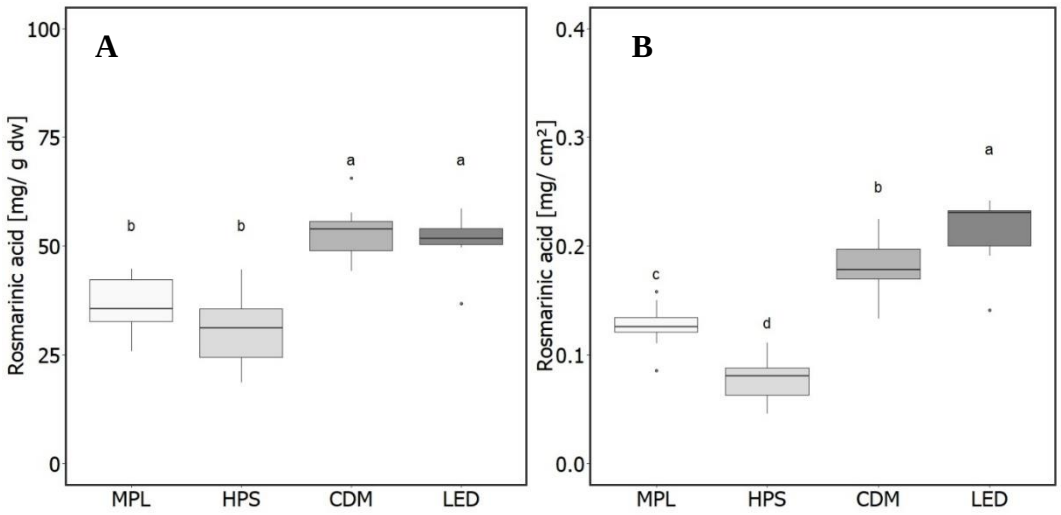


Fig. 5.5: Rosmarinic acid content in *P. scutellarioides* leaves. A: mg Rosmarinic acid per g dw. B: mg Rosmarinic acid per cm² leaf area. Unequal letters indicate significant difference, $p<0.05$; n=8.

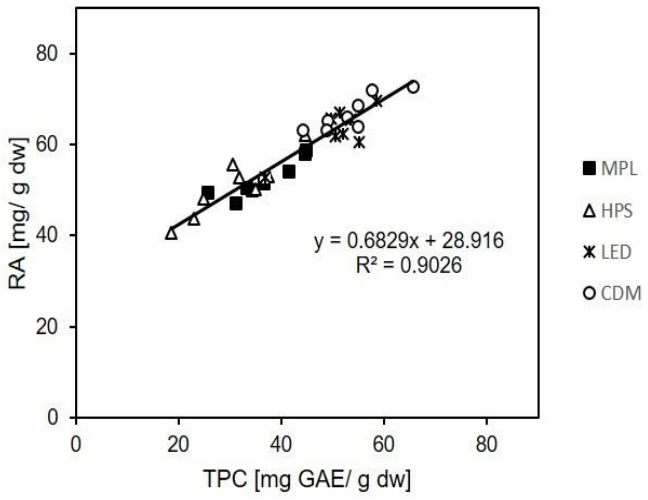


Fig. 5.6: Correlation of TPC [mg GAE/ g dw] measured by colorimetric method and RA [mg/g dw] quantified by HPLC of analyzed leaf samples from plants grown under MPL, HPS, CDM or LED light; n=8.

Tab. 5.6: Content of detected flavone glucoside derivatives in *P. scutellarioides* leaves from plants grown under MPL, HPS, CDM and LED light measured by HPLC-DAD. Values are means ± sd and are expressed as arbitrary units (a.u) per g dw or a.u. per cm² leaf area. Unequal letters in a row indicate significant difference, $p<0.05$; n=8.

| Flavone derivatives | | MPL | HPS | CDM | LED |
|---|---|---|---|---|---|
| Luteolin hexoside | [a.u./g dw] | 351.2±37.2$^{c}$ | 266.8±21.4$^{d}$ | 438.1±52.7$^{b}$ | 489.8±48.6$^{a}$ |
| | [a.u. /cm²] | 1.22±0.14$^{c}$ | 0.67±0.07$^{d}$ | 1.48±0.18$^{b}$ | 2.04±0.23$^{a}$ |
| Luteolin glucuronide | [a.u. /g dw] | 325.5±35.6$^{a}$ | 271.6±20.1$^{b}$ | 374.4±76.4$^{a}$ | 320.6±49.7$^{a}$ |
| | [a.u./cm²] | 1.13±0.16$^{b}$ | 0.68±0.07$^{c}$ | 1.26±0.23$^{ab}$ | 1.33±0.17$^{a}$ |
| Apigenin-X | [a.u. /g dw] | 770.8±63.0$^{b}$ | 762.3±47.6$^{b}$ | 951.3±106.6$^{a}$ | 796.0±86.3$^{b}$ |
| | [a.u. /cm²] | 2.66±0.16$^{b}$ | 1.91±0.17$^{c}$ | 3.20±0.24$^{a}$ | 3.30±0.26$^{a}$ |

### *5.4.6 PCA Analysis*

To estimate the correlation between the influence of leaf temperature and proportion of blue light on the content of phenolic ingredients a principal component analysis (PCA) was performed. Therefore, data from the quantitative secondary metabolites and leaf mass per area were analyzed. The first two principal components are describing about 85.5% of the variance of the data set (Scree Plot, Fig. S5.10). Samples grouped along Dim1 and Dim2 in individuals plot according their light treatment (Fig. 5.7A). The separation in Dim1 was more pronounced and describes about 68.5% of the variance, whereas Dim2 only describes about 17% of the data and separation of the groups was less clear. Variable plot shown in Fig. 5.7B indicate that Dim1 is mainly represented by content of phenolic ingredients such as RA and results from colorimetric methods (TPC, $TFC_{415\,nm}$ and $TFC_{510\,nm}$). A high correlation of the results from colorimetric methods with RA was previously shown in univariate analysis (Fig. 5.6). A correlation of luteolin hexoside with RA and results from colorimetric methods was observed in PCA analysis. These secondary metabolites showed a high negative correlation with higher leaf temperatures, which is mainly responsible for separation of MPL and HPS (high leaf temperatures, low content of RA) and LED and CDM treatments (low leaf temperatures, high RA). Apart from that, blue light which led to an

increased LMA showed a minor correlation with phenolic compounds. Apigenin-X and luteolin glucuronide were almost not influenced by leaf temperature or blue light and are contributing for Dim2 and the separation of LED and CDM. HPS and MPL were less affected by Dim2.

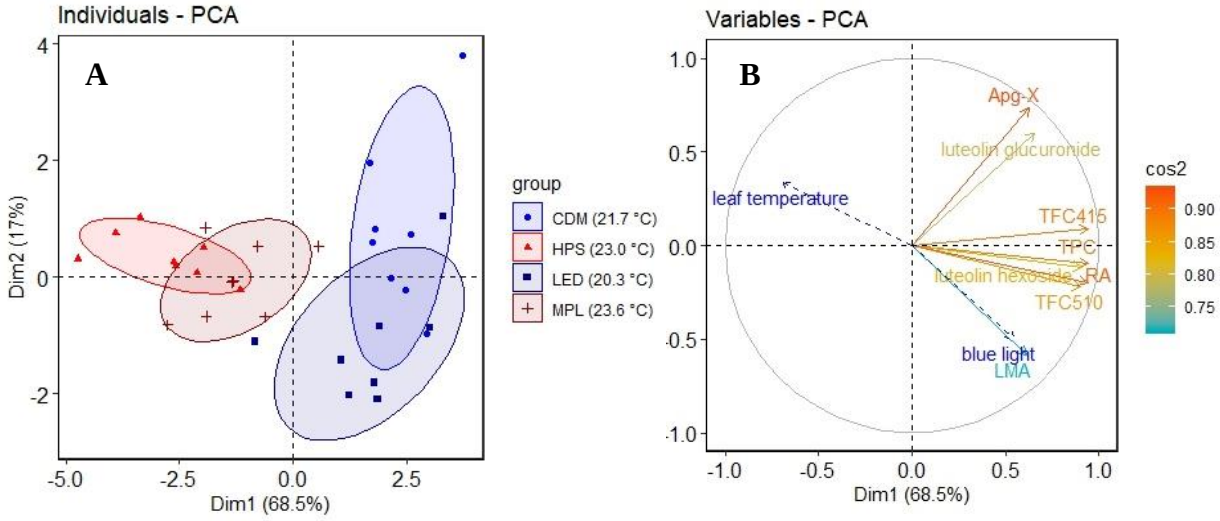


Fig. 5.7: Principal component analysis using quantitative data of phenolic compounds obtained from colorimetric methods and HPLC analysis and leaf mass per area data. A: Individuals plot. B: Variables plot; variables are colored according to their quality of representation (cos2); leaf temperature and blue light content were added as supplementary quantitative variables (dashed blue line).

## 5.5. Discussion

### *5.5.1 Plant growth was mainly affected by FR light and infrared radiation*

Plants grown with MPL and LED light showed increased plant height, longer internodes and lower proportions of leaf weight, compared to plants grown with CDM or HPS lamps. Light from MPL showed the lowest R:FR ratio (1.0). A low R:FR ratio is attributed to shade avoidance syndrome, inducing elongated growth (Smith, 1982). Plant height was linear increased with a decreasing R:FR ratio as demonstrated with geranium, petunia, snapdragon and impatiens by using B, R and FR LEDs (Park and Runkle, 2017). However, plants grown with CDM light showed shorter internode length compared to plants grown with LED light, although the R:FR ratio was almost equal (3.2 and 3.3).

This leads to the assumption that not only the R:FR ratio but also the absolute amount of FR radiation might increase stem elongation. The absolute amount of FR light in the area of the

absorption maxima of phytochromes (725–735 nm) was distinctly higher in the LED treatment (~2.6 µmol/m²s) compared to CDM treatment (~1.4 µmol/m²s).

Plants grown under MPL light contained the highest number of leaves and highest total fresh and dry weight. An increased leaf unfolding rate and higher biomass production were found in cucumber plants grown with an artificial sunlight spectrum (Hogewoning et al., 2010a). We additionally assume that increased infrared radiation was also responsible for faster plant development. Plants grown under LED and CDM light displayed lower leaf temperatures under the respective light source compared to plants grown with MPL or HPS light (Fig. 5.2, Tab. 5.2) and formed almost one leaf pair less on average. In an energy balance model, it was calculated that non-water stressed leaves under LED light showed only a 1.3 °C lower leaf temperature compared to leaves under HPS lamps. However, when leaves are water stressed, differences in leaf temperature under the respective light source can be significantly higher (Nelson and Bugbee, 2015). In our experiments, plants were not water stressed and displayed much higher differences in leaf temperature. A lower leaf temperature was also found in cucumber plants grown with LEDs in comparison to HPS-grown plants, leading to lower fruit yield (Särkkä et al., 2017). Additionally, a lower biomass production of rose and tomato plants grown with LEDs was attributed to a lack of heat emission from the LED light source in comparison with HPS-grown plants, where leaf temperature was approximately 1–2 °C higher (Bergstrand et al., 2016). This leads to the assumption that the impact on leaf temperature should not be unattended when LEDs are compared to high discharge lamp systems.

### *5.5.2 Blue light increased leaf thickness*

Leaves grown with LEDs showed a LMA approximately 1.7 higher compared to leaves grown with HPS lamps (Tab. 5.4). LMA is a good indicator that leaf morphology, e.g., leaf thickness and cell density, has changed (Poorter et al., 2009).

Leaves with higher LMAs showed both a thicker leaf and more pronounced palisade parenchyma detected in leaf cross sections (Fig. 5.4). Leaves with higher LMA and leaf thickness showed higher tolerance against high sunlight intensity and drought stress (not published data). The increase in LMA and leaf thickness is accompanied by an increase in blue light content in the light spectra of the examined lamp systems. Blue light is attributed to stimulate the development of sun-adapted leaves (Buschmann et al., 1978). When plants were grown with blue-deficient light sources, such as HPS lamps, they formed shade leaves

with lower LMA and reduced leaf thickness (Terfa et al., 2013), which we also found in the leaves of *P. scutellarioides*.

### *5.5.3 Rosmarinic acid and phenolic compounds were mainly influenced by leaf temperature*

Flavonoids and other phenolic compounds protect leaves from high UV radiation from sunlight, which can damage proteins, nucleic acids and cell membranes (Mierziak et al., 2014). Phenolic acids absorb harmful light in the UV and blue regions and emit energy as heat. Because LMA and leaf thickness were different under the respective light treatments, the amount of phytochemicals was measured in relation to g dw and leaf area (Tab. 5.5, Tab. 5.6, Fig. 5.5).

It was expected that in *P. scutellarioides* plants, phenolic ingredients were increased when plants were grown with lamps containing higher amounts of blue light because it was shown that increased blue light content led to higher phenolic compounds such as basil, campanula, chrysanthemum and rose leaves (Ouzounis et al., 2014; Bantis et al., 2016). In our study, we observed that infrared radiation, resulting in different leaf temperatures, showed a higher influence on the phenolic compounds per g dw compared to the influence of spectral light quality in *P. scutellarioides* leaves. Leaves with lower temperature due to CDM and LED light showed the highest TPC and $TFC_{415\,nm}$ content. Lower leaf temperature induced transcriptional activity in genes involved in the phenylpropanoid pathway (Christie et al., 1994), supporting our observations. However, the amount of blue light, leading to increased leaf thickness, is responsible for a higher accumulation of TPC per leaf area, as shown by the comparison of TPC per $cm^2$ in the light treatment between the MPL and HPS and between the LED and CDM lamps.

Colorimetric methods only give an overall impression of all phenolic compounds, including anthocyanins, flavonoids and phenolic acids. Especially the TPC method is unspecific because nonphenolic agents such as ascorbic acids are also reacting with the F-C reagent (Singelton and Rossi, 1965; Huang et al., 2005).

For this reason, qualitative and quantitative HPLC analysis were performed. In addition to rosmarinic acid, apigenin and luteolin derivatives were detected, which, to our knowledge, have not yet been described in *P. scutellarioides*. A luteolin hexoside, luteolin glucuronide and apigenin with an unknown residue (referred as X) appeared in larger quantities. Luteolin

glucoside and luteolin glucuronide were also found in other members of the *Lamiaceae* family, including rosemary, thyme, oregano and basil (Hossain et al., 2010).

TPC is correlated with RA ($R^2$=0.90); therefore, the dependency of leaf temperature on RA content was found. In experiments with *Mentha spicata,* it was demonstrated that RA content and TPC decrease when plants are exposed to increased heat stress (Fletcher et al., 2005). This supports our observations in which RA per g dw decreases with higher leaf temperatures. Leaves grown with MPL and HPS light might be stressed by excess infrared radiation, leading to a lower total amount of RA per dw. However, the higher LMA caused by the blue light content led to a higher RA concentration per $cm^2$ leaf area in MPL- and LED-treated leaves. In the principal component analysis, it was visualized, that leaf temperature is the main influence factor of the accumulation of secondary metabolites in leaves of *P. scutellarioides*. A high correlation of RA and results from colorimetric methods was found. Also luteolin hexoside showed a negative correlation to leaf temperature. A part from that, Apigenin-X and luteolin glucuronide seem to be unaffected by leaf temperature or blue light content.

*P. scutellarioides* leaves of the examined cultivar contained the highest amount of RA per g dw in leaves grown with CDM (53.5±6.5) or LED (51.0±6.4) light. In a comparative analysis of several members of the family *Lamiaceae,* a higher content of RA (58.5 mg/ g dw) was measured only in spearmint (*Mentha spicata*) (Shekarchi et al., 2012). Due to the high accumulation of RA in *P. scutellarioides,* approaches to produce RA in *P. scutellarioides* cell cultures still appeared in the late 1970s, with RA contents of 80–110 mg /g dw being accumulated (Razzaque and Ellis, 1977). Additionally, hairy root cultures from *P. scutellarioides* are promising for RA production in bioreactors (Bauer et al., 2015). We assume that RA production in *P. scutellarioides* cell cultures can also be induced by lower temperatures.

Flavonoid aglycones accumulate in the upper epidermis (Wollenweber and Dietz, 1981; Tattini et al., 2000) and act as screens against UV radiation. The examination of flavonoid content per leaf area is therefore a good indicator of the amount of phytoprotectors synthesized by plants. However, flavonoid glycosides and phenolic acids occurring in *P. scutellarioides* in higher quantities are also found in vacuoles in mesophyll tissue due to their high solubility (Agati et al., 2009). These compounds protect plant cells by reducing reactive oxygen species (ROS) due to their high antioxidant properties (Takahama and Oniki, 1997).

### *5.5.4 Influence of light sources on the content of anthocyanins*

Three cyanidin derivatives were found in *P. scutellarioides* leaves: they all contain one coumaroyl residue and two hexose residues. Two of them are malonylated containing one or two malonyl residues. The malonylated cyanidin glycosides appeared in higher quantities. Cyn-3,5-diglucoside acylated with p-coumaric acid was also detected in *P. scutellarioides* leaves by thin layer chromatography (Lamprecht et al., 1975; Lamprecht and Powell, 1977). The cyanidin diglucosides with a coumaroyl or a coumaroyl and a malonyl residue were found in red-colored leaves of *Perilla frutescens*, which also belongs to the *Lamiaceae* and are referred as shisonin and malonylshisonin (Meng et al., 2006). The same anthocyanin glycosides were found in red basil (Strazzer et al., 2011).

When *P. scutellarioides* is used as an ornamental plant, the anthocyanin content is important for the ornamental value of the plants. The anthocyanin content per g dw was the highest under CDM light. Anthocyanin synthesis is also influenced by temperature. Transcription of genes involved in anthocyanin synthesis is increased by lower temperature (Christie et al., 1994). However, leaves developed under HPS light, which displayed a higher leaf temperature, showed a higher content of anthocyanins compared with leaves grown with LED and MPL light. It was also shown that increased far-red light leads to decreased anthocyanin production in lettuce and might also be responsible for lower TAC per g dw in LED- and MPL-treated leaves (Li and Kubota, 2009). Far-red light is a signal that leaves are shaded. Shaded leaves of *P. scutellarioides* showed a decreased accumulation of anthocyanins in the leaf surface (Nguyen and Cin, 2009).

## 5.6 Conclusion

Most of the published results comparing different light systems are mainly focused on the spectral quality of light. We demonstrated that leaf temperature changes when different lamp systems are compared, and this had a higher effect on the production of RA and flavone derivatives in *P. scutellarioides* leaves than the blue light content. Leaves grown with LEDs or CDM lights showed the highest total RA per g dw. The high amount of blue light in experiments using LEDs is often attributed to the high accumulation of TPC. Nevertheless, when LEDs are compared with gas discharge lamps, the influence of infrared radiation is often neglected, leading to an overestimation of the blue light effect in many studies where leaf temperature is not measured. However, a higher content of blue light in the emission

spectra led to thicker leaves with more pronounced palisade parenchyma, resulting in a higher accumulation of phenolic compounds in relation to the leaf area.

## Competing interests

The authors declare no competing interests.

## Acknowledgements

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# 5.7 Supplemental Material

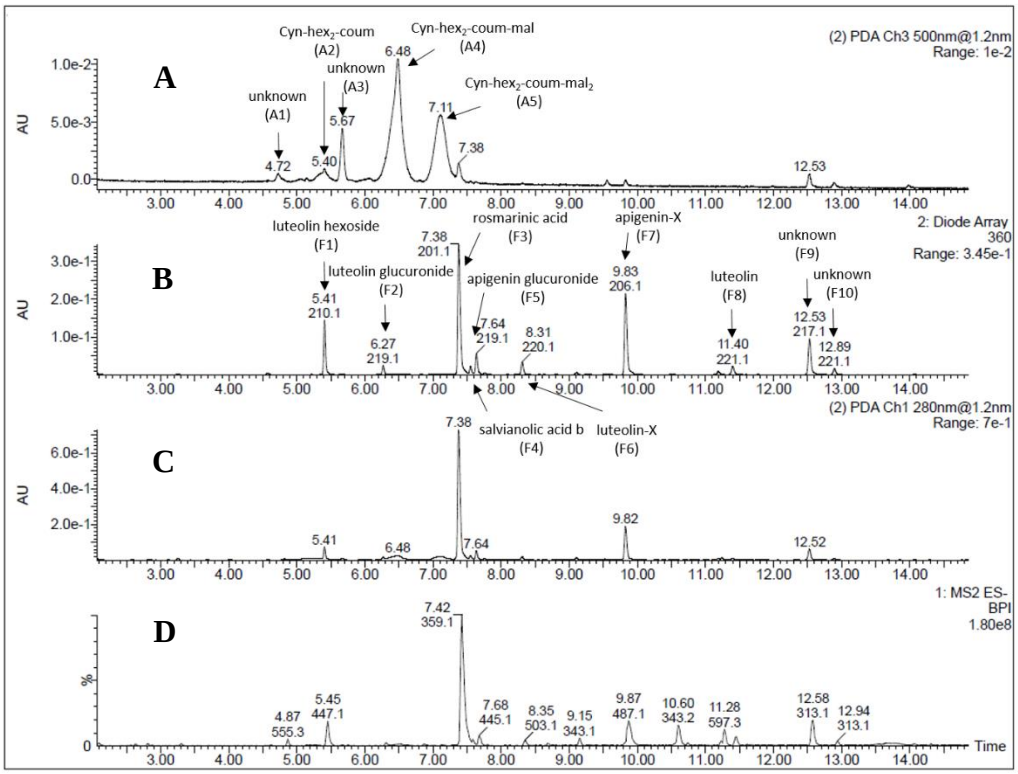


Fig. S5.8: UHPLC chromatograms of qualitative analysis of phenolic compounds from *P. scutellarioides*. A: chromatogram recorded at 500 nm showing anthocyanins. B: chromatogram recorded at 360 nm showing flavones and phenolic acids. C: chromatogram recorded at 280 nm showing phenolic acids. D: chromatogram of $MS^1$ scan from m/z 150 to 700 in negative mode.

Tab. S5.7: Identification of the detected peaks at 360 nm from methanolic *P. scutellarioides* leaf extract.

| PDA360 nm<br>**Class** | **Peak No.** | **Compound** | **RT [min]** | *m/z*<br>**[M–H]$^-$** | *m/z*<br>**Main Fragment** | *m/z*<br>**Other Fragments[1)]** | **$UV_{max}$ [nm]** |
|---|---|---|---|---|---|---|---|
| Fon | F1 | Luteolin hexoside | 5.41 | 447 | 285 | 151, 133, 199, 217, 175, 256 | 339 |
| Fon | F2 | Luteolin glucuronide | 6.27 | 461 | 285 | 151, 107, 175, 117, 199, 115, 133 | n.dtm. |
| HCA | F3 | Rosmarinic acid | 7.38 | 359 | 161 | 197, 179, 135, 123 | 328, 288sh |
| HCA | F4 | Salvianolic acid b | 7.55 | 717 | 537 | 519, 493, 673, 321, 475, 295, 179 | n.dtm. |
| Fon | F5 | Apigenin-glucuronide | 7.64 | 445 | 269 | 113, 85, 175 | 335, 267 |
| Fon | F6 | Luteolin-X | 8.31 | 503 | 285 | 218 | 348, 253, 268 |
| Fon | F7 | Apigenin-X | 9.82 | 487 | 269, | 113, 85, 139, 155, 218 | 336, 267 |
| Fon | F8 | Luteolin | 11.40 | 285 | 151 | 133, 199, 175, 217, 149, 241, 243 | 345, 266 |
| unknown | F9 | unknown | 12.52 | 313 | 161 | 151, 133, 109, 123 | 338, 250, 306sh |
| unknown | F10 | unknown | 12.89 | 313 | 161 | 151, 133, 109, 123 | 338 |

[1)] in order of their abundance; *Abbreviations:* RT, retention time; HCA, hydroxycinnamic acid and derivatives; Fon, flavone glycosides; X, unknown residue; sh, shoulder; n.dtm., not determined.

Tab. S5.8: Identification of the detected peaks at 500 nm from methanolic *P. scutellarioides* leaf extract.

| PDA500 nm<br>**Class** | **Peak No.** | **Compound** | **RT [min]** | *m/z*<br>**[M+H]$^+$** | *m/z*<br>**Main Fragment** | *m/z*<br>**Other Fragments[1)]** | **$UV_{max}$ [nm]** |
|---|---|---|---|---|---|---|---|
| unknown | A1 | unknown | 4.73 | 533 | no fragments detected | | n.dtm |
| Anth | A2 | Cyn-hex$_2$-coum | 5.40 | 757 | 595 | 287, 449, 147, 137, 207 | 530 |
| Anth | A3 | | 5.67 | 843 | n.dtm. | | n.dtm |
| Anth | A4 | Cyn-hex$_2$-coum-mal | 6.48 | 843 | 535 | 595, 287, 147, 109 | 530 |
| Anth | A5 | Cyn-hex$_2$-coum-mal$_2$ | 7.11 | 929 | 621 | 595, 287, 577, 885 | 530 |

[1)] in order of their abundance; *Abbreviations:* RT, retention time; Anth, anthocyanin; Cyn, cyanidin; hex, hexoside; coum, coumaric acid; mal, malonic acid n.dtm., not determined.

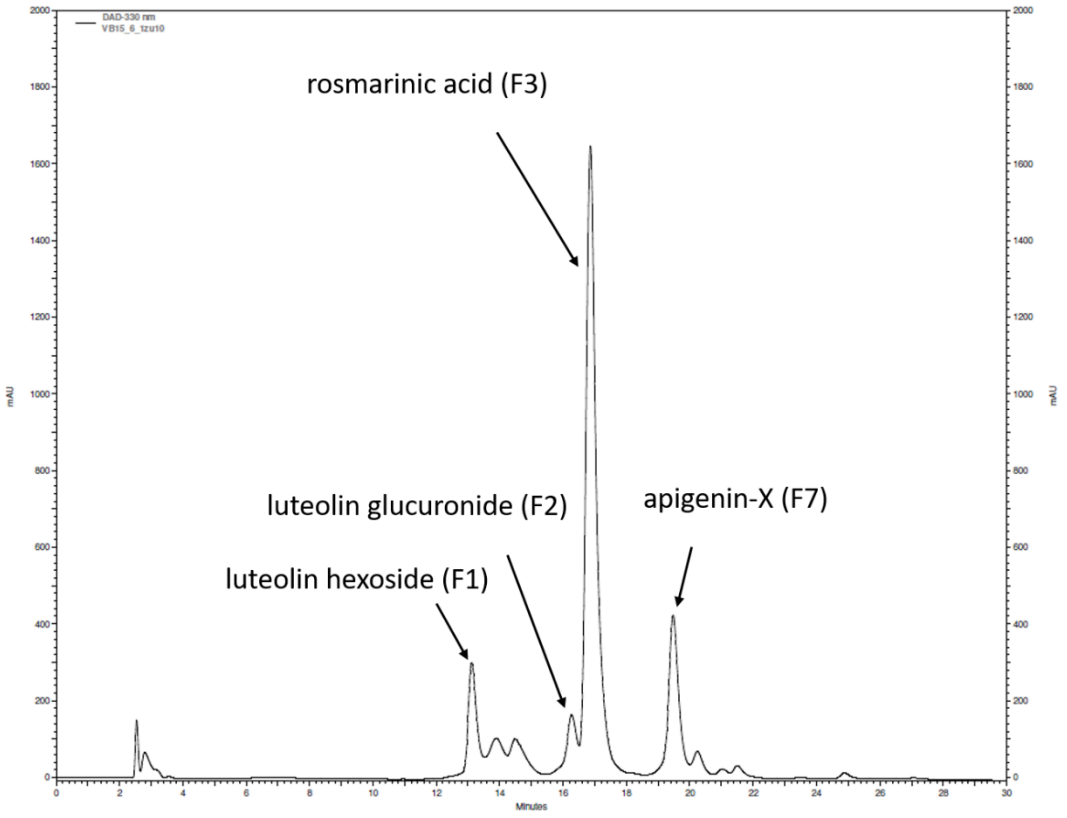


Fig. S5.9: HPLC Chromatogram (PDA 330 nm) of quantitative analysis of selected compounds from *P. scutellarioides*.

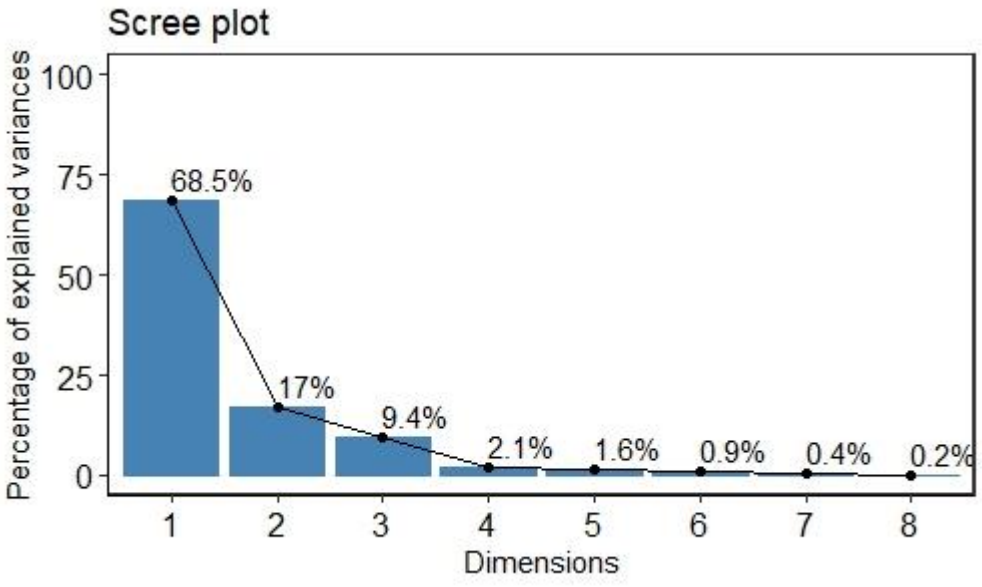


Fig. S5.10: Scree plot of PCA using quantitative data of phenolic compounds obtained from colorimetric methods and HPLC analysis and leaf mass per area data.

# 6. Influence of different light treatments on physiological performance of *Plectranthus scutellarioides* (L.)

## 6.1 Abstract

This chapter shows additionally results concerning *P. scutellarioides* (L.) influenced by different assimilation lamp systems. In a light stress experiments it was observed that plants precultivated with HPS lamps in climate chamber showed high susceptibility under direct sunlight. This was attributed to a low leaf thickness caused by a reduced blue light content in the emission spectrum from HPS lamps. Leaves developed with blue light enriched environments were more resistant to light stress and showed higher photosynthetic capacity. The emission spectrum of artificial sunlight from MPL induced stem elongation in the presence of global irradiance in the greenhouse. Cultivar dependency was also tested. Plant architecture was affected by increased far-red light content in the emission spectrum of MPL in all examined cultivars of *P. scutellarioides*. Cultivar dependency were primarily observed regarding the content of secondary metabolites.

## 6.2 Introduction

In chapter 5 it was shown that blue light increased leaf thickness. Furthermore, higher leaf temperatures caused by HPS and MPL treatment reduced the amount of phenolic secondary metabolites in *Plectranthus scutellarioides* (L.) of the cultivar 'Golden Dreams'.

This chapter describes the additional investigation about plant physiological performance of *P. scutellarioides* (L.) grown under artificial sunlight from MPL and other lamp systems.

In light stress experiments (experiment 1) *P. scutellarioides* plants were grown in climate chamber as previously described (chapter 5) and were further transferred under direct sunlight in summer 2018 with high light intensities up to 2000 µmol/m²s. The aim was to evaluate whether plant grown under different spectral light qualities showed an altered resistance against high irradiance due to different content of secondary metabolites or leaf thickness.

In experiment 2 *P. scutellarioides* were grown in smaller growth chambers with three different light treatments: MPL emitting artificial sunlight, commercial HPS lamps and LEDs emitting predominately blue and red light. The aim was to investigate the

photosynthetic capacities of these plants which can be altered due to leaf histological changes. Also phenolic compounds were quantified by colorimetric methods. Since the LEDs used in this experiment did not emit far-red light, morphological parameters were analyzed to verify if the lack of far-red light is leading to reduced elongation growth.

In experiment 3 the climate chamber experiment (chapter 5) was conducted with the red leaf cultivar 'Velvet Lace' of *P. scutellarioides* to estimate cultivar dependency on the response to the respective light sources.

Experiment 4 deals with the subject of the production of three cultivars of *P. scutellarioides* with MPL and commercial HPS lamps in a greenhouse. The aim was to examine the impact of increased far-red light in the emission spectrum of the artificial sunlight from MPL in the presence of global irradiance.

## 6.3 Material and methods

### *6.3.1 Experiment 1: Light stress response from plants grown in a climate chamber*

Eight plants were grown in climate chamber for five weeks under MPL, HPS, CDM and LED light as described in chapter 5 (Dörr et al., 2019) and were further transferred under direct sunlight in July 2018 for one week. During this time, daily light integral (DLI) was about 52.5±8.1 mol/m²d with light intensities up to around 2000 µmol/m²s. Leaf damages were documented and were categorized as low (beginning of chloroses), medium (distinct leaf chloroses) or high (strong chloroses, leaf burnings).

### *6.3.2 Experiment 2: Morphology, phenolic content and photosynthetic capacity from plants grown in climate chambers under MPL, HPS and LED light*

*Growth conditions*

Plant material was precultivated as described in chapter 5 (Dörr et al., 2019) before light treatment was applied. Afterwards plants were cultivated in small growth chambers for five weeks at about 21 °C and 60% rel. humidity. MPL (1300 W, Plasma International, Seligenstadt, Germany), HPS (600 W, DH Licht GmbH, Wülfrath, Germany) and two LED-KE-300 (300 W, DH Licht GmbH) were used. Spectral properties from MPL and HPS lamps are shown in chapter 5 (Dörr et al., 2019). Normalized light spectrum of LEDs was recorded by Jaz spectrometer and is shown in Fig. 6.1. Evaluated plants received in average following

light intensities for 20 h per day: MPL: 81.5±12.9, HPS: 82.7±10.6 and LED: 83.9±17.0 µmol/m²s. Differences in the average light intensity were not significant ($p<0.05$).

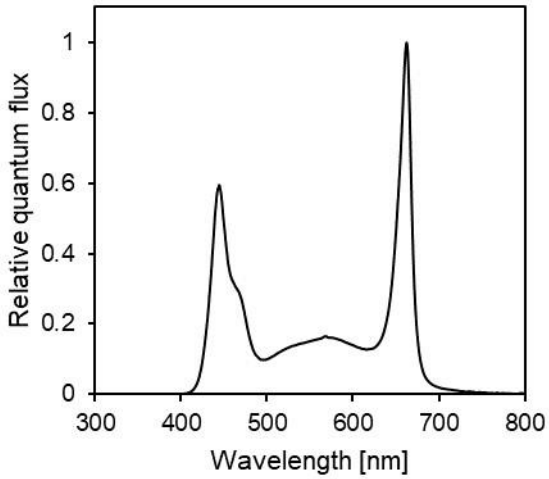


Fig. 6.1: Relative emission spectra of the used LED system. Spectrum was measured at 100 µmol/m²s and was normalized at 663 nm for better visualization.

*Morphological parameters*

Morphological parameters of 40 plants per light treatment were analyzed as described in chapter 5 (Dörr et al., 2019).

*Colorimetric methods*

Total phenolic content (TPC), total flavonoid content ($TFC_{415\ nm}$) and total anthocyanin content (TAC) was measured from eight leaf samples per light treatment as described in chapter 5 (Dörr et al., 2019).

*Gas exchange measurements*

Light response curves were recorded from four plants per light treatment using a portable gas exchange system (GFS 3000, Walz, Effeltrich Germany). The plants used for the measurement were grown for three weeks under the respective light source (MPL, HPS or LED) with about 100 µmol/m²s for 20 h per day.

The $CO_2$ concentration was ambient, and the relative humidity was 60%. The flow rate from the measuring head was set at 750 µmol/s, and the impeller was set to 5. After 30 min of equilibration at the light intensity of the measuring head of 2000 µmol/m²s, the

photosynthesis rate remained constant and the value was recorded. The light intensity was reduced afterwards to 1500, 1000, 750, 500, 400, 300, 200, 100, 50 and 0 µmol/m²s. For each light intensity step, 5 min of equilibration time were applied before the photosynthesis rate was recorded. Measurements were carried out alternately on the same day.

*Data analysis*

Univariate statistical analysis was performed as described in chapter 5(Dörr et al., 2019).

### *6.3.3 Experiment 3: Climate chamber experiment with the cultivar ˈVelvet Laceˈ*

The climate chamber experiment presented in chapter 5 was conducted with the red leaf cultivar ˈVelvet Laceˈ (Kientzler, Gensingen, Germany) of *P. scutellarioides*. The experiment was applied as described in chapter 5 (Dörr et al., 2019). For the cultivar ˈVelvet Laceˈ the phenolic compounds were only quantified by colorimetric methods. Leaf mass per area was determined as an indicator for altered leaf histology and to calculate the content of phenolic compounds in relation to leaf area. Average light intensity, number of used lamp systems and power per cultivation area during the experiments in climate chamber are shown in Tab. 6.1. The light distribution of the climate chamber experiments using MPL, HPS, CDM and LEDs is shown in Appendix 2 – Light distribution.

Tab. 6.1: Number of the four different lamp systems required for experiments in climate chamber, power per area and average light intensity on an area of about 7.5 m² .

| Lamp system | number | Total power [W] | Area [m²] | Power per area [W/m²] | PPFD [µmol/m²s] |
|---|---|---|---|---|---|
| MPL (1300 W) | 4 | 5200 | 7.5 | 693 | 107±10 |
| HPS (600 W) | 3 | 1800 | 7.5 | 240 | 104±21 |
| CDM (315 W) | 4 | 1260 | 7.5 | 168 | 98±13 |
| LED (300 W) | 4 | 1200 | 7.5 | 160 | 98±24 |

### *6.3.4 Experiment 4: Greenhouse experiment*

*General growth conditions and light treatment*

Light experiments were conducted from February to March (calendar weeks 8-13, 2017) in a greenhouse in Geisenheim (49° 59' 11.161" N 7° 58' 0.099" E, Germany) with three cultivars of *P. scutellarioides*: ˈGolden Dreamsˈ, ˈBlack Princeˈ and ˈSplit Fireˈ (Kientzler,

Gensingen, Germany). Plant material was precultivated as described in chapter 5 (Dörr et al., 2019) with the difference that coleus mother plants were cultivated with additional artificial light from HPS lamps with an intensity of about 85 µmol/m²s for 20 h per day.
During the experiment, the daily light integral (DLI) of global irradiance inside the greenhouse was on average about 8.8±4.4 mol/m²d; standard deviation indicated the variation of DLI during the experiment depending on weather conditions.
Plants were cultivated for five weeks with about 85±20 µmol/m²s ($DLI_{artifical}$: 6.1 mol/m²d) with MPL and HPS lamps for 20 h per day. Light intensity of artificial light was measured per individual pot at a height of 20 cm with a quantum sensor (LI-190R, LI-COR, Lincoln, Nebraska, USA). Differences in the average light intensity were not significant ($p<0.05$). Plants cultivated without additional artificial light were used as control. Day/night temperature inside the greenhouse was kept constant and was on average about 23±2 °C. Relative humidity was about 65±10%.

*Morphological parameters and determination of leaf mass per area (LMA)*
Morphological parameters of 45 plants per light treatment and LMA of eight leaves grown under the respective light source were analyzed as described in chapter 5 (Dörr et al., 2019).

*Data analysis*
Univariate statistical analysis was performed as described in chapter 5 (Dörr et al., 2019).

## 6.4 Results

### *6.4.1 Experiment 1: Light stress response from plants grown in a climate chamber*

Plants precultivated with HPS light showed sensitivity to high light stress indicated by an increased degree of chloroses and leaf burnings (Fig. 6.2, Fig. 6.3). First signs of leaf burnings were already visible after one day of incubation in many plants produced with HPS lamps (Fig. 6.4). Contrary, plants cultivated with MPL light showed almost no sign of leaf damages (Fig. 6.4). After additional seven days of incubation under direct sunlight all plants showed beginning of chloroses. However, plants cultivated with HPS lamps showed a higher degree of leaf burnings and an increased pigment degradation compared to plants precultivated with the other light sources (MPL, CDM and LED). Even some plants grown

with MPL showed medium degree of leaf damages, these plants were generally in a better shape compared to plants grown with the other light systems (Fig. 6.2).

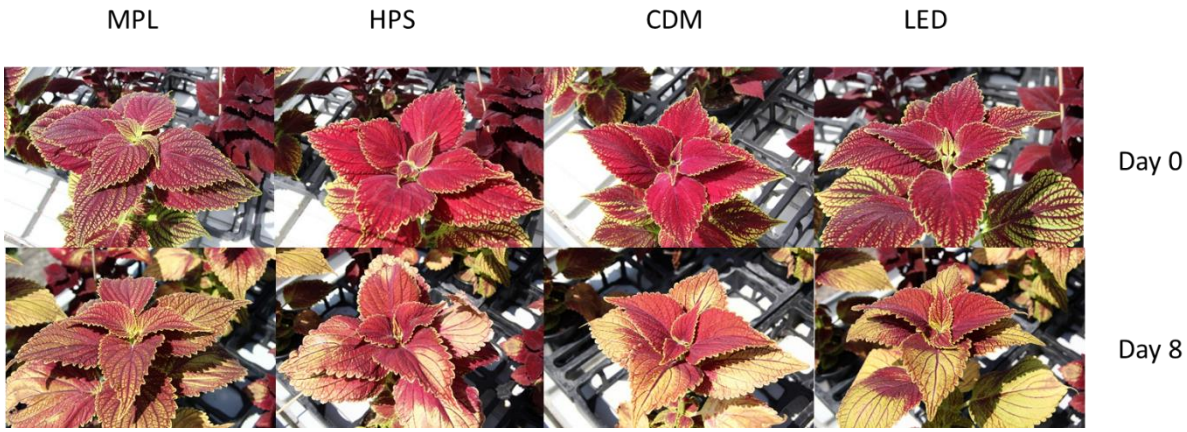


Fig. 6.2: Representative *P. scutellarioides* ('Golden Dreams') grown with MPL, HPS, CDM and LED before (Day 0) and after (Day 8) incubation for eight day under direct sunlight (DLI: 52.5±8.1 mol/m²d, PPFD up to 2000 µmol/m²s) in July 2018.

Fig. 6.3: Leaf from HPS grown plant incubated for 4 day under direct sunlight (DLI: 52.5±8.1 mol/m²d, PPFD up to 2000 µmol/m²s).

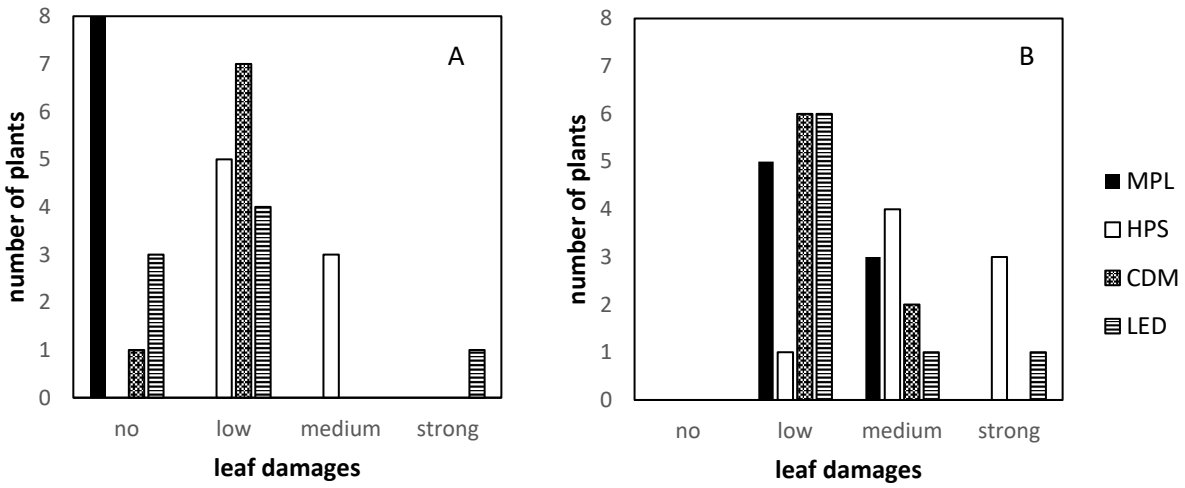


Fig. 6.4: Number of *P. scutellarioides* ('Golden Dreams') indicated as no, low, medium or strong leaf damages after 1 day (A) and 8 days (B) under direct sunlight (DLI:52.5±8.1 mol/m²d, PPFD up to 2000 µmol/m²s).

### *6.4.2 Experiment 2: Morphology, phenolic content and photosynthetic capacity from plants grown in climate chambers under MPL, HPS and LED light*

*Morphological parameters*

Plants grown with artificial sunlight from MPL showed the highest elongation growth, indicated by an increased plant height, an increased internode length, and the highest percentage of stem FW. Contrary, plants grown with LEDs showed the highest compactness. Total FW and number of leaf pairs were not significantly different under the respective light sources (Fig. 6.5, Tab. 6.2).

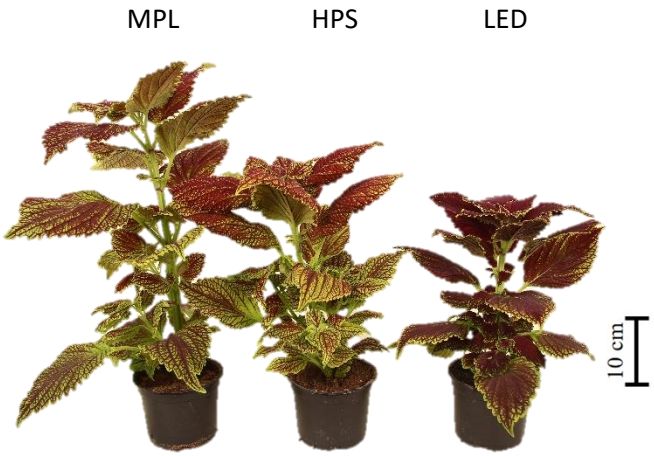


Fig. 6.5: Representative *P. scutellarioides* 'Golden Dreams' grown for five weeks in climate chambers with MPL, HPS, or LED light for 20 h with about 80 µmol/m²s per day.

Tab. 6.2: Morphological parameters of *P. scutellarioides* ('Golden Dreams') grown for five weeks under three different lamp systems (MPL, HPS, and LED) in climate chambers. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=40 plants.

| Morphological parameters | MPL | HPS | LED |
|---|---|---|---|
| Height [cm] | 34.7±5.0$^{a}$ | 25.9±4.4$^{b}$ | 23.1±3.5$^{c}$ |
| Number of leaf pairs | 6.9±0.6$^{a}$ | 6.6±0.8$^{a}$ | 6.7±0.5$^{a}$ |
| Internode length [cm] | 5.1±0.6$^{a}$ | 3.9±0.6$^{b}$ | 3.5±0.5$^{c}$ |
| Total FW [g] | 57.5±10.7$^{a}$ | 54.6±12.5$^{a}$ | 56.3±11.0$^{a}$ |
| Total DW [g] | 4.5±1.2$^{a}$ | 4.0±1.2$^{b}$ | 4.2±1.0$^{ab}$ |
| Stem FW [%] | 36.6±4.3$^{a}$ | 27.4±4.5$^{b}$ | 26.9±4.7$^{b}$ |

*Content of phytochemicals*

Plants grown with LEDs showed the most colorful leaves compared to plants grown under the other light sources (Fig. 6.5). This observation correlates also with total anthocyanin content (TAC) in leaves developed under LED light (Tab. 6.3).

Total phenolic content (TPC) and total flavonoid content (TFC) were also highest in plants grown under LED light. However, TPC was not significantly different compared to leaves grown with HPS lamps due to high variance in LED treatment. Total chlorophyll content (Chla+b) was not significantly different between plants developed under the three lamp systems (Tab. 6.3).

Tab. 6.3: Phenolic compounds measured by colorimetric methods in *P. scutellarioides* leaves ('Golden Dreams') of plants grown with light from MPL, HPS, and LED lamps. TPC= total phenolic content, expressed as gallic acid equivalents; TAC= total anthocyanin content expressed as cyanidin-3,5-O-diglucoside equivalents; $TFC_{415\ nm}$= total flavonoid content, expressed as quercetin equivalents. Chla+b= total chlorophyll content. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=8 leaf samples.

| Phenolic compounds | | MPL | HPS | LED |
|---|---|---|---|---|
| TPC | [mg/g dw] | 22.6±5.8$^{b}$ | 24.3±4.5$^{ab}$ | 33.2±9.0$^{a}$ |
| TAC | [mg/g dw] | 3.7±1.2$^{c}$ | 7.6±2.2$^{b}$ | 11.9±3.9$^{a}$ |
| $TFC_{415\ nm}$ | [mg/g dw] | 10.5±0.8$^{b}$ | 10.7±0.5$^{b}$ | 12.8±1.3$^{a}$ |
| Chla+b | [mg/g dw] | 1.7±0.1$^{a}$ | 1.5±0.3$^{a}$ | 1.8±0.5$^{a}$ |

*Light response curves*

To evaluate photosynthetic capacity, light response curves of plants grown in separate climate chambers either with MPL, HPS or LED lamps were recorded (Fig. 6.6). After an irradiance of 300 µmol/m²s, the net assimilation rate ($A_{net}$) was significantly higher ($p<0.05$)

in leaves of plants that were developed under artificial sunlight from MPL compared to leaves developed under HPS light. At a light intensity of more than 500 µmol/m²s, HPS plants displayed no increase in $A_{net.}$ Light saturation was reached at a higher light intensity of approximately 750 µmol/m²s in MPL and LED plants. At higher irradiances, photo-inhibition was observed. Leaves which developed under LED light showed even a higher photosynthetic capacity compared to MPL grown leaves.

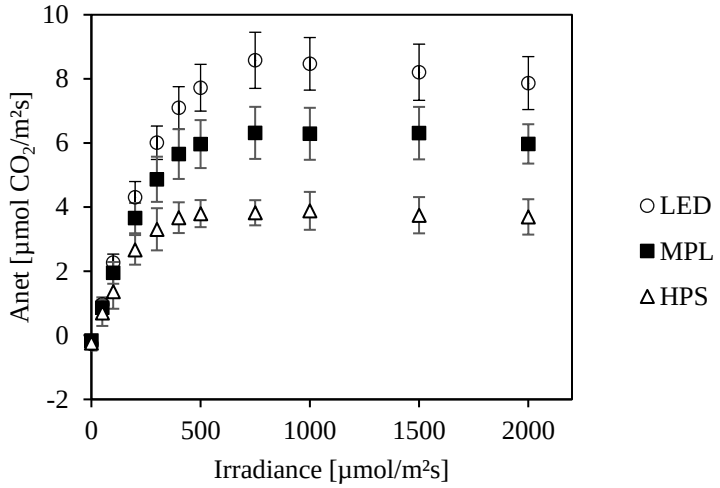


Fig. 6.6: Light response curves of *P. scutellarioides* ('Golden Dreams') grown under MPL, HPS or LED light. The plants were grown for three weeks under the respective light source with about 100 µmol/m²s for 20 h per day. Measurements were carried out alternately on the same day. Error bars indicate sd. (n=4 plants).

### *6.4.3 Experiment 3: Climate chamber experiment with the cultivar 'Velvet Lace'*

*P. scutellarioides* plants from the cultivar 'Velvet Lace' showed the highest leaf temperature under MPL systems. Leaves under CDM and LED light showed a distinct lower leaf temperature (Tab. 6.4).

Tab. 6.4: Average leaf temperature of *P. scutellarioides* ('Velvet Lace') under MPL, HPS, CDM and LED light measured with a thermal imaging camera. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=8 plants.

| Leaf temperature | MPL | HPS | CDM | LED |
|---|---|---|---|---|
| [°C] | 24.2±0.3$^{a}$ | 22.9±0.4$^{b}$ | 20.6±1.1$^{c}$ | 21.3±0.4$^{c}$ |

Representative plants grown under MPL, HPS, CDM and LED light are shown in Fig. 6.7. Plants grown with artificial sunlight from MPL showed significantly increased plant height and internode length. Light of CDM lamps led to most compact plants. No significant difference in total FW was determined between the four light treatments. However, total DW was significantly higher in plants grown with MPL and LEDs. Furthermore, an increased proportion of stem FW was measured in plants which developed under the light of MPL and LED (Tab. 6.5).

Tab. 6.5: Morphological parameters of *P. scutellarioides* ('Velvet Lace') grown for five weeks in a climate chamber under the four different lamp systems: MPL, HPS, CDM and LED. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=30 plants.

| Morphological parameters | MPL | HPS | CDM | LED |
|---|---|---|---|---|
| Height [cm] | 25.0±2.1$^{a}$ | 22.3±2.3$^{b}$ | 18.5±2.3$^{c}$ | 22.5±2.4$^{b}$ |
| Number of leaf pairs | 7.8±0.6$^{ab}$ | 7.9±0.7$^{a}$ | 7.7±0.7$^{ab}$ | 7.5±0.5$^{b}$ |
| Internode length [cm] | 3.2±0.2$^{a}$ | 2.8±0.2$^{c}$ | 2.4±0.2$^{d}$ | 3.0±0.3$^{b}$ |
| Total FW [g] | 42.4±6.2$^{a}$ | 41.6±5.8$^{a}$ | 40.2±7.6$^{a}$ | 43.4±5.7$^{a}$ |
| Total DW [g] | 5.6±0.7$^{a}$ | 4.2±0.7$^{b}$ | 4.0±0.7$^{b}$ | 5.5±1.5$^{a}$ |
| Stem FW [%] | 31.3±2.9$^{a}$ | 28.2±3.8$^{b}$ | 26.7±3.7$^{b}$ | 31.2±3.9$^{a}$ |


Fig. 6.7: Representative *P. scutellarioides* ('Velvet Lace') grown for five weeks in the climate chamber with MPL, HPS, CDM or LED light for 20 h with about 100 µmol/m²s per day.

LMA was significantly lower in leaves developed under HPS light in comparison to leaves grown with other light treatments. Between MPL, CDM and LED treatment no significant difference in LMA was determined (Tab. 6.6).

Tab. 6.6: Average leaf mass per area (LMA) from *P. scutellarioides* grown for five weeks in a climate chamber under the four different lamp systems: MPL, HPS, LED and CDM. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=8.

| Leaf mass per area | MPL | HPS | CDM | LED |
|---|---|---|---|---|
| LMA [mg dw/cm²] | $3.5\pm0.4^{a}$ | $2.7\pm0.3^{b}$ | $3.5\pm0.2^{a}$ | $3.6\pm0.3^{a}$ |

The TPC and TAC in relation to g dw was significantly lower in leaves developed under artificial sunlight from MPL compared to leaves grown with the other light sources (Tab. 6.7). Leaves developed under HPS, CDM and LED light treatments showed no significant differences in TAC and TPC in relation to g dw. Furthermore, no significant difference in TFC was found in leaves grown with the four light treatments. Since LMA was distinctly lower in leaves grown with HPS light, TFC in relation to leaf area was significantly lower in HPS grown plants compared to leaves developed under the other light sources. Consequently, phenolic compounds were highest in leaves grown with LED and CDM light in relation to leaf area.

Tab. 6.7: Phenolic compounds measured by colorimetric methods in *P. scutellarioides* leaves of plants grown with light from MPL, HPS, CDM and LED lamps. The content of phenolic compounds is expressed as mg per g dw or µg per cm² leaf area. TPC= total phenolic content, expressed as gallic acid equivalents; TAC= total anthocyanin content expressed as cyanidin-3,5-O-diglucoside equivalents; $TFC_{415\ nm}$= total flavonoid content, expressed as quercetin equivalents. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=8 leaf samples.

| Phenolic compounds | | MPL | HPS | CDM | LED |
|---|---|---|---|---|---|
| TPC | [mg/g dw] | $36.5\pm2.3^{b}$ | $51.2\pm5.3^{a}$ | $49.0\pm6.6^{a}$ | $45.5\pm5.4^{a}$ |
| | [µg/cm²] | $128.5\pm17.5^{c}$ | $139.4\pm19.0^{bc}$ | $169.5\pm20.7^{a}$ | $165.6\pm20.2^{ab}$ |
| TAC | [mg/g dw] | $17.1\pm2.0^{b}$ | $29.0\pm5.9^{a}$ | $31.2\pm3.2^{a}$ | $27.7\pm3.4^{a}$ |
| | [µg/cm²] | $59.8\pm7.2^{c}$ | $78.5\pm16.1^{b}$ | $107.9\pm11.3^{a}$ | $101.0\pm14.4^{a}$ |
| $TFC_{415\ nm}$ | [mg/g dw] | $14.9\pm1.0^{a}$ | $16.5\pm1.1^{a}$ | $15.8\pm1.5^{a}$ | $15.5\pm0.9^{a}$ |
| | [µg/cm²] | $52.3\pm3.7^{a}$ | $43.3\pm3.2^{b}$ | $54.8\pm4.4^{a}$ | $56.5\pm3.7^{a}$ |

### *6.4.4 Experiment 4: Greenhouse experiment*

Artificial lighting with HPS and MPL led to about threefold higher biomass FW and faster plant development including a higher number of leaf pairs. *P. scutellarioides* of all three cultivars grown under artificial sunlight from MPL showed increased elongation growth in

comparison to plants grown with HPS light indicated by a significantly higher plant height, internode length and increased proportion of stem FW (Tab. 6.8).

Tab. 6.8: Morphological parameters of three cultivars of *P. scutellarioides* plants grown for four weeks under the MPL and HPS light in a greenhouse. Plants cultivated without artificial light were used as control. The values are means ± sd. Unequal letters in a row indicate significant differences, $p<0.05$; n=45. Statistical analysis was only performed between the supplemental light treatments.

| Morphological parameters | cultivar | MPL | HPS | control |
|---|---|---|---|---|
| Height [cm] | 'Golden Dreams' | 29.7±3.2$^a$ | 18.6±2.0$^b$ | 11.4±1.3 |
| | 'Black Prince' | 41.4±3.7$^a$ | 32.5±3.5$^b$ | 17.4±2.1 |
| | 'Split Fire' | 23.1±3.3$^a$ | 15.7±1.9$^b$ | 9.5±1.6 |
| Number of leaf pairs | 'Golden Dreams' | 9.6±0.7$^a$ | 9.1±0.7$^b$ | 7.6±0.7 |
| | 'Black Prince' | 9.3±0.8$^a$ | 9.3±0.9$^a$ | 7.2±0.7 |
| | 'Split Fire' | 9.8±1.0$^a$ | 9.5±0.9$^a$ | 6.8±0.9 |
| Internode length [cm] | 'Golden Dreams' | 3.1±0.3$^a$ | 2.0±0.2$^b$ | 1.5±0.2 |
| | 'Black Prince' | 4.5±0.4$^a$ | 3.5±0.5$^b$ | 2.4±0.4 |
| | 'Split Fire' | 2.4±0.4$^a$ | 1.7±0.2$^b$ | 1.4±0.3 |
| Total FW [g] | 'Golden Dreams' | 67.9±10.5$^a$ | 60.7±7.3$^b$ | 20.1±2.7 |
| | 'Black Prince' | 60.5±7.3$^a$ | 58.7±7.0$^a$ | 18.0±3.0 |
| | 'Split Fire' | 60.0±7.3$^a$ | 60.4±9.6$^a$ | 18.0±4.8 |
| Total DW [g] | 'Golden Dreams' | 4.5±1.0$^a$ | 3.7±0.5$^a$ | 1.5±0.6 |
| | 'Black Prince' | 4.7±0.7$^a$ | 4.4±0.5$^a$ | 1.3±0.2 |
| | 'Split Fire' | 4.8±1.0$^a$ | 4.7±0.7$^a$ | 1.4±0.7 |
| Stem FW [%] | 'Golden Dreams' | 31.0±3.1$^a$ | 24.1±3.4$^b$ | 18.5±2.2 |
| | 'Black Prince' | 44.4±3.9$^a$ | 35.3±3.3$^b$ | 30.5±4.8 |
| | 'Split Fire' | 39.6±7.8$^a$ | 32.5±6.5$^b$ | 29.1±7.6 |

Leaf mass per area was significantly higher in leaves of the cultivar 'Golden Dreams' grown with MPL light. For the other cultivars no significant difference in LMA was detected between the MPL and HPS light treatments. Overall, plants grown without supplemental artificial light developed lower LMA (Tab. 6.9).

Tab. 6.9: The average leaf mass per area from three different cultivars of *P. scutellarioides* grown under MPL and HPS light for five weeks in a greenhouse. Plants cultivated without artificial light were used as control. The values are means ± sd. Unequal letters in a row indicate significant difference, $p<0.05$; n=8. Statistical analysis was only performed between the supplemental light treatments.

| Leaf histology | cultivar | MPL | HPS | control |
|---|---|---|---|---|
| LMA [mg dw/cm²] | 'Golden Dreams' | 3.1±0.2[a] | 2.7±0.3[b] | 2.2±0.2 |
| | 'Black Prince' | 3.1±0.2[a] | 3.0±0.3[a] | 2.1±0.1 |
| | 'Split Fire' | 3.3±0.1[a] | 3.1±0.3[a] | 2.1±0.1 |

# 6.5 Discussion

### *6.5.1 Increased leaf thickness resulted in a higher adaptation against high irradiance and increased photosynthetic capacity*

Leaves developed in climate chamber under HPS light showed a high sensibility under direct sunlight with DLI of about 52.5±8.1 mol/m²d and light intensities up to 2000 µmol/m²s in summer 2018. As previously shown (chapter 5), leaf thickness was lowest under HPS light (Fig. 5.4, Tab. 5.4) resulted in shade adapted leaves which were not adapted to resist to light stress conditions (Fig. 6.2, Fig. 6.3, Fig. 6.4). Leaves which developed under blue light enriched light environments (MPL, CDM and LED) showed reduced degree of leaf damages due to robust leaf morphology. In general, blue light is assumed to be responsible for the formation of "sun-like" leaves indicated by an increased LMA, smaller leaf size or increased amount of stomata shown in light experiments with lettuce, roses, tomatoes and campanulas (Li and Kubota, 2009; Abidi et al., 2013; Terfa et al., 2013; Ouzounis et al., 2014; Bergstrand et al., 2016). It might be also that blue light increased the thickness of cuticle, which is supposed to protect leaves against higher irradiances. Secondary metabolites played probably just a minor role in light protection since leaves developed under MPL showed distinct lower content of phenolic compounds but quite the same resistance against light stress like LED or CDM treatment.

LMA was also significantly lower in a red leaf cultivar of *P. scutellarioides* grown under HPS light in climate chamber experiment (Tab. 6.6). These plants also showed high degree of leaf burning when they were transferred under direct sunlight (data not shown).

No significant difference in LMA caused by light treatment of two other cultivars of *P. scutellarioides* was found in the greenhouse experiment (Tab. 6.9). Blue light from global irradiance might be sufficient to enable full leaf development. However, also cultivar differences can be a reason.

Plants grown with LEDs, which emitted the highest amount of blue light, showed the highest photosynthesis rate at higher light intensities (Fig. 6.6). In cucumber plants it was also observed that blue light stimulates photosynthetic capacity (Hogewoning et al., 2010b). The increased photosynthetic capacity might be explained by the induction of sun-adapted leaves as previously discussed. Lowest photosynthetic capacity was measured in leaves under HPS light, due to a low blue light content in the emission spectrum which caused the formation of shade-adapted leaves. Furthermore the absence of blue light can lead to morphologically abnormalities like leaf curling as observed in roses (Ouzounis et al., 2014). This phenomenon is called the "red light syndrome" and can also lead to lower photosynthetic performance as demonstrated in cucumber plants grown without blue light (Trouwborst et al., 2016).

In almost all plants leaf damages were observed after about one week of incubation under direct sunlight (Fig. 6.4). Coleus plants are usually produced with a DLI around 10 mol/m²s (Garland et al., 2010). The plants grown in the climate chamber were cultivated with a DLI of about 7.2 mol/m²s (100 µmol/m²s for 20 h). Although plants grown with blue enriched light environments (MPL, LED and CDM) showed higher resistance due to increased leaf thickness, chlorosis and leaf burning occurred in these plants as well. Light response curves showed a maximum light saturation of 750 mol/m²s for coleus plants grown in climate chamber (Fig. 6.6). At higher irradiance, photoinhibition was observed which can lead to photo-oxidation of chlorophyll and permanent leaf damages.

### *6.5.2 A lack of far-red light led to a compact plant growth*

In the earlier presented study (chapter 5), LEDs were used emitting a distinct amount of far-red light (Fig. 5.1) leading to an increased elongation growth. In experiment 2 in this chapter, LEDs did not emit far-red light (Fig. 6.1) and resulted in a compact growth with short internode length (Fig. 6.2, Tab. 6.2). Plants developed under MPL showed the highest elongation growth due to lowest R:FR ratio as described in chapter 5 (Dörr et al., 2019). The increased elongation growth under MPL was also observed in three different cultivars of *P. scutellarioides* in a greenhouse experiment using HPS lamps as reference (Tab. 6.8).

*6.5.3 Cultivar dependency of the experiments*

Overall with regard to morphological response all cultivars showed increased elongation growth under artificial sun light spectrum from MPL. In climate chamber experiments it was shown that phenolic constitutions were reduced in green leaf cultivar 'Golden Dreams' due to the influences of infrared light under MPL and HPS light (chapter 5). *P. scutellarioides* from the red leaf cultivar 'Velvet Lace' grown with MPL also showed the lowest total anthocyanin and total phenolic content (Tab. 6.7). Nevertheless, in the red leaf cultivar 'Velvet Lace' cultivar differences in the accumulation of phenolic constitutions were observed compared the cultivar 'Golden Dreams'. Plants grown with HPS lamps showed no significant differences of phenolic compounds in relation to dry weight compared to plants grown with LED and CDM (Tab. 6.7). In these experiments a significantly lower leaf temperature was measured in plants grown under HPS lamps compared to plant grown under MPL (Tab. 6.4).

For the experiment presented in chapter 5 and the climate chamber experiment using the red leaf cultivar 'Velvet Lace' around 700 W/m² of MPL, 240 W/m² of HPS, 170 W/m² of CDM and 160 W/m² of LED were installed to get about the same PPFD and an almost homogeneous light distribution (Tab. 6.1, Appendix 2 – Light distribution). In addition to the increased infrared radiation of MPL and HPS also the heat from convection might have resulted in higher leaf temperatures and caused different microclimates in the climate chamber. In further experiments when different lamp systems are tested, an increased amount of climate sensors measuring abiotic influencing factors should be used sensing room temperature, relative humanity and $CO_2$ concentration for individual pots. Especially *P. scutellarioides* turned out to be very thermosensitive.

Additionally, anthocyanins are the predominantly phenolic compounds in plants from the cultivar 'Velvet Lace'. The colorimetric methods used for quantification of phenolic compounds just give an overall impression of the content of secondary metabolites and these methods are very unspecific as discussed in chapter 5. With the TPC method also anthocyanins are taken in account. HPLC-analysis conducted with the cultivar 'Golden Dreams' (chapter 5) indicated that not all phenolic acids or flavonoids are influenced by leaf temperature or light color.

The altered composition of phenolic compounds might be reason for cultivar dependency. Particularly *P. scutellarioides* showed high variability in color and leaf shapes (Suva et al., 2015). The classification of coleus plants to the genus *Plectranthus* and *Solenostemon* is not

always clear and therefore leading to high genetic variability. Classifications according to the occurrence of secondary metabolites are an approach to reproduce the genetic origin as conducted for several plants from genus *Plectranthus* (Grayer et al., 2010).

## 6.6 Conclusion

Overall, the additional experiments presented in this chapter showed that blue enriched environments, are leading to increased photosynthetic capacity and reduced susceptibility against high irradiance. Furthermore, the increased far-red light from artificial sunlight from MPL resulted in an increased elongation growth in all examined cultivars of *P. scutellarioides* in comparison to HPS light treatment. This influence was also observed in the presence of global irradiance in a greenhouse experiment. Cultivar dependency was primarily observed with regard to the content of secondary metabolites.

# 7. Plant architecture and phytochemical composition of basil (*Ocimum basilicum* L.) under the influence of light from microwave plasma and high-pressure sodium lamps.

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**What are the contributions of the doctoral candidate and his co-authors?**

**(1) Concept and design**
OSD: 80%
HM: 20%

**(2) Conducting tests and experiments**
OSD: 80% (colorimetric measurements, sample processing, extraction of samples, collection of morphological data, chlorophyll and polyphenol meter measurements, measurement of leaf temperature)
SB: 20% (GC-MS analysis)

**(3) Compilation of data sets and figures**
OSD: 80% (compilation of GC-MS, morphological, and colorimetrical data, data visualization)
SB: 20% (compilation of GC-MS data)

**(4) Analysis and interpretation of data**
OSD: 90% (statistical analysis, analysis of GC-MS, morphological and colorimetrical data, interpretation of data)
SB: 10% (analysis of GC-MS data)

**(5) Drafting of manuscript**
OSD: 85%
SB: 5%
DR: 5%
HM: 5%

## 7.1 Abstract

Potted herbs such as basil are in high year-round demand in Central Europe. To ensure good quality in winter, artificial light is required. Many horticulturists, who want to replace their high-pressure sodium (HPS) lamps with light-emitting diodes (LEDs) to save electricity energy, struggle with high investment costs. In addition, switching to LEDs can overwhelm many smaller horticultural enterprises since there is a requirement of adjusting individual light recipes and furthermore cultivation problems can occur due to the lack of infrared radiation.

In this study, the influence of light from microwave plasma lamps (MPLs), acting as alternative light sources, on secondary metabolites and plant morphology of basil plants (*Ocimum basilicum* L.) was tested.

Basil plants were grown in a climate chamber with MPLs with two different light bulbs emitting either artificial sunlight (AS) or broad white light with increased blue and green light content (sulfur plasma light; SPL). The effect of these new lamp types was compared to standard commercial HPS lamps. In addition to morphological parameters such as height, internode length and fresh weight, plant secondary metabolites were examined. Essential oils and monoterpenes were quantified by GC-MS analysis, whereby phenolic compounds were analyzed calorimetrically.

Elongation growth and biomass production was increased under the AS spectrum in comparison to HPS-grown plants. Increased stem elongation was attributed to a higher content of far-red light in the AS spectrum.

Furthermore, basil plants grown under the AS spectrum contained the highest total phenolic and total flavonoid content compared to plants grown under the SPL and HPS lamps, probably due to the higher content of UV-A radiation. The lowest content of phenolic compounds was observed when HPS light was used, which was assumed to be caused by a low blue light content in the emission spectrum. An impact of the different light spectra on essential oil composition was observed. A significantly increased content of linalool was determined in basil leaves developed under both tested MPL spectra compared to HPS-grown plants. The total yield of the four major essential oils was lowest under HPS treatment.

**Key words:** artificial sunlight, microwave plasma lamps, basil, GC-MS, phenolic compounds, essential oils.

**Abbreviations**

AS, artificial sunlight; Chl, chlorophyll; Dim, dimension; EO, essential oils; FW, fresh weight; FR, far-red light; HPS, high-pressure sodium; LEDs, light emitting diodes; MPL, microwave plasma lamp; R:FR, red to far-red ratio; sd., standard deviation; SPL, sulfur plasma light; TFC, total flavonoid content; TPC, total phenolic content; PBAR, photo-biologically active radiation; PCA, principal component analysis; ROS, reactive oxygen species.

## 7.2 Introduction

Basil (*Ocimum basilicum* L.) is a popular culinary herbal plant due to its richness in distinctive aromatic flavors. Its taste and scent are mainly caused by monoterpenes and phenylpropanoids typical for members of the family of *Lamiaceae* including other herbal plants such as rosemary, oregano, and thyme. The essential oils accumulate in glandular hairs distributed in vegetative and reproductive organs and protect plants against herbivores and pathogens (Werker, 1993).

In European basil, linalool is the major monoterpene, whereby eugenol and estragol present the main phenylpropanoids that characterize the taste and aroma (Nitz and Schnitzler, 2004). However, the chemical constitution is altered according to species or cultivar (Marotti et al., 1996; Carović-Stanko et al., 2010).

In addition to monoterpenes and phenylpropanoids, basil plants contain high amounts of flavonoids, including flavanol and flavone derivatives or phenolic acids, such as rosmarinic acid and caffeic acid (Hossain et al., 2010). Rosmarinic acid was found to be the main phenolic compound (Lee and Scagel, 2009; Taulavuori et al., 2016), but chicoric acid can also appear in high quantities depending on the species (Kwee and Niemeyer, 2011). In addition to the use of basil as a culinary herbal plant, basil leaves are also used for traditional medical proposes (Feo and Senatore, 1993; Chiang et al., 2005) due to their high antioxidant and antimicrobial properties (Carović-Stanko et al., 2010; Kwee and Niemeyer, 2011) caused by their high content of secondary metabolites.

The demand for fresh herbs such as basil is high throughout the year in central Europe.

In year-round production, seasonal changes affect plant growth and quality including the chemical composition (McGimpsey and Douglas, 1994; Hussain et al., 2008). In basil, it was

measured that the essential oil content was higher when plants were grown at 25 °C compared to when plants were grown at 15 °C (Chang et al., 2005).

In addition to temperature, light is a major influencing factor on plant quality, especially in winter when, in the northern hemisphere, artificial light is required to enable year-round production of herbal plants. In basil, the content of phenolic compounds and fresh weight production were positively correlated with increasing daily light integral from 9.3 to 17.8 mol/m²d of plants grown in a controlled environment (Dou et al., 2018). Higher light intensities in the greenhouse led to higher quantities of essential oil production including linalool and eugenol in a greenhouse experiment using different shading materials (Chang et al., 2008). Additionally, higher light intensities provided by supplemental light from HPS lamps were found to increase essential oil production in basil (Nitz and Schnitzler, 2004).

As recent reviewed plant quality can also change according to spectral light quality, which has a high impact on plant morphology and secondary metabolism (Bantis et al., 2018).

With the development of LEDs, experiments with monochromatic light appeared numerous. At the beginning of LED development, the technology was focused on the emission of blue and red light to induce photosynthesis in plants. Nevertheless, for full plant development, other wavelengths are often required: far-red light is known to affect flowering, stem elongation or germination in several species (Demotes-Mainard et al., 2016). Additionally, UV-B light is supposed to induce secondary metabolism, not only increasing the nutritional impact for the human diet (Schreiner et al., 2012) but also increasing stress tolerance in plants. Furthermore, green light can be beneficial for growth, as observed in lettuce when supplemental green light was added to red and blue LEDs (Kim et al., 2004).

However, the responses to spectral light quality always depend on the species, cultivar and environmental conditions, making general statements difficult.

Although consumers may want a replacement for high-pressure sodium lamps, the use of multispectral LEDs is quite difficult for consumers because, for each species, an individual light recipe should be used. Additionally, broad white LEDs, lacking in the emission of infrared radiation, make the change from HPS lamps difficult since the full cultivation program has to be adjusted.

In this study, we tested new microwave plasma lamps (MPL) with two different light bulbs emitting full broad light, including infrared radiation: a light bulb emitting light with similar properties to sunlight (artificial sunlight; AS) and a sulfur-containing light bulb (sulfur plasma light; SPL) emitting broad white light with increased blue and green light content.

The aim of the study was to evaluate whether both spectra serve as beneficial light source alternatives to HPS lamps. In addition to morphological parameters, secondary metabolites, including phenolic content and essential oil content, were examined.

## 7.3 Materials & methods

### *7.3.1 Plant material and general cultivation conditions*

Basil seeds (Genovese type, ˈEleonoraˈ, Enza Zaden, Dannstadt-Schauernheim, Germany) were individually germinated in 288 Miniplug trays in summer 2018 in a greenhouse (Geisenheim University, Germany) at approximately 21 °C and 60% rel. humidity. Young seedlings were further cultivated for two weeks until the first leaf pair stage was reached. Afterwards, three plants with a height of approximately 5 cm were planted in one 12 cm pot (1 L) with a common substrate (LAT-Terra Standard P, pH: 5.9, N: 120 mg/L, $P_2O_5$: 120 mg/L, $K_2O$: 170 mg/L, Mg: 120 mg/L, HAWITA GRUPPE GmbH, Vechta, Germany). Pots were placed in a climate chamber and were further cultivated for four weeks with assimilation light as described below. Irrigation was applied with low tide tables. The day/night temperature was set to 21.0±1.0 °C with a relative humidity of 60±10%.

### *7.3.2 Light treatments*

The climate chamber was divided into three compartments with opaque material. In each compartment, different light spectra were used: microwave plasma lamps containing two different light bulbs (1300 W, Aurion Anlagen Technik, Seligenstadt, Germany) emitting artificial sunlight (AS) or white light with increased blue and green light (sulfur containing light bulb referred as sulfur plasma light; SPL). Commercial high-pressure sodium lamps (600 W, DH Licht GmbH, Wülfrath, Germany) were used as reference light source. Plants were grown with a photoperiod of 20 h per day. For each pot, the light intensity was measured at an approximate height of 20 cm with a LI-190R Quantum Sensor (LiCOR, Lincoln, Nebraska USA). The average light intensity from the evaluated pots was as follows: AS, 97.6±9.1 µmol/m²s; SPL, 88.4±3.9 µmol/m²s; and HPS, 98.7±9.8 µmol/m²s. Light spectra were recorded with a JAZ spectrometer (Ocean Optics, Ostfildern, Germany) and analyzed with spectroscopic software (Spectra Suite 6.2, Ocean Optics, Ostfildern, Germany). The normalized spectra from different lamp systems are shown in Fig. 7.1. The

proportions of light in percent of photo-biologically active radiation (PBAR, 280-800 nm) were categorized as follows: UV-B (280-315 nm), UV-A (315-400 nm), blue (400-500 nm), green (500-550 nm), yellow (550-600 nm), red (600-700 nm) and far-red (700-800 nm). The R:FR ratio (red:far-red) was calculated according to Smith (1982) (Tab. 7.1).

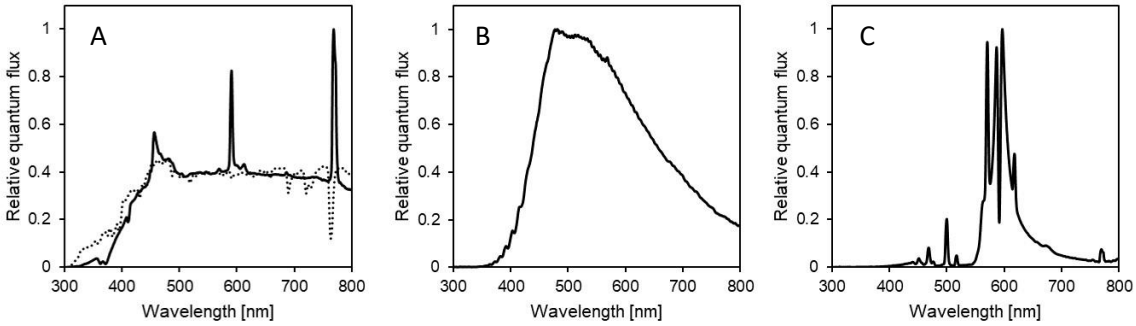


Fig. 7.1: Relative emission spectra of the used light sources. A: Emission spectra from a MPL emitting AS (solid line) vs. sunlight spectra (dotted line) measured on a cloudless day in Geisenheim (May 2018, 49° 59' 11.161" N 7° 58' 0.099" E, 90 m above sea level). B: Emission spectra from an MPL emitting SPL. C: Emission spectra from an HPS lamp. Spectra from MPL with AS, sunlight and HPS lamp were adapted from Dörr et al. (2019).

Tab. 7.1: Spectral properties of AS, SPL and HPS lamps. Values are the percentage of photo-biologically active radiation (280–800 nm). For AS and HPS data were used from Dörr et al. (2019). R:FR ratio was calculated according Smith (1982).

| Light Source | [nm] | UV-B 280-315 | UV-A 315-400 | blue 400-500 | green 500-550 | yellow 550-600 | red 600-700 | far-red 700-800 | R:FR ratio |
|---|---|---|---|---|---|---|---|---|---|
| AS | % | - | 2.3 | 22.9 | 12.1 | 13.7 | 24.3 | 25.1 | 1.05 |
| SPL | % | - | 0.9 | 27.2 | 20.4 | 17.7 | 22.8 | 11.0 | 1.66 |
| HPS | % | - | 0.2 | 4.5 | 2.1 | 48.8 | 38.0 | 7.0 | 2.9 |

### *7.3.3 Measurement of leaf temperature*

Leaf temperature was measured under the respective light source with a thermal imaging camera (H2640, emissivity: 0.96, InfReC, InfRec, Nippon Avionics, Tokyo, Japan). Images of eight pots per treatment were recorded and analyzed by thermal imaging camera software (InfReC Analyzer NS9500, 2.7A, InfReC, InfRec, Nippon Avionics). The average leaf

temperature was measured at three leaf pairs in the upper part of the plants (Fig. S7.6, Tab. S7.5)

### *7.3.4 Morphological parameters*

Twenty pots were evaluated per treatment. Means from three plants per pot were calculated. Plant height, stem and leaf weight were measured. The average internode length was calculated from the plant height divided by the number of leaf pairs.

### *7.3.5 Determination of flavonol and chlorophyll content with a chlorophyll and phenol meter*

To determine the content of flavonoids and chlorophyll content on the leaf surface, a polyphenol and chlorophyll meter (Dualex Scientific+™, Force-A, Orsay, France) was used. After the cultivation time, measurements were taken at the upper leaf surface from three fully developed leaf pairs from the top of eight pots per treatment, and an average value was calculated per pot.

### *7.3.6 Sample processing*

The same leaves that were measured with the chlorophyll and phenol meter were harvested and frozen immediately in liquid nitrogen, and the samples were stored at -80 °C. Eight leaf samples were ground with a mortar and pestle after freezing with liquid nitrogen, and ground plant material was further processed as described below.

### *7.3.7 Colorimetric analysis*

For colorimetric determination of phenolic compounds and chlorophyll content, approximately 100 mg of ground plant material was weighed and extracted with 2 mL of 100% (v/v) methanol for two days at RT in the dark under constant shaking.

*Total phenolic content*

Total phenolic content (TPC) and total flavonoid content (TFC) were measured by colorimetric methods optimized for a microplate reader as described in Dörr et al. (2019). These methods were further adapted for basil leaf extract (Köttner, 2019).

*Chlorophyll content*

Twenty microliters of leaf extract were diluted with 80 µL of methanol (100%) in round-base 96-well microplates (Sarstedt, Nümbrecht, Germany), and the absorbance was measured at 652 nm and at 665 nm with an infinite M200 microplate reader controlled with Magellan 7.2 Software (TECAN, Männedorf, Switzerland). Three technical replicates were measured for each sample.

Total chlorophyll content (Chl a+b) was calculated according to Lichtenthaler and Buschmann (2001) for pure methanol solvent:

$$\text{Chl a+b } (\mu g/mL) = (16.72A_{665\ nm} - 9.16A_{652\ nm}) + (34.09A_{652\ nm} - 15.28A_{665\ nm})$$

*7.3.8 Analysis of monoterpenes and phenylpropanoids via GC-MS*

For extraction, 1 mL of distilled dichloromethane (≥ 99.5 % p. a.; Carl Roth Karlsruhe, Germany) was added to approximately 100 mg of ground plant material. A total of 5 µL of internal standard (adamantan, 10.51 g/L in DCM, Sigma-Aldrich, St. Louis, United States) was added, and the solution was sonicated in an ultrasonic bath for 5 min at RT (Gershenzon et al., 2000; Errenst, 2012). The solution was then incubated at RT for 30 min. Samples were shaken every 5 min. Afterwards, the samples were passed through quartz wool filters containing sodium sulfate. Samples were analyzed by GC/MS using a GC 7890A gas chromatograph (Agilent Technologies, Santa Clara, United States) coupled with an MSD 5977B mass spectrometer (Agilent Technologies, Santa Clara, United States). Monoterpenes and phenylpropanoids were separated with a 30 m × 250 µm × 0.5 µm Stabilwax column (Restek, Bellefonte, United States). Injections were made in split mode with a split ratio of 1:10, a split flow of 12 mL/min and a 40 °C initial temperature with an increase of 12 °C/s to 230 °C. The final temperature of 230 °C was held constant for 5 min. The Carrier gas was helium with a flow rate of 1.2 mL/min. The oven temperature was held at 40 °C for 2 min

in the beginning and increased by 8 °C/min to 165 °C and then by 18 °C/min to 220 °C. The final temperature was maintained for 10 min.

Compounds were identified and quantified in SIM mode by comparisons of mass spectral data and retention time with eucalyptol (quantifier ion: m/z 81; qualifier ions: m/z 108, 154), linalool quantifier ion: m/z 71; qualifier ions: m/z 93, 121, methyl eugenol (quantifier ion: m/z 178; qualifier ions: m/z 147, 163, eugenol (quantifier ion: m/z 164; qualifier ions: m/z 131, 149 and estragol (quantifier ion: m/z 117; qualifier ions: m/z 133, 148) reference standards (Sigma-Aldrich, St. Louis, United States). Estragol did not appear in high quantities in the examined cultivar and was not further analyzed. The total yield of the four major EO was calculated as grams of oil per 100 g fresh weight and is expressed as % (w/w) EO.

### *7.3.9 Data analysis*

Statistical analysis was performed using R (R 3.4.3). Data were analyzed for normality with the Shapiro-Wilk test. Levene's test was used to assess the equality of variance. If the conditions were fulfilled, one-way analysis of variance (ANOVA) ($p \leq 0.05$) was performed with Tukey's range test as a post hoc analysis. If the data were not normally distributed and/or were heterogeneous, the nonparametric Kruskal-Wallis test ($p \leq 0.05$) was used. Repetitions were based on individual pots. The experiment was repeated independently. Box plots were created with R package ggplot2. Principal component analysis was performed using the R package FactoMineR and factoextra for visualization based on ggplot2. The ellipse.level setting was set to 0.68 for the individual plots. Average leaf temperature, blue light proportion and UV-A content were used as supplementary quantitative variables.

## 7.4 Results

### *7.4.1 Leaf temperature*

The leaf surface temperature measured by the thermal imaging camera was altered under the respective light sources (Fig. S7.6, Tab. S7.5). Basil plants under an MPL with the AS spectrum showed an average leaf surface temperature of about 23.6 °C which was approximately 3 °C higher in comparison to plants under HPS light (20.5 °C). The leaf temperature of plants grown under SPL (21.2 °C) resulted in approximately 1 °C higher leaf temperatures compared to those of leaves grown under HPS lamps.

### *7.4.2 Morphological parameters*

Basil plants grown under the three different light sources showed different morphological appearances (Fig. 7.2, Tab. 7.2). Plant height was significantly higher under light from both MPL with AS and SPL in comparison to the height of plants grown with HPS lamps. The lowest plant height was seen when basil plants were grown with HPS light. Additionally, the average internode length was significantly higher under AS and SPL. Furthermore, plant development was faster under AS and SPL, as shown by the formation of an additional leaf pair in the same cultivation time compared to the leaf pairs on plants grown with HPS light. Total fresh weight per plant was highest under AS, followed by SPL and HPS light. However, plants grown with HPS light showed a significantly higher percentage of leaf weight compared to plants grown with both MPLs.

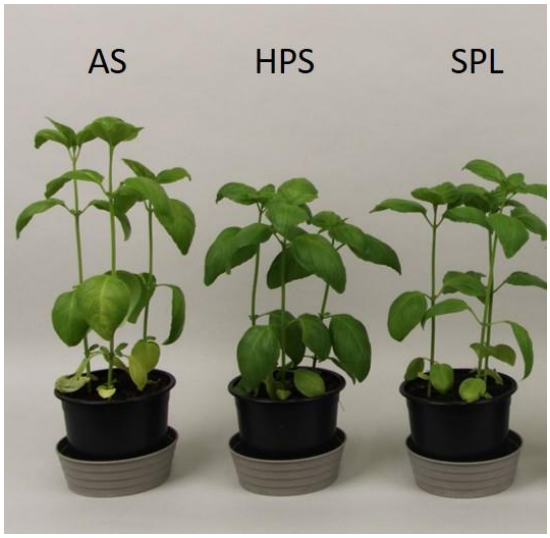


Fig. 7.2: Representative basil plants grown for four weeks in a climate chamber under AS, HPS or SPL.

Tab. 7.2: Morphological parameters of basil plants grown under the three different light qualities: AS, SPL and HPS. The values are means ± sd. Unequal letters in a row indicate a significant difference, $p<0.05$; n=20.

| Morphological Parameters | AS | SPL | HPS |
|---|---|---|---|
| Height [cm] | 23.7±1.9$^{a}$ | 22.2±1.9$^{a}$ | 17.3±2.9$^{b}$ |
| Number of Leaf Pairs | 5.0±0.2$^{a}$ | 4.8±0.4$^{a}$ | 4.3±0.4$^{b}$ |
| Internode length [cm] | 4.8±0.3$^{a}$ | 4.6±0.5$^{a}$ | 4.0±0.6$^{b}$ |
| Total FW [g] | 5.4±0.6$^{a}$ | 4.8±0.7$^{b}$ | 3.8±0.7$^{b}$ |
| Leaf FW [%] | 66.3±2.1$^{b}$ | 66.8±4.4$^{b}$ | 71.3±6.3$^{a}$ |

### *7.4.3 Phenolic compounds*

Basil leaves developed under AS showed the highest total phenolic content (Tab. 7.3). The lowest TPC was measured in basil leaves grown under the HPS light source. Similar results were obtained for the total flavonoid content measured by colorimetric methods (Fig. 7.3A). However, no significant differences were found in the TFC in leaves grown between both full-spectrum light sources (AS and SPL). The flavonol value measured by the chlorophyll and phenol meter at the leaf surface indicated that flavonols accumulated in the highest quantities in leaves grown under the AS source and in the lowest quantities in leaves developed under HPS light (Fig. 7.3B).

Tab. 7.3: Phenolic content of basil leaves grown under different light qualities: AS, SPL and HPS. The values are means ± sd. letters in a row indicate a significant difference, $p<0.05$; n=8.

| Phenolic compounds | AS | SPL | HPS |
|---|---|---|---|
| TPC [mg/g fw] | $7.1\pm0.4^{a}$ | $6.1\pm1.1^{b}$ | $4.9\pm0.5^{c}$ |

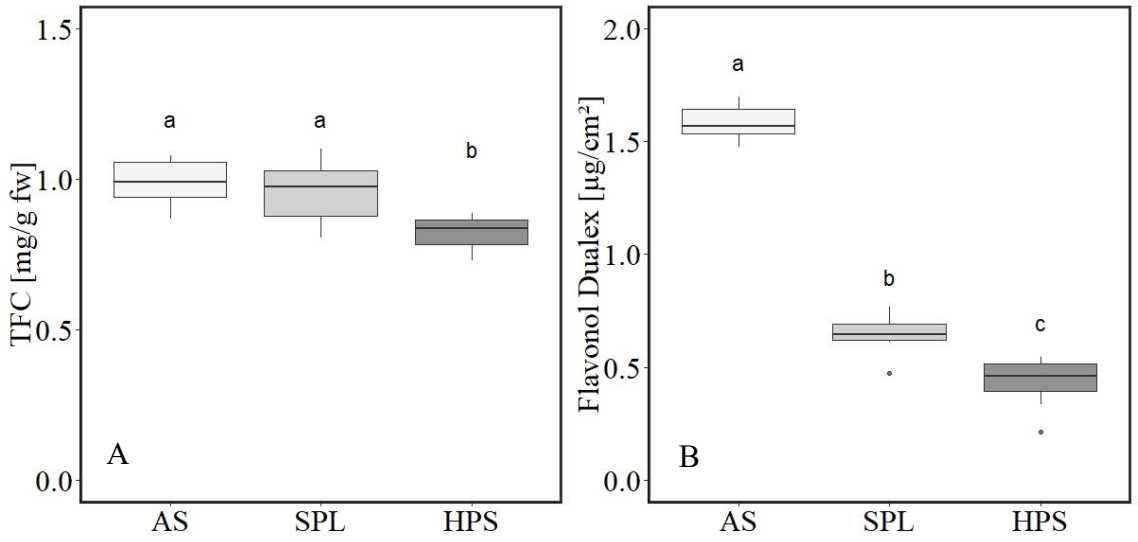


Fig. 7.3: Total flavonoid content [mg/g fw] measured by colorimetric methods (A) and flavonol values [µg/cm²] measured by a chlorophyll phenol meter (B) from plants grown under AS, SPL or HPS light. Unequal letters indicate a significant difference, $p<0.05$; n=8.

### *7.4.4 Chlorophyll content*

Under the AS source, basil plants showed the significantly lowest chlorophyll content per fresh weight as measured by colorimetric methods (Fig. 7.4A). The chlorophyll content of

plants grown with HPS and SPL did not show significant differences. Chlorophyll measurements in terms of g fresh weight were consistent with the chlorophyll values measured by the chlorophyll phenol meter at the leaf surface (Fig. 7.4B).

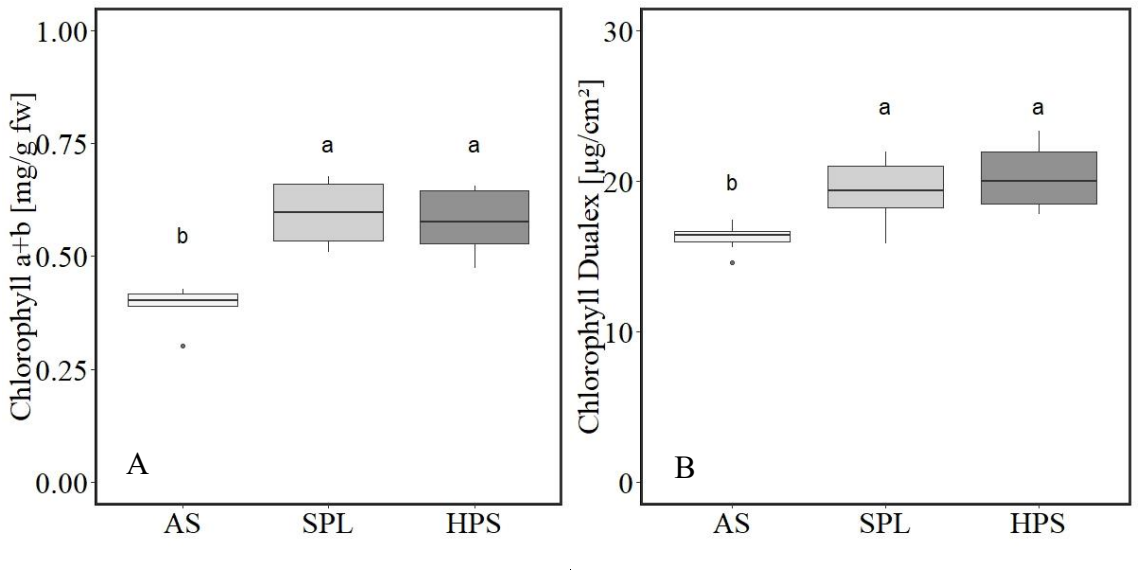


Fig. 7.4: Chlorophyll content [mg/g fw] measured by the colorimetric method (A) and chlorophyll value [µg/cm²] measured by a chlorophyll phenol meter (B) from plants grown under AS, SPL or HPS light. Unequal letters indicate a significant difference, $p<0.05$; n=8.

### *7.4.5 Monoterpenes and phenylpropanoids*

Four different monoterpenes and phenylpropanoids measured by GC-MS analysis appeared in higher quantities in the examined basil cultivar: two monoterpenes, eucalyptol and linalool, and two phenylpropanoids, methyl eugenol and eugenol. Eugenol showed the highest contribution to the essential oil content. Estragol, also common in basil, did not appear in high quantities and was not further analyzed.

The monoterpene and phenylpropanoid contents were altered in leaves grown under the respective light sources (Tab. 7.4). Eucalyptol content was significantly higher in leaves that developed under MPL emitting AS or SPL compared to the content in basil plants grown with HPS light. Additionally, linalool and eugenol contents were higher under AS and SPL compared to the contents in plants grown with HPS light; nevertheless, no significant difference was detected due to high variability. Methyl eugenol content was lowest in leaves grown under the AS and HPS lights. The highest methyl eugenol content was significantly

detected in leaves grown with SPL. The total yield of the four essential oils was lowest in leaves grown under the light from HPS lamps.

Tab. 7.4: Content of monoterpenes and phenylpropanoids from basil leaves grown under different light qualities: AS, SPL and HPS. The values are means ± sd. Unequal letters in a row indicate a significant difference, $p<0.05$; n=8.

| GC-MS results | AS | SPL | HPS |
|---|---|---|---|
| Eucalyptol [µg/g fw] | 76.3±19.7$^{a}$ | 74.0±9.4$^{a}$ | 51.6±13.1$^{b}$ |
| Linalool [µg/g fw] | 110.2±37.6$^{a}$ | 107.7±21.7$^{a}$ | 85.5±31.2$^{a}$ |
| Methyl eugenol [µg/g fw] | 10.1±5.5$^{b}$ | 26.9±18.6$^{a}$ | 16.8±12.9$^{ab}$ |
| Eugenol [µg/g fw] | 474.0±100.9$^{a}$ | 490.6±54.0$^{a}$ | 404.9±88.7$^{a}$ |
| EO yield [% ] | 0.67±0.15$^{ab}$ | 0.70±0.08$^{a}$ | 0.56±0.13$^{b}$ |

### *7.4.6 Principal component analysis*

For multivariate analysis of the chemical ingredients in the basil plants, a principal component analysis (PCA) was performed with leaf temperature, blue light and UV-A proportions as supplementary quantitative variables. Approximately 71% of the variance of the data is described by the first two principal components (Fig. S7.7). In the individual plot (Fig. 7.5A), samples are grouped according to their light treatment in dimension 1 & 2 (Dim 1&2). The separation of the AS treatment from HPS and SPL was more pronounced, while the HPS and SPL groups overlapped. In the variable plot (Fig. 7.5B), it appears that the separation of AS from HPS and SPL treatment is caused by the content of phenolic compounds (TFC, TPC, and flavonoid in leaf surface from Dualex measurements) and chlorophyll content (by the colorimetric method and Dualex measurements), which were negatively correlated. Methyl eugenol content also showed a negative correlation with phenolic ingredients, while the quality of representation (cos2) was lower. It seems that UV-A and leaf temperature were positively correlated with phenolic ingredients, while chlorophyll and methyl eugenol contents were negatively affected. However, the impact from leaf temperature might be a spurious relationship since the leaf temperature and UV-A proportions from the light treatments occur simultaneously. Additionally, the blue light proportion is related to TPC and TFC. Eugenol, linalool and eucalyptol seemed to be unaffected by leaf temperature and UV-A content. The contents of these correlated monoterpenes and phenylpropanoids are responsible for the high dispersion of the AS group

and the slight separation of it from the HPS and SPL treatments. Blue light content seemed to slightly and positively affect linalool, eugenol and eucalyptol content.

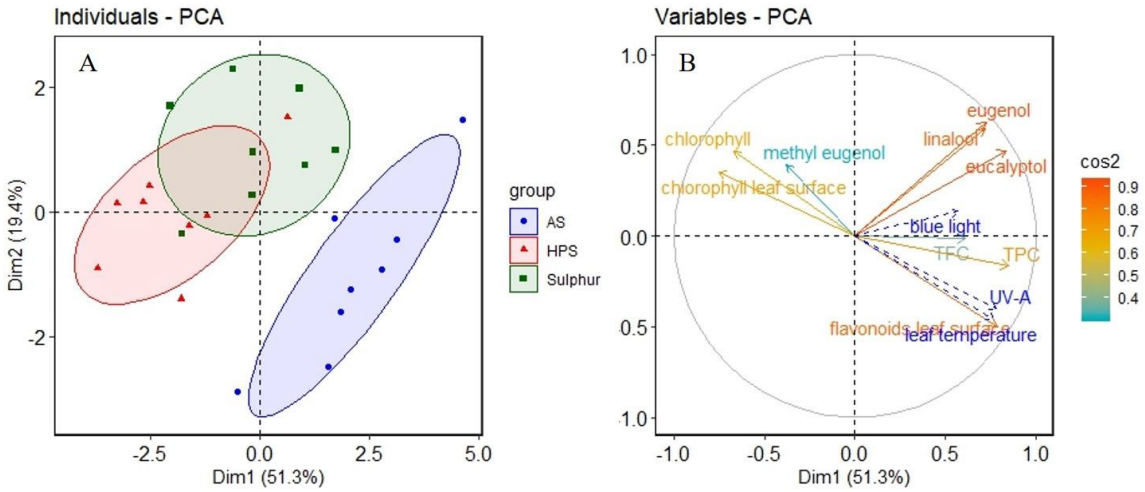


Fig. 7.5: Principal component analysis using quantitative data of phenolic compounds, essential oils and chlorophyll content A: Individuals plot. B: Variables plot; variables are colored according to their quality of representation (cos2); leaf temperature, blue light and UV-A light content were added as supplementary quantitative variables (dashed blue line).

## 7.5. Discussion

### *7.5.1 Plant morphology was mainly affected by a lower R:FR ratio and leaf temperature*

Elongation growth from plants grown with AS or SPL was increased compared to plants grown with HPS light, indicated by a higher internode length (AS: ↑36%, SPL ↑27%), higher plant height (AS: ↑16%, SPL ↑11%) and lower proportion of leaf weight (AS: ↓7%, SPL ↓6%). An increased internode length was, in general, more pronounced when plants received a light spectrum with a low R:FR ratio (Smith, 1982). The R:FR ratio from the AS (1.05) and SPL spectra (1.66) were distinctly lower compared to the HPS light spectrum (2.9). Cucumber plants grown with an artificial solar spectrum resembling MPL in climate chambers also showed higher biomass production, increased elongation growth and a higher leaf unfolding rate compared to those grown with HPS or fluorescent lamps (Hogewoning et al., 2010a).

Nevertheless, plants grown with the three light treatments received different spectral properties of light (

Tab. *7.1*), and different leaf temperatures were measured with a thermal imaging camera (Fig. S7.6, Tab. S7.5). Different leaf temperatures were obtained by varying infrared radiation not only in the emission spectra of the different lamp types but also in the power

of the lamps needed to emit almost the same light intensities (note: for MPL, no reflector was available). In addition to light quality, temperature also affects plant growth. Basil plants grown at 25 °C produced more biomass and leaves and showed increased plant height compared to plants grown at 15 °C (Chang et al., 2005). A temperature dependency was observed in our experiments. Basil plants grown with AS showed the highest leaf temperature, resulting in the highest plant height, fresh weight, and leaf number. In contrast, plants that were grown with HPS light showed the lowest values in the previously mentioned morphological parameters due to a lower leaf temperature.

### *7.5.2 Phenolic ingredients were increased by increased blue light and UV-A radiation*

The total phenolic content was highest under AS, whereas no significant increase in TFC was measured in basil plants grown with SPL. Both spectra contained high amounts of blue light and a distinct amount of UV-A.

Higher blue light content and UV in broad white LEDs increased phenolic compounds in basil in a climate chamber experiment (Bantis et al., 2016). The blue light dependency of phenolic compound production in basil was also observed in the greenhouse experiment when interlighting LEDs were used in addition to HPS light (Taulavuori et al., 2016). In recent study with basil callus cultures it was shown that blue light was preliminary responsible for the increase of TPC (Nadeem et al., 2019).

Shiga et al. (2009) reported that rosmarinic acid is a major phenolic compound in basil and contributes the most to the total phenolic content. Contradictory to previous statements, rosmarinic acid and TPC were increased under predominantly red illumination (Shiga et al., 2009; Lobiuc et al., 2017). We could not find a tendency for red-dominated light to increase phenolic compounds since plants grown with HPS light (high red light, low blue light) contained the lowest amount of TPC and TFC. In our previously published study, we demonstrated that leaf temperature had a higher impact on rosmarinic acid and other phenolic compounds compared to spectral light quality in *Plectranthus scutellarioides* (Dörr et al., 2019). However, in basil plants, we could not find the same indication, assuming that in basil, UV-A had a higher influence on the accumulation of phenolic ingredients.

Flavonoid content measured at the leaf surface on basil leaves grown under the respective light source showed a similar trend compared to TFC measurements by colorimetric methods; however, differences between the three light treatments were more pronounced in the leaf surface measurements. Flavonols accumulated in the upper leaf epidermis to protect

plant cells against harmful UV radiation (Wollenweber and Dietz, 1981; Mierziak et al., 2014). The accumulation of flavonols in basil leaves is induced by blue light (Bantis et al., 2016). This supports our observations that under AS and SPL, higher amounts of flavonols were detected compared to those detected in plants grown under HPS light treatment. The flavonol index measured on the leaf surface was higher in AS leaves compared to leaves grown with SPL. In principal component analysis, it was demonstrated that UV-A content had a higher impact on the flavonol index compared to blue light. Additionally, leaf mass per area was significantly higher in leaves grown with AS compared to those grown with SPL (not published), probably resulting in higher flavonol values measured by the phenol meter.

### *7.5.3 Artificial sunlight decreased the chlorophyll content*

The results from chlorophyll determination using the chlorophyll meter and colorimetric methods were consistent. The lowest chlorophyll content was observed when artificial sunlight was used, which also produced the highest content of phenolic compounds of plants grown under the examined light sources.

According to the carbon nutrient balance hypothesis (Bryant et al., 1983), carbon and nitrogen are in competition in the use of phenolic compounds or proteins that enable growth and photosynthesis because both are sharing L-phenylalanine as a common precursor (Margna, 1977). To counteract the harmful behavior of reactive oxygen species (ROS) induced by UV light, plants produce secondary metabolites acting as antioxidants to reduce the amount of ROS. The plant cells in basil plants under AS, therefore, may invest more resources in the synthesis of plant protectors instead of enzymes involved in primary metabolism. Under HPS light, where the energy-rich wavelength was lowest, the chlorophyll content was highest.

### *7.5.4 Essential oil production was altered under different light sources*

In addition to the content of phenolic compounds and chlorophyll content, essential oils were also altered according to the respective light source. Eugenol, linalool and eucalyptol contents were higher in plants grown under MPL with AS and SPL spectra compared to plants grown with HPS light. However, only the eucalyptol content was significantly higher. It was speculated that the increase in eucalyptol was due to the increased leaf temperature.

A temperature dependency of essential oil production was previously reported by Chang et al. (2005). Basil plants grown at 25 °C showed three times higher volatile oil contents compared to basil plants grown at 15 °C, whereas eugenol content increased with increasing temperature. However, principal component analysis showed that linalool, eucalyptol and eugenol were unaffected by leaf temperature or UV-A radiation.

Chang et al. (2005) also assumed that linalool and eucalyptol were unaffected by temperature changes, concluding that the difference in eucalyptol and linalool content observed in our experiments was probably due to other external influences.

In a climate chamber experiment, it was determined that essential oils including linalool and eucalyptol were increased when monochromatic blue light was used compared to when white LEDs were used. The lowest amount of essential oil was detected under monochromatic red light (Amaki et al., 2011). Blue light also increases eugenol content in basil callus cultures (Nadeem et al., 2019). Furthermore, monochromatic blue light from LEDs also increased the content of monoterpenes such as myrcene and limonene content observed in leaves from the medicinal plant *Lippa rotundifolia* (Hsie et al., 2019).

We also observed a positive effect of blue light on essential oil production. It was observed in several studies that supplemental UV-B has a positive contribution to essential oil production in basil (Johnson et al., 1999; Nitz and Schnitzler, 2004; Chang et al., 2009). Interestingly, the methyl eugenol content was decreased when UV-B was implemented (Nitz and Schnitzler, 2004). Methyl eugenol is thought to be carcinogenic; therefore, applications to decrease the harmful phenylpropanoid are beneficial. It was assumed that UV-B inhibited the activity of O-methylation of eugenol by o-methyltransferase. In our experiment, basil grown with AS light showed the lowest content of methyl eugenol, assuming that an artificial solar spectrum emitting UV-A might be beneficial for a reduction of the amount of methyl eugenol.

Besides abiotic conditions like light intensity, light quality and temperature also the proportion of methyl eugenol in basil leaves are depending on the cultivar. In our cultivar (Genovese type, ˈEleonoraˈ) the proportions of methyl eugenol altered under the respective light sources from 1.5% (AS), 3.0% (HPS) to 3.8% (SPL). In the cultivar ˈBagecoˈ methyl eugenol was the major EO with a proportion up to around 65% (Nitz and Schnitzler, 2004).

In further investigations, when reflectors for MPL are available, experiments will also be conducted in greenhouses to estimate if the new light source can be efficient in the commercial horticultural industry. Park et al. (2018) assumed that plasma lighting are an

suitable alternative light source for greenhouses and plant factories due to the increase of growth rate and improving flowering in tomatoes. Besides the horticultural industry the MPL emitting artificial sunlight might be also an interesting light source for other fields. Nevertheless, an evaluation of the light efficiency and economic usability of micro wave plasma lamps is required and will be investigated in further experiments.

## 7.6 Conclusion

Basil plants grown with full-spectrum light sources provided by MPL showed better plant quality compared to HPS-grown plants, as indicated by faster plant development, an increased content of essential oil and higher flavonoid accumulation due to higher blue light and UV-A content in the emission spectra. In addition, plant growth was beneficially affected by higher leaf temperature. In particular, artificial sunlight spectra might be an interesting light source for plant production in enclosed environments when a balanced light spectrum, including UV-A and near-infrared radiation, is required.

**Competing interest**

The authors declare no competing interests.

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# 7.7 Supplemental material

Tab. S7.5: Average leaf temperature measured by a thermal imaging camera from basil leaves under three different light sources: AS, SPL and HPS. The values are means ± sd. Unequal letters in a row indicate a significant difference, $p<0.05$; n=8.

| Leaf surface temperature | AS | SPL | HPS |
|---|---|---|---|
| [°C] | 23.6±0.5[a] | 21.2±0.3[b] | 20.5±0.3[c] |

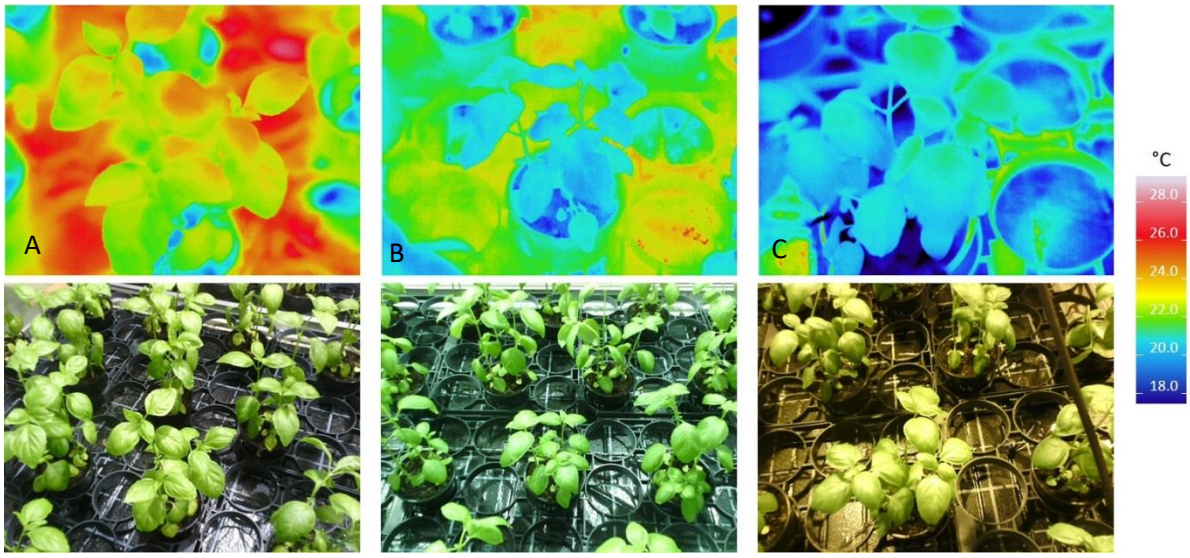


Fig. S7.6: Representative thermal images of basil pots under three different light qualities: AS (A), SPL (B), and HPS (C).

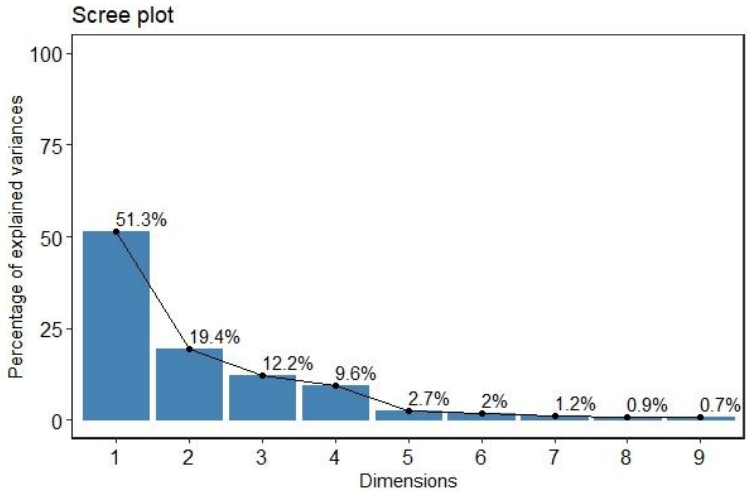


Fig. S7.7: Scree plot of PCA using quantitative data of phenolic compounds, essential oils and chlorophyll content

# 8. Investigation on morphology and physiology of potted roses grown with light from microwave plasma and high-pressure sodium lamps

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Declaration of author contributions to the manuscript

**Status: accepted for publication in European Journal of Horticultural Science**

**What are the contributions of the doctoral candidate and his co-authors?**

**(1) Concept and design**
OSD: 70%
HM: 30%

**(2) Conducting tests and experiments**
OSD: 100% (establishment of colorimetric methods, sample processing, collection of morphological data, analysis of photosynthesis rate, chlorophyll and phenol meter measurements)

**(3) Compilation of data sets and figures**
OSD: 100% (compilation of all data sets, data visualization)

**(4) Analysis and interpretation of data**
OSD: 80% (statistical analysis, analysis of colorimetrical data, interpretation of data)
HM: 20% (interpretation of data)

**(5) Drafting of manuscript**
OSD: 90%
HM: 10%

## 8.1 Abstract

In this study, microwave plasma lamps (MPL) that emit sun-like light were tested. Plant cultivation experiments using potted roses were conducted with supplemented assimilation light in greenhouse and climate chamber to investigate the influence of artificial sunlight on external and internal plant quality. Light from HPS lamps was used as a reference. Plant growth parameters were recorded, and the chlorophyll and flavonoid contents in rose leaves were determined. Furthermore, the photosynthetic rate and soluble sugar content were measured in the climate chamber.

Potted roses grown under MPL light showed one week earlier flowering but displayed reduced branching and consequently a lower number of flowers and buds compared with plants grown under HPS light. In potted roses grown under MPL light photosynthesis rate at growth light intensity of leaves grown in climate chamber was increased, whereby the contents of fructose and glucose were not significantly different.

**Key words:** flavonoid content, spectral quality of light, photomorphogenesis, artificial sunlight

**Abbreviations**

DLI, daily light integral; FR, far-red light; GH, greenhouse; HPS, high-pressure sodium; LEDs, light emitting diodes; MPL, microwave plasma lamp; NIR, near infrared radiation; R:FR, red to far-red ratio; sd., standard deviation; PBAR, photo-biologically active radiation; UV, ultraviolet.

## 8.2 Introduction

Plants have evolutionary adaptations to a wide range of lighting conditions, depending on season, time of day, weather or growing environment (Smith, 1982). The spectral quality of light from UV-light to far-red light (280-800 nm) is perceived by various photoreceptors, leading to morphological and physiological changes (Briggs and Olney, 2001). In the process of photomorphogenesis, the main photoreceptors (phytochromes, cryptochromes, phototropins and UVR8 receptors) allow plants to adapt to changing light environments.

Phytochromes absorb red (R) and far-red (FR) light and occur in two different interchangeable forms, the inactive ($P_R$) and active ($P_{FR}$), depending on the R:FR ratio of the light spectra (Smith, 1982). Under shade conditions, lower R:FR ratios lead to shade avoidance syndrome, resulting in stem elongation, reduced branching and formation of larger and thinner leaves (McLaren and Smith, 1978; Casal, 2013; Park et al., 2016). Contrary, blue light can inhibit elongation growth and lead to the formation of thicker "sun-like" leaves (Hogewoning et al., 2010b; Xiaoying, 2012; Cope and Bugbee, 2013).

Blue light is also supposed to increase the content of secondary metabolites. A higher content of phenolic constitutions was measured in basil, roses, chrysanthemums, campanula, and arugula grown with LEDs emitting a high content of blue light (Ouzounis et al., 2014; Bantis et al., 2016; Taulavuori et al., 2018).

Another group of blue and UV-A light absorbing photoreceptors are the phototropins, which are responsible for phototropism, chloroplast movement and stomatal opening (Christie, 2007). UVR8 receptors cover the UV-B range and are supposed to be involved in chloroplast differentiation (Wargent et al., 2009) and in the accumulation of UV-B-absorbing secondary metabolites (e.g., flavonoids and phenolic acids), that counteract cell damage from harmful UV radiation (Kliebenstein et al., 2002; Favory et al., 2009).

For plant cultivation experiments under controlled and natural conditions it is necessary to adjust abiotic conditions, such as temperature, relative humidity, $CO_2$ content and light intensity. However, the production of sun-like light with today's lamp systems is limited.

Light from high-pressure sodium (HPS) lamps is still the major lighting source in greenhouses due to their moderate light yield and low acquisition cost (van Ieperen and Trouwborst, 2008; Nelson and Bugbee, 2014; Ouzounis et al., 2018). Nevertheless, the spectral properties from HPS lamps differs to sunlight; they emit predominately orange and red light, with a low portion of blue light.

In experiments, where natural conditions are simulated, the usage of unnatural light such as light from HPS lamps can lead to wrong experimental conclusions, because of the strong influence of spectral light quality on the external and internal plant quality (Olle and Viršile, 2013; Ouzounis et al., 2015; Bantis et al., 2018). However, the responses are depending on species, growth environment and experimental conditions (Bantis et al., 2018).

The most common LEDs (Light-emitting-diodes), which might be an alternative light source for plants, principally emit high proportions of blue and red light. Even white LEDs still emit light with different spectral properties compared to natural sunlight. Due to their lack of UV light emission and near infrared radiation (NIR), most LEDs are unsuitable for experiments with environmental natural conditions.

To date, studies using a full spectrum of artificial sunlight have only been performed on young cucumbers and tomato plants and have been limited to climate chamber experiments (Hogewoning et al., 2010a; Hogewoning et al., 2012). (Hogewoning et al., 2010a) published the first studies using an artificial spectrum that resembled a realistic solar spectrum. Artificial sunlight was provided using a sulfur plasma lamp, a filter to reduce the intensity of green light and a quartz-halogen lamp to provide near-infrared irradiance.

In this study, microwave plasma lamps (MPL) were tested, which emit sun-like light including UV-A and NIR without modification. The electrodeless lamp systems have a power of about 1300 W and are supposed to have a high longevity.

Potted roses were used as a model plant to represent a market-relevant ornamental plant. In the northern hemisphere, potted roses are usually produced from October to March with assimilation light occurring at an intensity between 100 and 200 µmol/m²s for approximately 20 h per day (Paradiso et al., 2011). The production of potted roses in winter term is increased due to the high demand for Christmas, Valentine’s day and Mother’s day.

The main objective of this study was to investigate how plant morphology and plant physiology are altered depending on the influence of artificial sunlight by MPL compared to traditional HPS lamps.

In addition to experiments in climate chamber, further experiments were conducted in the greenhouse to investigate the influence of artificial sunlight in addition to global irradiance.

# 8.3 Materials and Methods

### *8.3.1 Plant material and general growth conditions*

Potted roses from the ˈApacheˈ (Kordes Roses, Klein Offenseth-Sparrieshoop, Germany) cultivar were provided by Rosa Danica® (Marslev, Denmark). Four cuttings were rooted in 12 cm pots and were already pinched twice in Denmark.

The plants were further cultivated in Geisenheim (49° 59' 11.161" N 7° 58' 0.099" E, Germany) in a climate chamber and greenhouse (see below) with a density of 20 pots per m². LAT-Terra Standard P was used as substrate (pH: 5.9, N: 120 mg/L, $P_2O_5$: 120 mg/L, $K_2O$: 170 mg/L, Mg: 120 mg/L, HAWITA GRUPPE GmbH, Vechta, Germany). Irrigation was applied with low tide tables. Fertilization was provided in irrigation water with 0.1% Ferti® Mega 3 (18 % N, 12 % $P_2O_5$, 18 % $K_2O$, Planta, Regenstauf, Germany).

### *8.3.2 Light treatments*

Artificial sunlight was provided using a MPL (1300 W, Plasma International, Mühlheim am Main, Germany) with a similar spectrum compared to sunlight (Fig. 8.1B). The influences from sun-like light from this MPL were compared to those of light from a commercial HPS lamp (600 W, DH Licht, Wülfrath, Germany) (Fig. 8.1A). Due the not jet available MPL reflector, light distribution was heterogeneous and therefore light intensity was measured per individual pot at a height of 20 cm with a quantum sensor (LI-190R, LI-COR, Lincoln, Nebraska, USA). Average light intensity of climate chamber and greenhouse experiment is shown below. The light spectra from HPS, MPL and natural sunlight outside and inside the greenhouse on a cloudless day (May, Geisenheim, Germany, 2018, 49° 59' 11.161" N 7° 58' 0.099" E, 90 m above sea level) were recorded using a UV-Vis Spectrometer (Jaz, Ocean Optics, Ostfildern, Germany). Light spectra were analyzed with spectroscopic software (Spectra Suite 6.2, Ocean Optics, Ostfildern, Germany). Spectra were categorized as follows: UV-B (280-315 nm), UV-A (315-400 nm), blue (400-500 nm), green (500-550 nm), yellow (550-600 nm), red (600-700 nm) and far-red (700-800 nm). For each section, the proportion of light was calculated as % photo-biologically active radiation (PBAR, 280-800 nm) (Tab. 8.1). The R:FR (red: far-red) ratio was calculated as the ratio between the absorption maxima of the inactive and active forms of phytochrome ($P_r$ and $P_{fr}$) according to Smith (1982): $R{:}FR=\frac{\mathrm{Pr}(655-665\ nm)}{Pfr(725-735\ nm)}$.

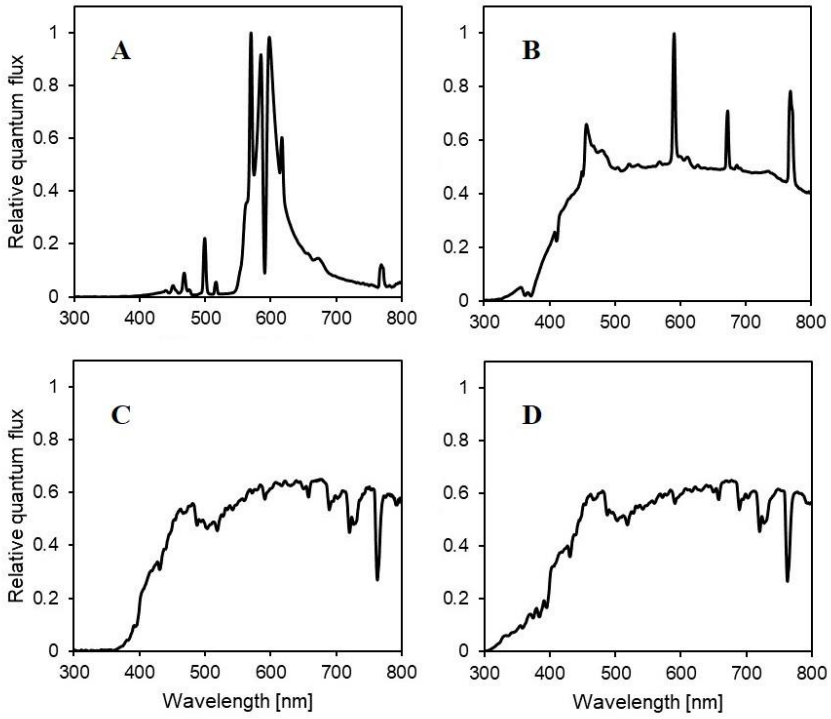


Fig. 8.1: Relative emission spectra from HPS lamp (A) and from microwave plasma lamp (B) and relative spectra from sun light measured inside (C) and outside the greenhouse (D) on a cloudless day (May, 2018, 49° 59' 11.161" N 7° 58' 0.099" E, 90 m above sea level). For better comparability, relative intensity of sun spectrum was adjusted in the range of the spectrum of MPL.

Tab. 8.1: Spectral characteristics from HPS lamp, MPL and representative sun spectra outside (Sun) and inside greenhouse (GH). Values are expressed as % PBAR (photo-biologically active radiation 280-800 nm). R:FR ratio was calculated according to Smith (1982).

| Light Source | [nm] | UV-B 280-315 | UV-A 315-400 | blue 400-500 | green 500-550 | yellow 550-600 | red 600-700 | far-red 700-800 | R:FR ratio |
|---|---|---|---|---|---|---|---|---|---|
| HPS | % | - | 0.2 | 4.5 | 2.1 | 48.8 | 38.0 | 7.0 | 2.79 |
| MPL | % | - | 2.3 | 22.9 | 12.1 | 13.7 | 24.3 | 25.1 | 1.05 |
| Sun | % | 0.1 | 3.9 | 21.0 | 11.4 | 12.8 | 26.6 | 24.2 | 1.21 |
| GH | % | - | 1.0 | 19.4 | 11.7 | 13.8 | 28.6 | 25.5 | 1.24 |

### *8.3.3 Greenhouse and climate chamber conditions*

For the greenhouse experiment 20 pots with potted roses were grown for five weeks (March to April, calendar weeks 9-14, 2016). Shade material was used in greenhouse to decrease the amount of natural light in the greenhouse for a targeted total daily light integral of about 12-14 mol/m²d, which is generally applied for potted rose production. During the experiment, the daily light integral (DLI) of global irradiance inside the greenhouse was on average

11.2±6.4 mol/m²d; standard deviation indicated the variation of DLI during the experiment depending on weather conditions. Plants were grown with additional artificial light from MPLs or HPS lamps with an intensity of 80±14 µmol/m²s for 20 h per day ($DLI_{artifical}$: 5.8±1.0 mol/m²d). Day/night temperature inside the greenhouse was on average 20.7±0.7/ 18.6±0.8 °C. Relative humidity was 57±9 %.

For the climate chamber experiment the climate room was split into four compartments with two replicates per treatment. Mixing of artificial light was prevented using opaque material. 60 potted roses were grown under MPL or HPS lamps for 20 h per day for six weeks. The light intensity from MPL and HPS lamps was 100±6 µmol/m²s ($DLI_{artifical}$: 7.2±0.4 mol/m²d). Room temperature was kept constant to 21±1 °C with 60±10 % relative humidity.

### *8.3.4 Measurement of leaf temperature*

Leaf temperature from potted roses was measured inside the greenhouse under HPS or MPL light in the absence of sunlight by thermal imaging camera (H2640, InfReC). The viewing angle and distance to the plants was kept constant. Emissivity value was set to 0.96. From each treatment three images were recorded and analyzed by thermal camera software (InfReC Analyzer NS9500, 2.7A, ATuS GmbH). Temperature was measured at eight leaves in the upper part of each plant (see supplemental material Fig. S8.5).

### *8.3.5 Morphological parameters*

Plant height and fresh weight from 20 (greenhouse) or 60 (growth camber) was measured from the pot level up to the vegetation point. The numbers of buds (>3 mm), flowers and primary shoots were counted per pot (four plants per pot). The portion of open flowers was calculated as the ratio of open and half open flowers divided by the total amount of flowers and buds. Primary shoots were counted when shoots grown from cuttings had a minimum length of 5 cm.

### *8.3.6 Leaf pigment analysis*

The chlorophyll and flavonoid contents were determined using a polyphenol and chlorophyll-meter (Dualex Scientific+™, Force-A, Orsay, France). Three leaves from 20 (greenhouse) or 60 (climate chamber) potted roses were measured at the upper leaf surface and an average value was calculated per pot.

### *8.3.7 Measurement of photosynthesis rate*

For measurement of photosynthesis rate another set of plants were grown in climate chambers. The photosynthesis rate was measured for 12 leaves per treatment either under HPS or MPL with an intensity of 100 µmol/m²s using a portable gas exchange system (GFS 3000, Walz, Effeltrich Germany). It was consciously decided against standard measuring light (blue and red LEDs) to prevented adaptation to another light environment. The $CO_2$ concentration was ambient (~550 ppm) and the relative humidity was about 60 %. The flow rate from the measuring head was set at 750 µmol/s and the impeller was set to 5. After 20 min of equilibration for each leaf the photosynthesis rate remained constant and the value was recorded.

### *8.3.8 Analysis of primary soluble sugars*

Leaf samples from 12 potted roses per treatment grown in climate chamber were harvested. Each sample contained six fully developed upper leaves. Leaf samples were stored at -20 °C for further processing. Leaf samples were freeze-dried for three days and plant material was ground with a mill. Approximately 25 mg of plant powder was extracted three times at 80 °C for 15 min. with 1.5 mL ethanol (80% v/v). Primary soluble sugars (glucose and fructose) were determined enzymatically according to Zhao et al. (2010) with slight modifications as described by Kirigia et al. (2018).

### *8.3.9 Data Analysis*

Statistical analysis was performed using statistical software R (R 3.4.3). Data were analyzed for normality with the Shapiro-Wilk test. Levene's test was used to assess the equality of variance. When the data were normally distributed and variance homogeneity was proved, one-way analysis of variance (ANOVA) test ($p \leq 0.05$) was performed with Tukey's range test as a post-hoc analysis. When the data were not normally distributed and/or heterogeneous, the non-parametric Kruskal-Wallis test ($p \leq 0.05$) was used. Repetitions were based on individual pots.

Statistical tests were only performed to indicate significant differences between the assimilation light treatments in the same growth environment due to difference between climate chamber and greenhouse experiments in light intensity, room temperature and

cultivation time (see above). Graphical images were created with R package ggplot2 (Wickham, 2016).

## 8.4 Results

### *8.4.1 Plant morphology*

Potted roses grown under the MPLs showed earlier flower opening in the greenhouse and climate chamber indicated by a significantly higher percentage of open flowers (Fig. 8.2; Fig. 8.3). However, the total number of flowers and buds in plants grown under HPS light in the climate chamber was significantly increased by about 50 % (Tab. 8.2).

Plants grown under HPS light in the climate chamber also showed a higher degree of branching, indicated by a significantly higher number of primary shoots accompanied by an increased number of flowering shoots. However, potted roses in the greenhouse experiment showed no significant difference in the number of flowers and buds, even though a significantly higher number of primary shoots in potted roses grown under sun-like light from the MPL was counted. This increase in the number of primary shoots is caused by the occurrence of blind shoots without a flower or bud (Tab. 8.2, Fig. 8.3).

No difference in fresh weight was measured in the greenhouse experiment, whereas potted roses grown under HPS light in the climate chamber displayed a significantly increased fresh weight by 19 %. In both growth environments potted roses grown under MPL light showed a reduced plant height of about 12-15 % compared to with the plant height from roses grown under HPS light (Tab. 8.2, Fig. 8.3).

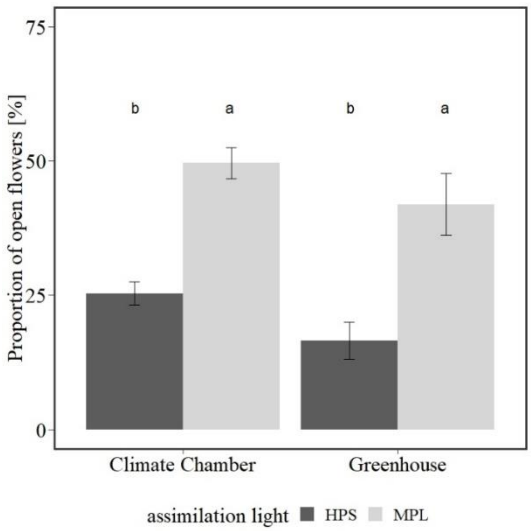


Fig. 8.2: Proportion of open flowers on the day of evaluation. Different letters indicate significant differences between assimilation light treatment in same growth environment, p≤0.05 n=20 (greenhouse) and n=60 (climate chamber). The values are averages ± SE.

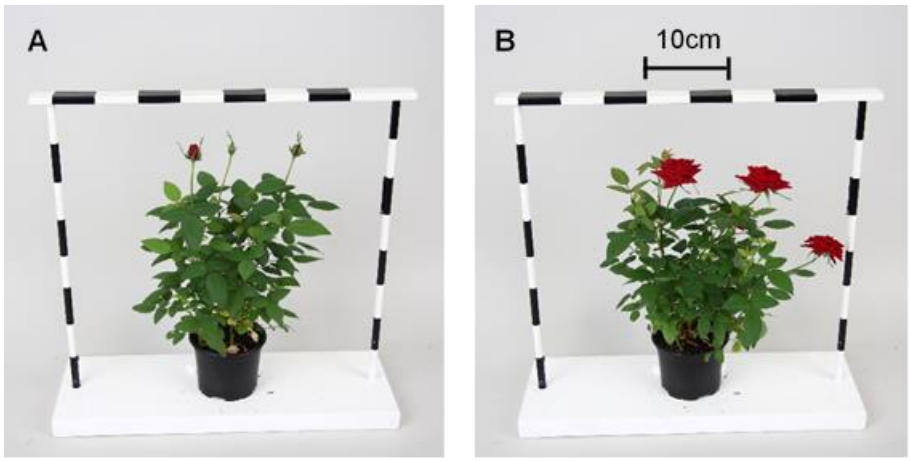


Fig. 8.3: Representative potted roses grown for 5 weeks in the greenhouse grown with HPS lamps (A) or artificial sunlight from MPLs (B). The visual appearance of potted roses grown in the climate chamber was similar (not shown).

Tab. 8.2: Morphological parameters of roses grown under HPS or MPL light in the greenhouse and climate chamber. The values are averages ± SE. Different letters indicate significant differences, p≤0.05, n=20 (greenhouse) and n=60 (climate chamber).

| Growth environment | Assimilation light | Number of flowers and buds | Fresh weight [g] | Height [cm] | Number of primary shoots |
|---|---|---|---|---|---|
| Greenhouse | HPS | 3.5±0.2$^{a}$ | 21.1±0.5$^{a}$ | 22.4±0.4$^{a}$ | 5.8±0.2$^{b}$ |
| | MPL | 3.8±0.2$^{a}$ | 21.5±0.4$^{a}$ | 19.7±0.5$^{b}$ | 7.6±0.4$^{a}$ |
| Climate Chamber | HPS | 8.8±0.2$^{a}$ | 34.6±0.6$^{a}$ | 23.7±0.4$^{a}$ | 9.6±0.4$^{a}$ |
| | MPL | 5.8±0.2$^{b}$ | 29.0±0.6$^{b}$ | 20.2±0.3$^{b}$ | 6.7±0.2$^{b}$ |

### *8.4.2 Leaf pigments*

The leaf chlorophyll content, measured with a chlorophyll and polyphenol-meter, was higher in plants grown in the climate chamber compared to the chlorophyll content of potted roses grown in the greenhouse (Fig. 8.4A). No difference in chlorophyll content was determined between plants grown under HPS or MPL in the greenhouse experiment. Nevertheless, a significantly lower chlorophyll content in plants grown under MPLs was measured in the climate chamber experiment.

In both growth environments the flavonoid content was significantly increased by 24 % (greenhouse) and 42 % (climate chamber) in leaves grown with light from the MPL compared with that of leaves grown under HPS lamps (Fig. 8.4B). However, the flavonoid content in potted roses grown under HPS or MPL light was higher in the greenhouse experiment compared with that of potted roses grown with the same light treatment in the climate chamber.

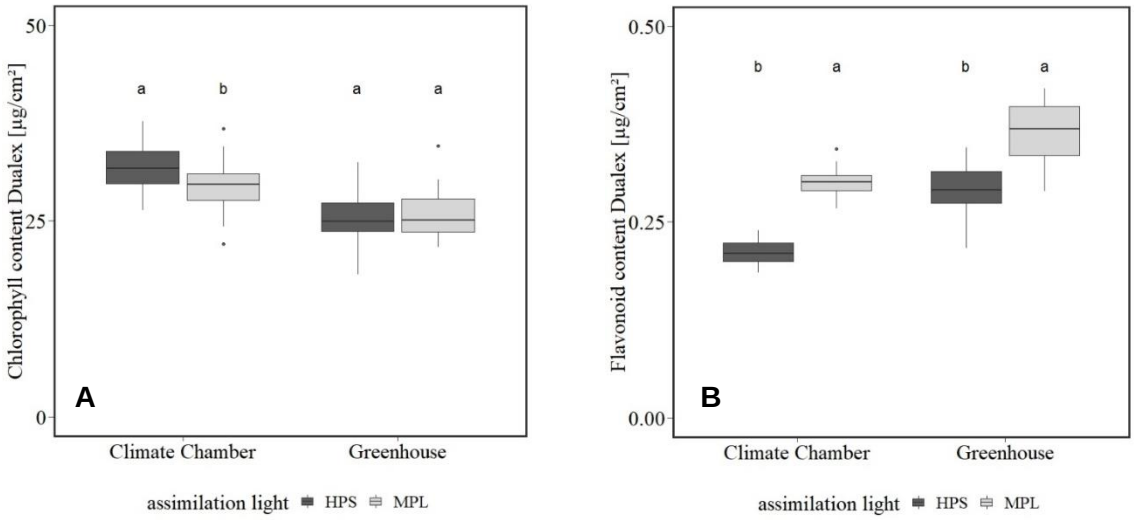


Fig. 8.4: Chlorophyll (A) and flavonoid (B) contents (Dualex values [µg/cm²]) from rose leaves grown under HPS or MPL light in the greenhouse or climate chamber. Different letters indicate significant differences between light treatments in the same growing environment, $p \leq 0.05$, n=20 (greenhouse) and n=60 (climate chamber).

### *8.4.3 Photosynthesis rate and sugar content*

Potted roses displayed a significantly higher photosynthetic rate under MPL light compared to roses grown under HPS light. Nevertheless, the content of primary sugars was not affected by a higher photosynthetic rate (Tab. 8.3).

Tab. 8.3: Net assimilation rate at growth irradiance (100 µmol/m²s) and primary leaf sugar content (mg/g dry weight) of potted roses grown under HPS or MPL light. The values are averages ± SE. Different letters indicate significant differences, p≤0.05, n=12 (Net assimilation rate) and n=16 (sugar content).

| Growth environment | Assimilation Light | $A_{net}$ [µmol/m²s] | Glucose [mg/g dw] | Fructose [mg/g dw] |
|---|---|---|---|---|
| Climate Chamber | HPS | 4.6±0.1[b] | 9.0±0.6[a] | 7.2±0.5[a] |
| | MPL | 5.1±0.1[a] | 9.5±0.4[a] | 7.9±0.3[a] |

## 8.5. Discussion

Investigations with artificial sunlight have only been performed on cucumbers and tomatoes so far and have been limited to climate chamber experiments (Hogewoning et al., 2010a; Hogewoning et al., 2012). In an investigation about the influence of spectral quality from LEDs on morphology and phytonutrients of red pak choi, a LED system emitting artificial sunlight was used as a reference (Mickens et al., 2019). Nevertheless, the term artificial sunlight for this light system is only partially correct since the LEDs are not emitting infrared radiation.

In this study, we examined the morphological and physiological responses of potted roses to artificial light from a new MPL, which emits sun-like light including infrared radiation without modifications.

The spectral properties of artificial sunlight from the MPL and HPS lamps were different (Fig. 8.1A, B) and caused significant (p≤0.05) changes in the morphological appearance of potted roses grown in greenhouse and climate chamber. Light from the HPS lamp contains a lower amount of blue light, a higher amount of red light and a higher R:FR ratio (2.79 to 1.05) compared to the light emission spectra of the MPL (Tab. 8.1).

Differences between climate and greenhouse experiments concerning light intensity, cultivation period and climatic conditions lead to a limited extent of comparability. In the greenhouse experiment the total DLI including artificial light and global irradiance was about 16 mol/m²d ($DLI_{artificial}$: ~35 %). In climate chamber the DLI of artificial light was about 7.2 mol/m²d. For potted rose production a DLI integral of about 12-14 mol/m²d is usually applied (Mortensen, 2004; Moe et al., 2006; Paradiso et al., 2011). However, potted roses displayed similar morphological and physiological responses in the process of photomorphogenesis in both growth environments.

### *8.5.1 Decreased plant height was probably caused by increased blue light from MPL*

Potted roses grown under artificial sunlight from MPL showed a smaller plant height in both growth environments compared to plants grown under HPS lamps, although a lower R:FR ratio is attributed to an increased stem elongation in the process of shade avoidance (Smith, 1982). An increased stem and leaf elongation by increased far-red light was already observed in lettuce and petunia (Lee et al., 2016; Park et al., 2016).

Regarding the plant height, the far-red light effects could be reversed by the higher proportion of blue light in the sun-like light spectrum compared to the spectrum of HPS light (22.9 % to 4.5 %). It was shown that potted roses grown under blue LED light had smaller plant heights and were more compact compared to those in HPS plants in the greenhouse and climate chamber experiments (Terfa et al., 2013; Bergstrand et al., 2016). Plant height from potted roses was reduced by increasing the blue light content in combination with red light in the presence of global irradiance in the greenhouse (Ouzounis et al., 2014). A higher proportion of blue light was decreased plant height as demonstrated in lettuce, poinsettia, tomato plants, chrysanthemums and cucumber seedling (Li and Kubota, 2009; Islam et al., 2012; Xiaoying, 2012; Ouzounis et al., 2014; Hernández and Kubota, 2016). (Cope and Bugbee, 2013) as demonstrated in radish, soyabean and wheat that not only the proportion of blue light but also the absolute amount of blue light plays a role in elongation inhibition.

### *8.5.2 Artificial sunlight from MPL led to earlier flowering but reduced branching degree*

An earlier flower opening was observed of plants grown under artificial sunlight in climate chamber and greenhouse. In long-day plants, such as *Arabidopsis*, petunia and eustoma, it was shown that far-red light promotes flowering (Cerdán and Chory, 2003; Cerny et al., 2003; Yamada et al., 2009; Park et al., 2016).

However, flower formation in roses is mainly controlled by temperature and irradiance, not by photoperiod (Zieslin and Mor, 1990). Although roses are considered to be facultative day-neutral plants, the earlier flowering time in this study could be attributed to the increased far-red light in the artificial sunlight spectrum of the MPL. However, the earlier flowering might be an indirect effect of far-red light, resulting in a lower degree of branching in the climate chamber experiment and a higher number of blind shoots in the greenhouse experiment.

It was shown that a low R:FR ratio caused by natural shading results in inhibited sprouting and reduced branching in roses (Mor and Halevy, 1984). Another reason for earlier flowering

is the lower number of main shoots on the plants grown under the MPL. Thus, more resources (e.g. nutrient and soluble sugar) were available per shoot, which have resulted in faster flower development. A lower branching degree and lower ornamental value induced by lower R:FR ratios were also observed in petunia (Park et al., 2016). It could be excluded that the earlier flowering was due to the increased blue light content in artificial sunlight compared with that in HPS. It was shown that the blue light from LEDs did not affect the time to flower opening in potted roses compared to plants grown under HPS light in greenhouse and climate chamber (Terfa et al., 2013). Nevertheless, flower development was three days faster under white light in climate chamber compared to plants grown under blue LEDs (Abidi et al., 2013). suggesting that white broad light is beneficial for flowering.

In experiments with different blue light proportions no differences in the number of flowers and buds have been observed in potted roses grown with LEDs (Ouzounis et al., 2014).

When potted roses were grown under blue LEDs a higher photosynthetic capacity, higher leaf mass per area and higher levels of soluble sugars were observed (Terfa et al., 2013). Artificial sunlight from the MPL also led to a higher photosynthesis rate in the climate chamber experiment; however, no significant difference in glucose and fructose contents in rose leaves were detected.

### *8.5.3 Blue light und UV-A increased flavonol content in rose leaves*

UV and blue light have a high energy content and can cause cell damage. Flavonoids are involved in light protection against excessive radiation energy and therefore can avoid cell damage (Mierziak et al., 2014). (Ouzounis et al., 2014) showed that a higher blue light content led to higher phenolic acid and flavonoid contents. It was also shown that total amounts of flavonoids measured by HPLC-MS (rutin and quercetin) were positively correlated with the flavonoid values by a Dualex device (Ouzounis et al., 2014). The transcription of flavonoid synthesis genes can be stimulated directly by UV and blue light (Kubasek et al., 1992). Blue light led to increased content of secondary metabolites in basil, roses, chrysanthemums, campanula, and arugula grown with LEDs (Ouzounis et al., 2014; Bantis et al., 2016; Taulavuori et al., 2018).

Therefore, the increased blue and UV-A light content from the MPL could be responsible for higher flavonoid formation compared to plants grown under HPS light. The flavonoid content was higher in the greenhouse experiment compared to the content in the climate chamber experiments, which shows that flavonoid formation is also regulated by the light

intensity. Secondary metabolites can enhance the intrinsic defense of plants against abiotic as well as biotic stress (Mierziak et al., 2014; Huché-Thélier et al., 2016).

### *8.5.4 Are MPL alternative lighting sources for horticultural industry?*

Compared to natural sunlight, artificial sunlight by MPL light displayed the same spectral characteristics, with the exception of a lower UV light content and a slight lower R:FR ratio (Tab. 8.1). Furthermore, four prominent peaks (457 nm, 590 nm, 673 nm and 769 nm) are present in artificial sunlight (Fig. 1), which do not occur in natural sunlight. Artificial sunlight was shown to have beneficial effects on biomass production, plant height and leaf unfolding rate of cucumber plants in comparison to HPL and fluorescent light treatments (Hogewoning et al., 2010a).

We recently published an investigation, in which MPL were tested for the cultivation of *Plectranthus scutellarioides* in a climate chamber (Dörr et al., 2019). *P. scutellarioides* plants grown with artificial sunlight from MPL showed increased leaf thickness, higher biomass and plant height in comparison to HPS grown plants. Nevertheless, the effects could also be achieved with other light sources and were dependent on the blue content and the influence of the infrared radiation on leaf temperature.

Due to the missing light reflectors in MPL, statements about energy efficiency cannot be made yet. After the development of an optimized reflector energy efficiency measurements will be conducted to determine if the new MPL are suitable for commercial horticulture industry. However, we already presume that MPL emitting artificial sunlight are less efficient and too expensive for replacing HPS lamps due to reduced light emission.

For potted rose productions LEDs are assumed to be an economic alternative due to their energy saving potential (Ouzounis et al., 2018). Nevertheless, Park et al. (2018) see a usability of MPL for horticultural industry. Their MPL emitted light, which was not as close to sunlight as our tested MPL. Anyway, the lamps might be more energy efficient, and also beneficial growth attributes were observed in tomatoes compared to HPS grown plants.

At least for research proposes our tested MPL might be an interesting light source because no other light system is able to emit light in almost the same spectral properties like sunlight.

## 8.6 Conclusion

Artificial sunlight from MPL altered plant morphology and increased the flavonoid content and photosynthetic rate. Nevertheless, a lower ornamental value due to decreased numbers of flowers, buds and shoots was observed in potted roses grown under MPL compared to plants grown under commercial HPS lamps.

With the development of LEDs studies using artificial light have frequently been performed; however, many experiments lack the use of suitable control conditions, because even broad white LEDs are lacking in an emission of UV and NIR. The tested new MPL can be used as a sun-like light reference in plant cultivation experiments under environmental natural conditions and might help understanding how plants react to the spectral quality of light.

**Competing interest**

The authors declare no competing interests.

**Acknowledgement**

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# 8.7 Supplemental Material

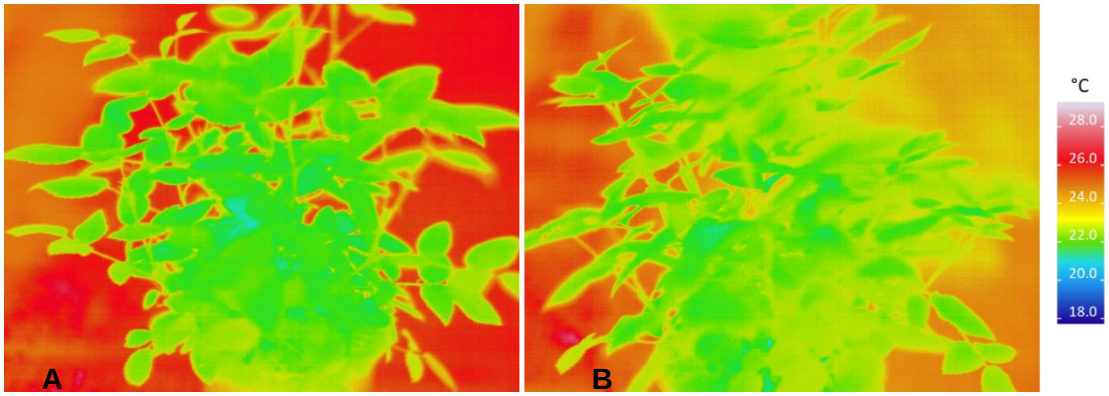


Fig. S8.5: Representative picture of thermal camera image from potted rose under HPS (A) or MPL (B) light inside the greenhouse in the absence of sunlight.

Potted roses under MPL light displayed an average leaf temperature of 22.4±0.8 °C. The leaf temperature of potted roses under HPS light was a bit lower with an average leaf temperature of 21.9±0.2 °C.

# 9. General discussion

In this thesis the impact of spectral light quality from MPL on physiological responses of three different plant species was examined. Overall, plant morphology and content of secondary metabolites were altered in coleus, basil and potted roses due to the usage of different light sources. This chapter summarizes the main findings and assigns them to a general context. Finally, the usability of the MPL system in terms of light efficiency for horticulture and plant research will be discussed.

## 9.1 Effects on morphology and secondary metabolite accumulation - A mixture of light color and infrared radiation

### *9.1.1 Plant architecture is strongly influenced by R:FR*

Depending on the light source, plant architecture of coleus, basil and potted roses has been changed. An increased amount of far-red light in artificial sunlight spectrum of the MPL led to either an induced elongation growth in coleus and basil plants or to a reduced branching degree observed in potted roses compared to plants grown under lamp systems emitting light with higher R:FR ratios.

The phytochrome mediated reaction to far-red radiation is well described and the induction of stem elongation by far-red light occurs in several plant species (Tab. 4.2).

### *9.1.2 Blue light - A trigger for plant to adapt against high irradiances*

With coleus it was demonstrated that blue light content is a strong influential factor for leaf morphology. Leaf thickness and leaf mass per area were positively correlated with the proportion of blue light (Fig. 5.4, Tab. 5.4). Leaves developed with blue deficient light source from HPS lamps were thinner and showed susceptibility against high irradiances (Fig. 6.2, Fig. 6.3).

Blue light indirectly resulted in a higher photosynthetic capacity due to increased leaf thickness and distinct formation of palisade parenchyma. In addition to the increased tolerance against high irradiance, also the resistance of plants against plant herbivores can rise. Thicker cuticle and increased cell wall thickness are the first defense mechanism against insect herbivores (War et al., 2012). The cuticle is furthermore a physical barrier against fungal pathogens (Serrano et al., 2014). Moreover, trichomes occurring for example in coleus are involved in the defense mechanism against insect herbivores (War et al., 2012).

The changing proportion of trichomes under the respective light source was not evaluated but might be an interesting research area for the future.

Flavonoids and phenolic acids can protect plants against harmful radiation. Energy rich UV radiation and blue light are causing light stress and can result in a higher accumulation of protective compounds (Kubasek et al., 1992; Favory et al., 2009; Mierziak et al., 2014; Bantis et al., 2016) (see also Tab. 4.2).

These results could be confirmed in this study, since the content of secondary metabolites of basil and potted roses was positively affected by blue light enriched environment (Fig. 7.3, Tab. 7.3, Fig. 8.4). However, in coleus increased blue light mainly indirectly resulted in a higher content of phenolic compounds per leaf area by increasing leaf thickness. (Fig. 5.5, Tab. 5.5, Tab. 5.6). Leaf morphology of rose and basil was not studied, but it could be altered by different light sources as well. Potted roses grown under red and blue LEDs showed increased LMA compared to plants grown with HPS light (Abidi et al., 2013; Terfa et al., 2013) which was attributed to higher blue light content. Likewise, basil plants developed a higher LMA under higher proportions of blue light (Jensen et al., 2018). Preliminary tests indicated that LMA was increased under the light from MPL compared to leaves grown with HPS lamps (data not shown).The increased flavonol value measured by polyphenol meter from basil plants and potted roses (Fig. 7.3B, Fig. 8.4) might be due to an increase of leaf thickness under the MPL.

### *9.1.3 The role of infrared radiation on plant morphology and secondary metabolites*

Experiments with coleus showed that in addition to light color, leaf temperature can have also influences the content of secondary metabolites and the plant architecture (chapter 5). Especially the comparison between LEDs and discharge lamps demonstrates that, leaf temperature can be changed. This can result in an overestimation of the influence of spectral light quality if the leaf temperature is not measured. Under LED light, lower leaf temperatures compared to leaves under discharge lamp as discussed in chapter 5 and 6 (see also chapter 4.5.6 Influence of infrared radiation on plant growth).

As a result of lower leaf temperature, a decreased growth performance can occur, resulting in lower biomass and reduced yield (Hernández and Kubota, 2015; Bergstrand et al., 2016; Särkkä et al., 2017). Kim et al. (2019) observed that higher leaf temperatures were accompanied by increased transpiration and higher photosynthesis rate. In this study, the

growth of basil and coleus plants was positively influenced by higher leaf temperatures causing in an increased biomass and faster plant development (Tab. 5.3, Tab. 6.5, Tab. 7.2.) Leaf temperature also depends on the leaf-position on the plant. In general, unshaded upper leaves which were directly exposed to the light sources, showed higher leaf temperatures under discharge lamps compared to shaded leaves (Fig. 5.2, Fig. S7.6). In plants under LED no distinct difference of leaf temperatures between shaded and unshaded leaves was observed (Fig. 5.2).

In a study with HPS lamps and LED interlights (lighting systems which are used between taller growing plants), leaf temperatures in upper leaves were about 2 °C higher under HPS light compared to LED illuminated leaves as observed in tomato plants (Kim et al., 2019). Shaded leaves of tomato plants under HPS light showed about 4 °C lower leaf temperatures compared to upper leaves, while leaf temperature remained constant through the whole plant under LED interlights. Plants grown with HPS lamps showed increased vegetative biomass and lower proportion of fruits compared to LED light treatment (Kim et al., 2019). The authors postulate that higher temperature in upper leaves changes biomass allocation and leads to smaller, thinner leaves and increased water transport from roots to upper leaves.

In this study increased leaf temperatures under MPL might be caused also by the installation of higher number of MPL devices, because for MPL systems no reflector was available. This resulted in a high total power per cultivation area (Tab. 6.1) to get an almost homogenous light distribution (Appendix 2 – Light distribution) as discussed in chapter 6.

In conclusion, the results indicated that the impact of light color and infrared radiation on morphology and secondary metabolites is not always clearly separable in experiments with differed lamps systems. In further investigation the usage of glass panes acting as infrared filter might help to separate these influences. Furthermore, more experiments have to be conducted to estimate the direct effect of near and long wave infrared radiation on morphology and secondary metabolites.

### *9.1.4 Differences between climate chamber and greenhouse experiments*

The results presented in this thesis were mainly conducted in climate chambers in the absence of global irradiance to obtain a clear effect of the respective light source without the impact of external influences. In greenhouse experiments, light intensity and light duration are fluctuating depending on changing weather conditions and season which complicates the reproducibility of the results. In addition, indoor cultivation has an increasing importance as

discussed below in the outlook chapter, therefore light experiments are often conducted in closed systems to study how plants grow in the absence of sunlight.

Nevertheless, greenhouse experiments are required to evaluate the light sources from the perspective of the application for today's horticultural industry. In this study greenhouse experiments using MPL and HPS lamps were conducted with potted roses (chapter 8) and coleus plants (chapter 6). Potted roses showed almost the same morphological appearance in greenhouse experiment compared to climate chamber experiment (Fig. 8.2, Fig. 8.3, Tab. 8.2) underlining the strong effect of increased far-red light on plant architecture as previously discussed. Like in climate chamber experiments, coleus plants grown with artificial light from MPL in greenhouse showed also an increased elongation growth, indicated by a significantly higher plant height and internode length in comparison to plants grown with HPS lamps (Tab. 6.8). Leaf mass per area was also significantly higher from plants of cultivar 'Golden Dreams' grown with MPL under greenhouse conditions (Tab. 6.9).

Analysis of secondary metabolites of coleus and roses in greenhouse experiments was not analyzed as extensively as in the climate chamber experiments. However, the flavonoid content on the leaf surface of potted roses was increased under MPL light source compared to HPS grown plants. Also the phenolic content of coleus plants was altered in plants grown in greenhouse experiment using MPL and HPS lamps, but no clear results were associated to different light source (data not shown). Moreover, external influences like room temperature, relative humanity, $CO_2$ and the orientation of greenhouse compartment might have a higher effect on secondary metabolites in coleus plants compared to the spectral quality of light.

In further research of light experiment in greenhouse, multivariate analysis (Fig. 5.7, Fig. 7.5) should include previous mentioned abiotic factors in addition to spectral quality parameters. Multivariate analysis can help to find the strongest influence factors for plant morphology and content of secondary metabolites in order to derive an optimized plant cultivation procedure. This implies an increased usage of sensors measuring the microclimate of individual plants. A high emissivity value of around 0.98 (López et al., 2012) enables measurements of leaf temperature with infrared thermography to control the crop temperature. An installation of thermal imaging cameras measuring the leaf surface temperature during the whole experiments might be helpful to estimate the changing of leaf surface temperature during the time and to indicate the water status of the plants.

However, the measurement of leaf temperature by thermal imaging cameras used in this thesis only evaluated the leaf surface temperature. In further approaches additional measurements on the lower leaf surface and inner side of the leaves should be taken in account.

### *9.1.5 Cultivar and species dependency on light responses*

The coleus cultivar 'Golden Dreams' was the main research object in this study, however also experiments with three different cultivars of *Plectranthus scutellarioides* were carried out in greenhouse and climate chamber. The responses to spectral light quality were similar especially with regard to increased elongation growth caused by lower R:FR ratio of the artificial sunlight from MPL compared to HPS treatment (Tab. 6.5, Tab. 6.8).

Like coleus also basil plants showed increased elongation growth induced by lower R:FR ratio. Basil and coleus relate to the same subfamily (*Nepetoideae*) of *Lamiaceae* and in general, elongation induced by far-red light is observed in several plant species as shown in Tab. 4.2 in the introduction part.

However, in potted roses, belonging to the family of *Rosacea*, a distinct elongation growth induced by lower R:FR ratio was not determined in this study. Lower R:FR ratio resulted in reduced branching degree and lower number of flowers (chapter 8).

With regard to the content of secondary metabolites, some cultivars (experiment 3 in chapter 6) and species dependencies were observed.

Except the induction of the content of phenolic compounds by blue and UV light, several secondary metabolites are individually altered according to substance class, plant species, light source and experimental conditions (Tab. 4.2). Secondary metabolites are not essential for plant growth but increase the fitness for attraction of pollinators or for plant defense against abiotic stresses, herbivores and plant diseases. The individual usage of these substances result in a high species dependency.

### *9.1.6 Light experiments with single light colors*

The usage of LEDs allows to estimate the effects of single light colors on plant physiology. The many possibilities of adjusting the light emission spectrum have the disadvantage that general statements about the influence of the light colors on plant physiological processes are imprecisely. In this thesis, blue light was defined from 400-500 nm. However,

experiments using different blue and green LEDs showed that distinct wavelengths of blue and green light have different influences on the accumulation of secondary metabolites and morphology as demonstrated in lettuce (Johkan et al., 2012; Samuolienė et al., 2012) (see also Tab. 4.2). Consequently, the indication of a specific wavelength is very important when single LEDs are used.

Also the interaction of single light colors and the interplay with the photoreceptors is uncertain. A defined artificial sun-like spectrum used as a control condition might be helpful to compare these effects. Whether the MPL, emitting an artificial sun-like light, is suitable for plant research or horticultural purposes will be discussed in the next chapter.

## 9.2 Artificial sunlight from MPL - an efficient light source?

In comparison to HPL light treatment, plants from all three investigated species were positively affected by artificial sunlight from MPL. Coleus showed increased biomass production, thicker leaves with higher photosynthetic capacity and increased stress tolerance against high irradiances (Tab. 5.3, Fig. 5.4, Fig. 6.2, Fig. 6.4, Fig. 6.6). Basil showed increased accumulation of essential oils and flavonoids and higher biomass (Tab. 7.2, Tab. 7.3, Tab. 7.4, Fig. 7.3) under artificial sunlight from MPL compared to HPS treatment. Furthermore, the carcinogenic methyl-eugenol was decreased under artificial sunlight from MPL (Tab. 7.4). In potted roses an increased flavonoid content in leaves and a higher photosynthesis rate was observed (Tab. 8.3, Fig. 8.4). As previously mentioned, these effects were mainly attributed to higher blue light content in comparison to HPS light which only contains about 5% blue light of PBAR radiation. In blue deficient light environments plant development can be negatively affected indicated by leaf formation or suppressed growth (Ouzounis et al., 2014; Trouwborst et al., 2016).

However, is a balanced sun-like light from MPL required for plant growth? A ceramic metal halide lamp and LEDs also positively affected plant quality parameters observed in coleus due to increased proportion of blue light. Furthermore, the lack of blue light in HPS can be compensated by global irradiance in greenhouse. Due to low R:FR ratio of MPL systems emitting artificial sunlight, negative plant quality parameters like increased elongation growth (coleus and basil) or lower branching degree (potted rose) were observed. For horticultural industry compact plant growth and higher branching degree is favored to maximize the production per area and optimize the packaging and transportation (Börnke

and Rocksch, 2018). However, for cultivation of cut flowers an increased elongation growth is preferred (Dole, 2015).

Nevertheless, investigation costs and energy efficiency of supplemental lighting are often more important than the light spectrum of lamp systems. Light efficiency of different lamps is defined as the amount of light emission per electricity power and is in general expressed as lm/W (lumen per Watt). The actual distributor of the MPL with artificial sunlight spectrum indicates that about 45-50 lm/W are emitted (personal communication). However, the measurement of the light intensity in lumen is not suitable for plants. The value lumen is adapted to the spectral sensitivity of human's eye which has a maximum in green light region. Consequently, since the definition of PAR and the development of LEDs, light efficiency for plant growth is defined as photosynthetic active photon flux per electricity power and is expressed as µmol/s per W which is equivalent to µmol/J (Nelson and Bugbee, 2014).To compare efficiency of different light sources, it must be considered that the energy of photons ($E_{Photon}$) depends on its wavelengths ($\lambda$) with the following relationship according to Planck's law including Planck's constant ($h$= 6.62607004·10$^{-34}$ Js) and the speed of light (c= 299792458 m/s) (according to Heldt and Piechulla (2015, 5. Edition, pp.45)):

$$E_{Photon} = \frac{hc}{\lambda}$$

The photon flux per electricity power (PFE) expressed as µmol/J is given by the $E_{Photon}$ and Avogadro's constant ($N_A$= 6.02214076·10$^{23}$ mol$^{-1}$) (own transformation):

$$PFE = \frac{1}{E_{Photon} * N_A}$$

Fig. 9.1 shows the PFE as a function of the wavelength.

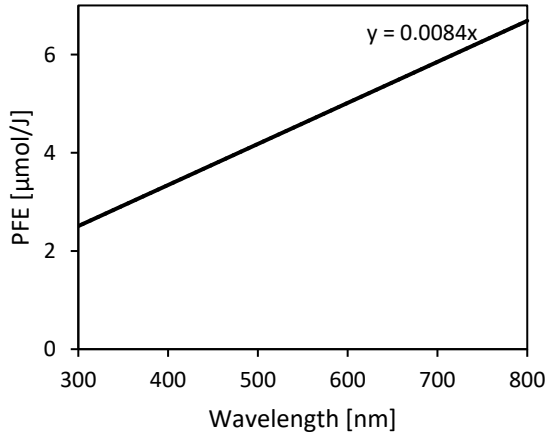


Fig. 9.1: Photon flux per electricity power (PFE) expressed as µmol/J a function of the wavelength (own graphic, own calculation).

Consequently, more photons per Joule can be generated when the wavelength is longer. The maximum amount of light emitted per Joule for several light colors is shown Tab. 9.1.

Tab. 9.1: Maximum PFE of different light colors. PFE from sunlight spectrum in the range of PAR or PBAR was calculated from spectral data (own calculation).

| Light color | Wavelength [nm] | Maximum PFE [µmol/J] |
|---|---|---|
| UV-B | 280-315 | 2.5 |
| UV-A | 315-400 | 3.0 |
| Blue | 400-500 | 3.8 |
| Green | 500-550 | 4.4 |
| Yellow | 550-600 | 4.8 |
| Red | 600-700 | 5.5 |
| Far-red | 700-800 | 6.3 |
| Sunlight PAR | 400-700 | 4.6 |
| Sunlight PBAR | 280-800 | 4.8 |

The maximum PFE of red light (600-700 nm) is about 5.5 µmol/J. With the same energy, a lower amount of blue light (400-500 nm) can be emitted (maximum PFE: 3.8 µmol/J). For a light source emitting the same spectral properties like sunlight from 280-800 nm the maximum PFE is about 4.8 µmol/J.

Tab. 9.2 shows an overview of current different light systems with regard to their light efficiencies in PAR region, color rendering index (CRI) and costs per photon flux (€/µmol). The amount of µmol/J was calculated by a conversion factor for the MPL light systems. Light sources with spectral quality close to sunlight (CRI=100) display a higher CRI. Light sources with high CRI such as MPL emitting artificial sunlight, CDM or LEDs emitting a broad white light, have the advantage that the plants appear more natural so that damage or leaf discoloration can be better recognized. LEDs are not emitting infrared radiation, therefore the efficiency assessment of the amount of PAR emitted per electricity power favors LED systems. LED devices, emitting predominantly red light, are the most efficient light sources to generate high amounts of photons. Blue and red LEDs are showing a light efficiency close to 70% ($PPE_{660\ nm\ LED}$: 3.8 µmol/J, $PPE_{660\ nm\ max}$: 5.5 µmol/J; $PPE_{450\ nm\ LED}$: 2.6 µmol/J, $PPE_{450\ nm\ max}$: 3.8 µmol/J; Tab. 9.2; Fig. 9.1). The generation of sun-like light from LEDs is less efficient since many different LED chips including inefficient green chips

have to be used to get an almost continuous light spectrum. The MPL emitting artificial light showed the lowest light efficiency compared to other light sources.

Tab. 9.2: Photon flux per electricity power (PFE), color rendering index (CRI) and costs of different lamps systems. The PFE for lamp systems is expresses as µmol/J in the PAR region. PFE from blue and red LED were obtained from data sheets of most efficient LEDs from OSRAM[1]. PFE from MPL was calculated from luminous efficiency (lm/W) by a conversion factor. Costs and CRI for single LEDs are not estimated (-). CRI for LED with 90% red (R) and 10% blue (B) is not estimated.

| Lamp system | CRI | PFE $_{400\text{-}700\ nm}$ [µmol/J] | Costs [€] | Costs per PF [€/µmol] |
|---|---|---|---|---|
| LED blue $_{450\ nm}$ [1] | - | 2.6 | - | - |
| LED red $_{660\ nm}$ [1] | - | 3.8 | - | - |
| LED 90% R, 10% B (300 W) [2] | - | 3.7 | 1200.00 | 1.08 |
| LED full spectra (300 W) [2] | 90 | 2.3 | 1800.00 | 2.61 |
| CDM (315 W) [2] | 90 | 1.7 | 540.00 | 1.01 |
| MPL artificial sunlight (1300 W) [3] | 97 | 0.8 | 4000.00 | 3.84 |
| MPL sulfur (1300 W) [2] | 82 | 1.7 | 1800.00 | 0.81 |
| HPS lamp (600 W) [2] | 25 | 1.8 | 250.00 | 0.28 |
| HPS lamp (1000 W) [2] | 25 | 2.0 | 445.00 | 0.22 |

[1] (OSRAM Opto Semiconductors, 2018) [2] (DH Licht GmbH, personal communication); [3] (UV Sytec GmbH, personal communication)

LEDs are more efficient in the emission of light compared to discharge lamps. However especially during winter periods in greenhouses, the lack of infrared radiation must be compensated by adjusting the temperature in the greenhouse (Ouzounis et al., 2018). Consequently, the calculated energy cost savings are reduced due to increased heating costs. Furthermore, because of the higher investment costs for LED fixtures compared to discharge lamps, the usage of LEDs in greenhouses cannot be economically (Nelson and Bugbee, 2014). However, nowadays with increasing light efficiency and lower costs, LEDs can be replace HPS systems even higher heating costs have to be taken in account (Ouzounis et al., 2018). Nevertheless Tab. 9.2 indicates that HPS lamps are still the cheapest light sources. Even if efficient LEDs are used that emit predominantly red light, it may take several years for the investment costs to pay off, which was not calculated in the previously mentioned study. Hybrid systems using a combination of LED and HPS light can be a solution to reduce the investment costs and save electricity energy in the greenhouse.

For horticultural industry the MPL emitting artificial sunlight is probably inappropriate due to high costs and low light efficiency. MPL from another distributor emitting white light

with a lower CRI (Tab. 9.2) are showing higher light efficiency and lower investment costs but they are very susceptible to failures. CDM also emitting broad white light (Fig. 5.1), however the light is not as continuous as the artificial sunlight from MPL. They are a better alternative technology for horticultural industry due to increased light yield and lower acquisition cost.

For plant research sun-like light conditions are often required. Especially in light experiments a standard sun-like light spectrum is useful for a better comparison of the results. LEDs are able to emit light with high CRI. However, the CRI is not the best indicator to compare the light quality with sunlight, because CRI only takes the color rendering of some colors in the visible light (380-780 nm) in account. LEDs which were referred to emit artificial sunlight are lacking in an emission of IR and their emission spectrum is not as continuous as sunlight (Mickens et al., 2019). Compared to other lamp systems, the MPL showed a much lower light yield, but they might be an valuable light source for experiments in greenhouse and climate chambers due to high similarity of their spectrum compared to sunlight (Fig. 5.1). Nevertheless, also the spectrum of sunlight is changing during the day, by weather conditions or latitude as shown in the introduction part. LEDs devices are better suitable to simulate such light situations since the MPL are only able to emit a fixed spectrum of light. Furthermore, an implementation of LED chips emitting long and near IR are required. In general, IR LED chips were not used due too low efficiency. However, with increasing efficiency, high power IR LED chips might be beneficial for horticultural industry to control the leaf temperature of the crops.

# 10. Outlook

This chapter gives an outlook how artificial light can be implemented in future cultivation systems. Besides higher plants also insects and micro algae are influenced by spectral light colors. In the end of this chapter the indoor controlled agriculture is briefly discussed with regard to sustainable and efficient production of horticultural crops.

## 10.1 Light as insect traps

The focus of this thesis lies on the direct reaction of plants to different light sources. However, spectral light quality also influences the orientation of insects. Insects are able to recognize UV, blue and green light but are also attracted to yellow light depending on the insect species (Shimoda and Honda, 2013). Insect pests like white flies, thrips or fungus gnats are generally captured by yellow and blue sticking cards to reduce the density of adolescent insects and monitor the occurrence of insect species. However, the attractiveness of the sticking cards to distinct species depends on the color of the card and brightness of the ambient light conditions (Johansen et al., 2011). Besides insect pests beneficial insects can be captured as well by the sticking cards. Approaches to trap insects with artificial light of LEDs are assumed to be more efficient and selective in capturing insect pests. It was shown that green (517 nm) LED traps were preferred compared to yellow sticking traps by white flies (Stukenberg et al., 2015). How the behavior of insect pests or beneficial insects like pollinators or natural enemies of insects change under different light sources could was not examined in this study. Intelligent pest management by the usage of artificial light might be an interesting approach for the future.

## 10.2 Micro algae- small factories for primary and secondary metabolites

Micro algae including photoautotroph organisms like *Chlorella*, *Spirulina* or *Haematococcus* are potential resources for pharmaceutical reagent and colorants for the food industry due to richness in carotenoids and other accessory pigments (Vuppaladadiyam et al., 2018). In closed cultivation systems, light from LEDs can be applied to increase the content of these secondary metabolites (Schulze et al., 2014). In *Haematococcus pluvilaes* it was observed that xanthophyll astaxanthin can be increased by violet-blue LEDs (380-470 nm) (Katsuda et al., 2004). Likewise, an increased light intensity has a positive effect

on the astaxanthin accumulation in *Chlorella zofingiensis* (Del Campo et al., 2004). Astaxantin has an economical importance as it is used as feeding additive for cultured salmon resulting in the typical pinkish color (Lorenz and Cysewski, 2000).
Spirulina is an autotroph bacterium producing phycocyanin which is used as blue colorant by the food industry. The accumulation of phycocyanin was induced by green light while red light led to increased biomass production of *Spirulina* (Prates et al., 2018).

## 10.3 Indoor controlled environment agriculture - the future of horticulture?

Feeding the world is going to be a major challenge in coming years, due to increasing urbanization, environmental pollution and high yield losses caused by the influence of climate change. The UN predicts that the population on our planet increases till year 2050 up to around 10 billion, whereby about two third of the humans will live in urban areas.
The production of crops in indoor controlled environments is one way to exclude outside environmental influences, enabling full controllable efficient plant factories.
In the early 90s, the MPL systems tested in this thesis were assumed to be an efficient light source for enclosed artificially-lighted plant growth factories (MacLennan et al., 1994). However, with the development of LEDs which are emitting no direct infrared radiation, plant cultivation in multilayer is possible leading to a new plant production system called “indoor vertical farms”. Indoor vertical farming is mainly applied for herbal plants, lettuce or microgreens due to their compactness (Beacham et al., 2019). Many indoor vertical farms are placed in Asian and North American high-density cities where high landscape pollution and urbanization impede field production of crops (Kalantari et al., 2018).
Indoor vertical farms are assumed to have the advantage to save water by integration of hydroponic or aeroponic systems. Hydroponic cultivation uses nutritional solution as growth media, while roots of plants in aeroponic systems are sprayed with mists of nutrient solutions (Al-Kodmany, 2018). However, also hydroponic systems are applied in greenhouses. In field conditions in Arizona, for 1 kg of lettuce about 250 L of water are required resulting in a yield of about 4 kg/m² per year. Applying hydroponic systems in a greenhouse, the yield is estimated up to around 40 kg/m² per year with a water usage of 20 L per kg (Barbosa et al., 2015). Due to efficient water recycling, the water use efficiency for vertical farming is even lower and is predicted to be close to 1 L per 1 kg (Kozai, 2013; Graamans et al., 2018). In

enclosed plant factories the usage of herbicides and pesticides is also reduced compared to field conditions (Benke and Tomkins, 2017). The fact, that vertical farms are integrated directly in urban structure shortens the transportation ways. With the multilayered approach, the yield of lettuces (kg/m² per year) in vertical plant factories is assumed to be around 2.3 higher in comparison to the yield of an efficient greenhouse in the Netherlands (Graamans et al., 2018). With special light recipes the cultivation of crops can be optimized leading to better taste by essential oils or increased leaf coloration by anthocyanins as shown in this thesis.

Besides the many advantages of indoor vertical farming, an often neglected fact is the high energy consumption due to the usage of artificial light which is required for indoor production of crops. (Graamans et al., 2018) estimated an electricity requirement of about 247 kWh for production of 1 kg lettuce (dry weight). Energy consumption for an efficient greenhouse in the Netherlands is much lower with about 70 kWh for the same amount of lettuce. Most of the energy in greenhouses is used for greenhouse heating. However, in higher latitudes artificial lighting becomes more necessary increasing the energy demands in greenhouses.

Additional to the high energy costs, the high investment costs for vertical facilities account for very high prices of horticulture products (Banerjee and Adenaeuer, 2013). Although costs for LEDs are decreasing and light technologies become more efficient, it is doubtful that such cultivation systems can be established in long term.

Vertical farms are only sustainable if regenerative energies are used. However, greenhouses using hydroponic systems and efficient lighting have the same advantages of a controllable and resource-saving cultivation of crops.

The sun is the best and most cost-effective light source we have available. Therefore, the cultivation of plants without sunlight in sun efficient seasons does not seem to be sustainable.

Overall cultivation systems of the future need further improvements of the yield, investment costs, land usage and resource consumption. New light systems have the potential to control light intensity and adjust the spectrum of light to fulfill the light demands of plants. That requires an increased application of sensors measuring the physiological status of the plants. This implement high technologized facilities producing the food of our future.

# 11. Conclusion

The light color is an important trigger for plant physiological processes. With artificial light the plant morphology and the content of secondary metabolites can be influenced in a targeted way. In this thesis, it was shown that, blue enriched environments result in the formation of sun-adapted leaves with a higher photosynthetic capacity, increased resistance against high irradiance and higher accumulation of secondary metabolites per leaf area. Blue light can influence the content of phenolic compounds directly. However, these responses are also dependent on species and cultivar.

The artificial sunlight from MPL resulted in increased elongation growth or lower degree of branching due to low R:FR ratio.

Furthermore, the results obtained in this this thesis indicate that that the impact of infrared radiation should not be neglected comparing different lamp systems.

Especially when LEDs are compared to discharge lamps an overestimation of light color can be a consequence since also infrared radiation is an influence factor for phenolic compounds and plant growth. The tested MPL, used in this thesis, might not be efficient enough for an implementation in horticultural industry. In comparison to discharge lamps, LEDs have a higher light efficiency. Furthermore, due to their flexible adjustment of light spectrum, LEDs are more suitable for future cultivation systems.

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# Appendix

## Appendix 1 - Standard curves

*Colorimetric determination of phenolic compounds*

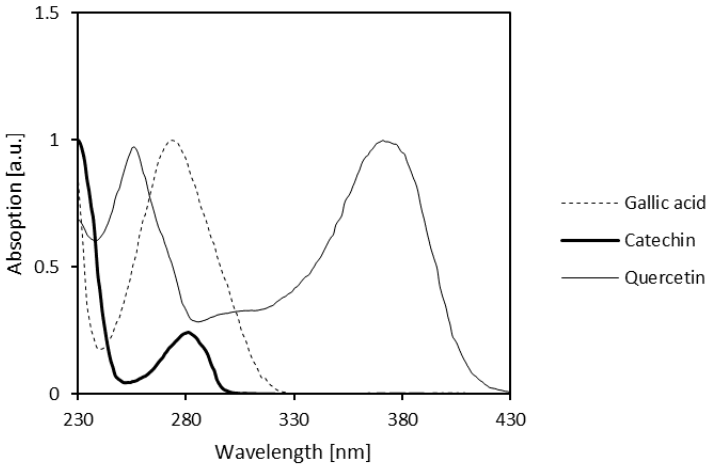


Fig. A1: Normalized absorption spectra of gallic acid, catechin and quercetin used as standard references for colorimetric methods. An UV translucent microplate was used (Eppendorf, Hamburg, Germany).

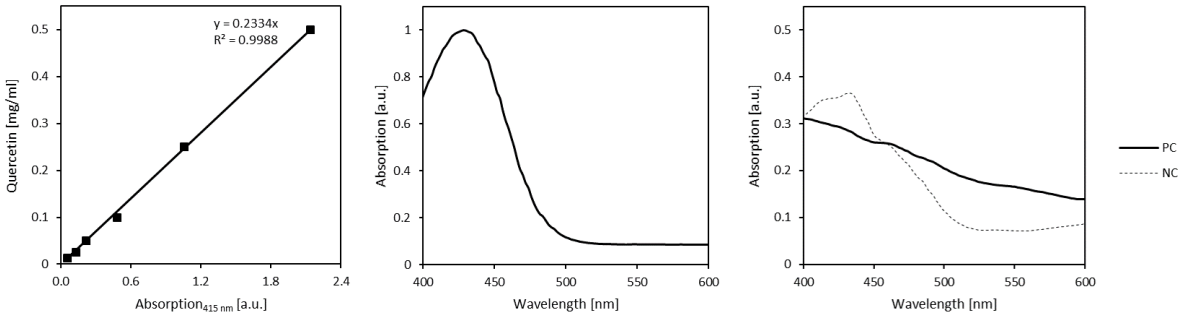


Fig. A2: $TFC_{415\ nm}$ standard curve and absorption spectra. A) Representative standard curve for quercetin standard used for total flavonoid method quantified at 415 nm. B) Normalized absorption spectrum of quercetin standard supplemented with $AlCl_3$ according to $TFC_{415\ nm}$ method described in Dörr et al. (2019) (chapter 5). C) Absorption spectra of positive (PC, $+AlCl_3$) and negative control (NC, $-AlCl_3$) of representative rose leaf extract.

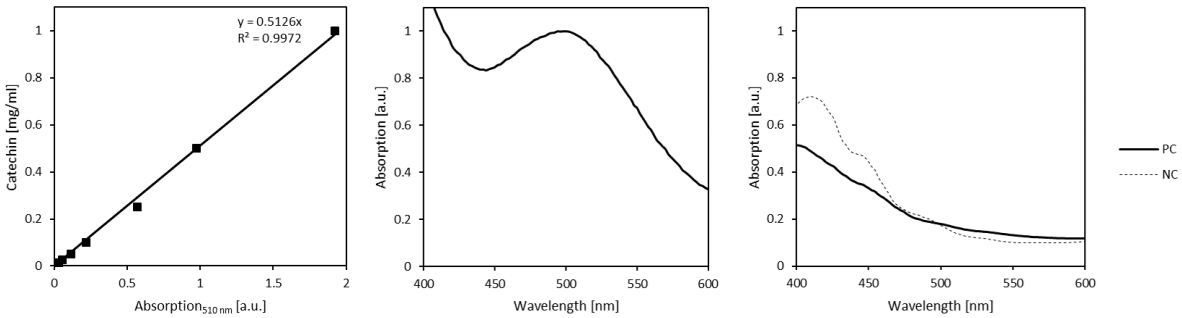


Fig. A3: $TFC_{510\ nm}$ standard curve and absorption spectra. A) Representative standard curve for catechin standard used for total flavonoid method quantified at 510 nm. B) Normalized absorption spectrum of quercetin standard supplemented with $AlCl_3$ according to $TFC_{510\ nm}$ method as described in Dörr et al. (2019) (chapter 5). C) Absorption spectra of positive (PC, +$AlCl_3$) and negative control (NC, -$AlCl_3$) of representative rose leaf extract.

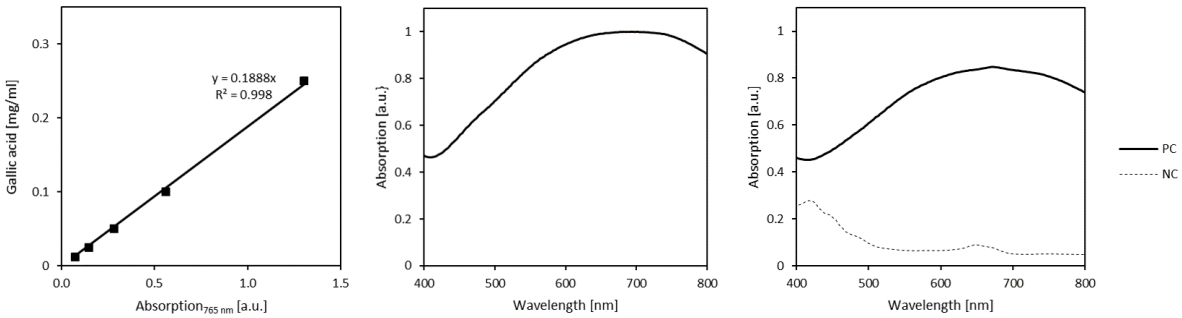


Fig. A4: TPC standard curve and absorption spectra. A) Representative standard curve for gallic acid standard used for total flavonoid method quantified at 765 nm. B) Normalized absorption spectrum of quercetin standard supplemented with FC-reagent according to TPC method as described in Dörr et al. (2019) (chapter 5). C) Absorption spectra of positive (PC, +F-C) and negative control (NC, -F-C) of representative rose leaf extract.

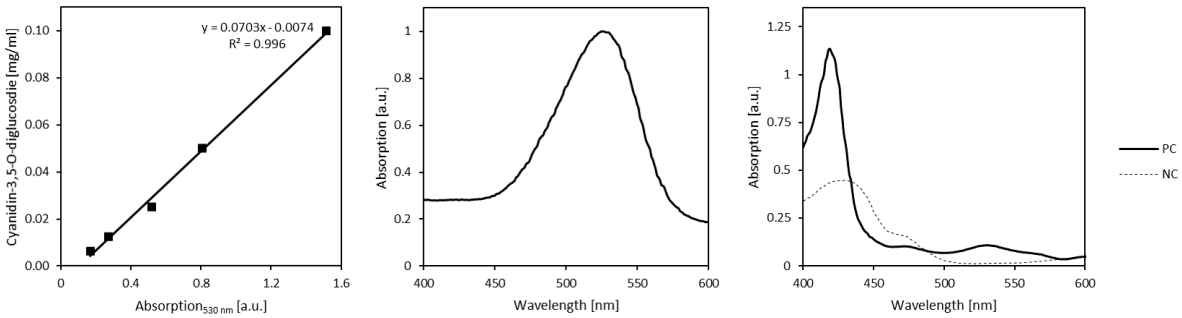


Fig. A5: TAC standard curve and absorption spectra. A) Representative standard curve for cyaniding-3,5-O-diglucoside standard used for total anthocyanin method quantified at 530 nm. B) Normalized absorption spectrum of cyaniding-3,5-O-diglucoside standard supplemented with HCl according to TAC method as described in Dörr et al. (2019) (chapter 5). C) Absorption spectra of positive (PC, +HCl) and negative control (NC, -HCl) of representative coleus leaf extract.

## *Standard curve for determination of soluble sugars*

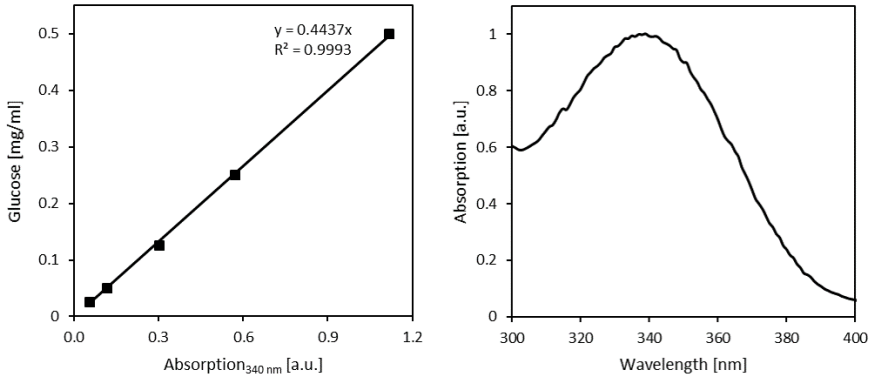


Fig. A6: Glucose standard curve and absorption spectra of NADPH. A) Representative standard curve for glucose standard used for determination of soluble sugars (chapter 8). Quantification occurred at 340 nm. B) Normalized absorption spectrum of NADPH according to GFS method (chapter 8).

## *Standard curve for quantitative determination of rosmarinic acid by HPLC*

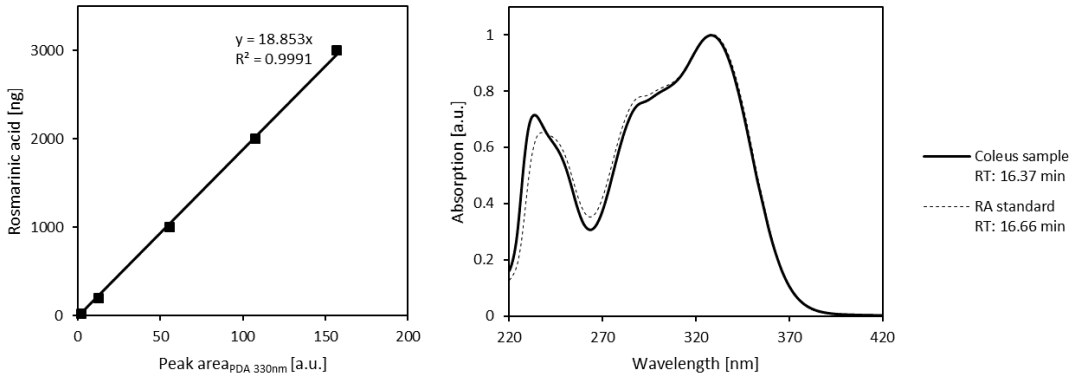


Fig. A7: Rosmarinic acid (RA) standard curve of quantitative HPLC analysis and normalized absorption spectra of RA standard and detected peak of representative coleus sample at PDA 330 nm. Quantitative HPLC method is described in Dörr et al. (2019) (chapter 5).

# Appendix 2 – Light distribution

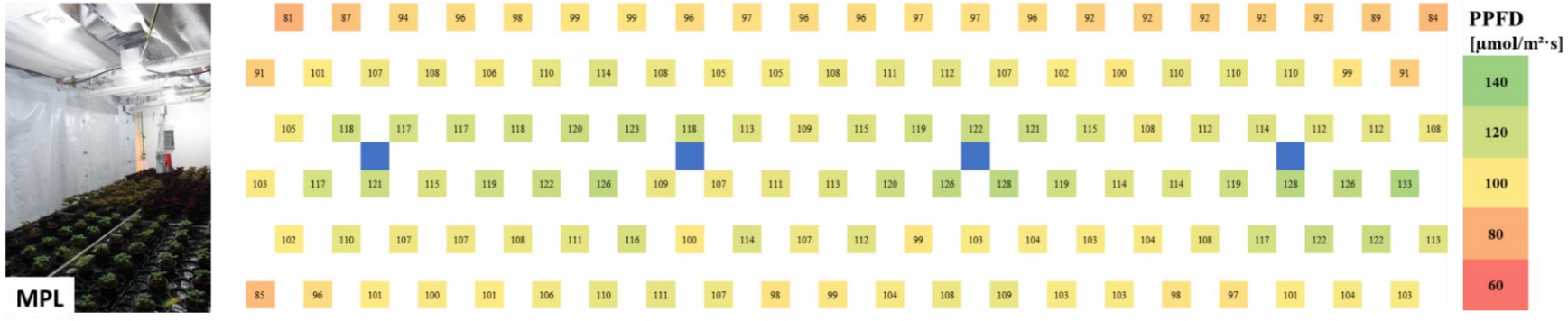


Fig. A8: Schematic light distribution diagram of climate chamber experiments using four MPL as light source. Light intensity (PPFD) was measured for each plant at a height of 10 cm above the pot edge with a quantum sensor (LI-190R, LI-COR, Lincoln, Nebraska, USA). The exposed area is approximately 7.5 m² (5x1.5 m). Blue spots indicate the approximate position of the lamp systems. Border areas are not displayed. Average light intensity for this area was 107±10 µmol/m²s.

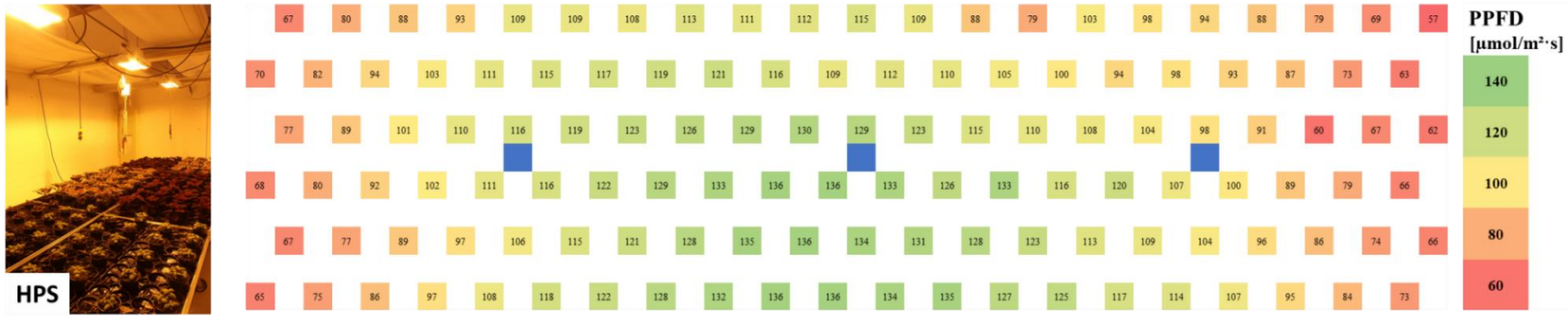


Fig. A9: Schematic light distribution diagram of climate chamber experiments using three HPS lamps as light source. Light intensity (PPFD) was measured for each plant at a height of 10 cm above the pot edge with a quantum sensor (LI-190R, LI-COR, Lincoln, Nebraska, USA). The exposed area is approximately 7.5 m² (5x1.5 m). Blue spots indicate the approximate position of the lamp systems. Border areas are not displayed. Average light intensity for this area was 104±21 µmol/m²s.

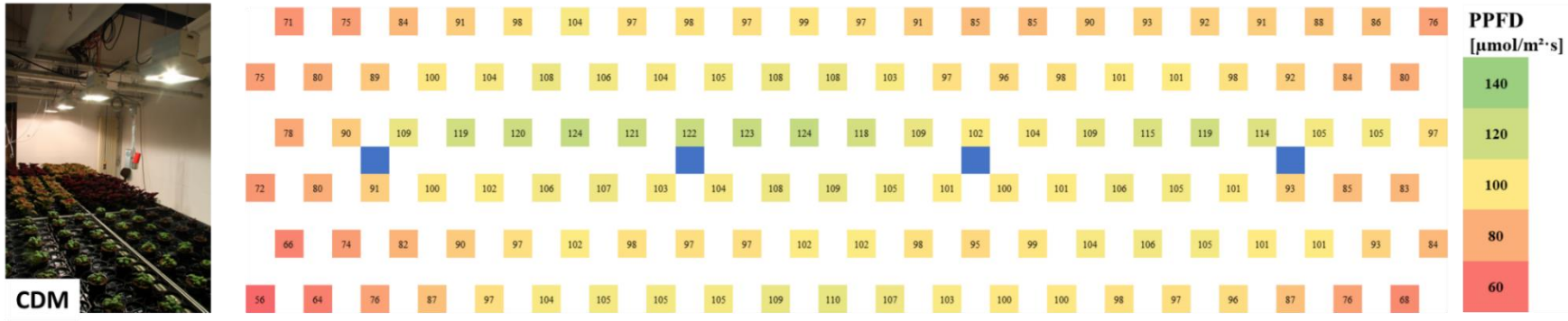


Fig. A10: Schematic light distribution diagram of climate chamber experiments using four CDM lamps as light source. Light intensity (PPFD) was measured for each plant at a height of 10 cm above the pot edge with a quantum sensor (LI-190R, LI-COR, Lincoln, Nebraska, USA). The exposed area is approximately 7.5 m² (5x1.5 m). Blue spots indicate the approximate position of the lamp systems. Border areas are not displayed. Average light intensity for this area was 97±13 µmol/m²s.

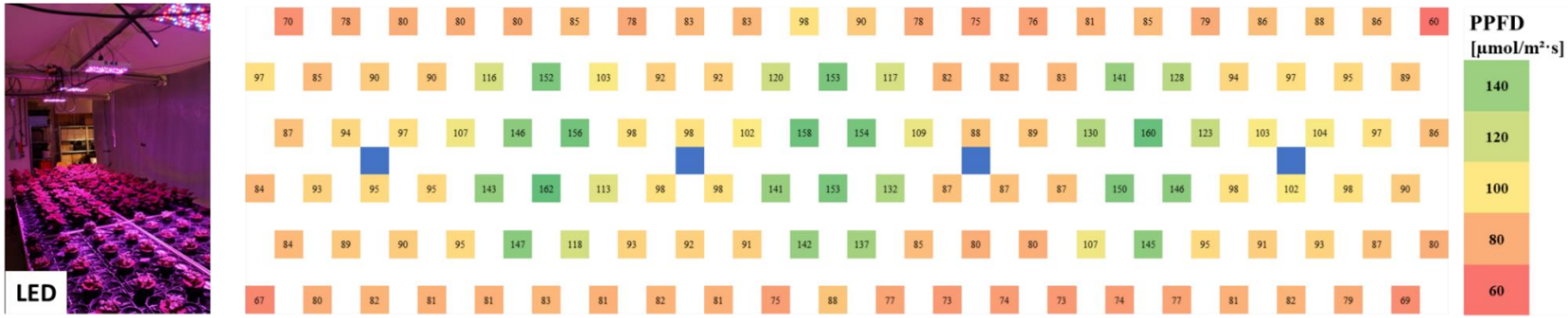


Fig. A11: Schematic light distribution diagram of climate chamber experiments using four LED lamps as light source. Light intensity (PPFD) was measured for each plant at a height of 10 cm above the pot edge with a quantum sensor (LI-190R, LI-COR, Lincoln, Nebraska, USA). The exposed area is approximately 7.5 m² (5x1.5 m). Blue spots indicate the approximate position of the lamp systems. Border areas are not displayed. Average light intensity for this area was 98±24 µmol/m²s.

# Declaration

I herewith declare that I have not previously participated in any doctoral examination procedure in a mathematics or natural science discipline.

*Frankfurt am Main, July 27, 2020*

Place, Date Oliver S. Dörr

Author's Declaration

I herewith declare that I have produced my doctoral dissertation on the topic of

"***Influence of artificial sunlight from a microwave plasma lamp on morphology and secondary metabolism of horticultural plants***"

independently and using only the tools indicated therein. In particular, all references borrowed from external sources are clearly acknowledged and identified. I confirm that I have respected the principles of good scientific practice and have not made use of the services of any commercial agency in respect of my doctorate.

*Frankfurt am Main, July 27, 2020*

Place, Date Oliver S. Dörr

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