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Investigations on the effect of light reduction on yield, growth, and secondary metabolites of lemon balm (*Melissa officinalis* L.)

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Abbreviations

4CL	4-coumarate-CoA ligase
a.i.	Active ingredient
AAPH	2,2'-azobis(2-amidinopropane) dihydrochloride
ABA	Abscisic acid
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
AUC	Area under the curve
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
C4H	*t*-cinnamic acid 4-hydroxylase
CAT	Catalase
DHAR	Dehydroascorbate reductase
DM	Dry matter
DMAPP	Dimethylallyl pyrophosphate
DOXP	1-deoxy-D-xylulose 5-phosphate
DPPH	Diphenyl-1-picrylhydrazyl
dt	Decitonne
DXP	1-deoxy-D-xylulose 5-phosphate
DXR	DXP reductoisomerase
DXS	DXP synthase
EC	Enzyme Commission number
EO	Essential oil
ET	Electron transfer
FC	Folin-Ciocalteu assay
FM	Fresh matter
FNR	Fachagentur Nachwachsende Rohstoffe (Agency for Renewable Resources)

FPP	Farnesyl pyrophosphate
FPPS	Farnesyl pyrophosphate synthase
FRAP	Ferric ion reducing antioxidant parameter
GAE	Gallic acid equivalents
GG	Gross-Gerau
GGPP	Geranylgeranyl pyrophosphate
GPP	Geranyl pyrophosphate
GPPS	Geranyl pyrophosphate synthase
GST	Glutathione S-transferase
ha	Hectare
HAT	Hydrogen atom transfer
HMGB1	High mobility group box-1
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HMGR	HMG-CoA reductase
HPLC	High-performance liquid chromatography
HPPR	Hydroxyphenylpyruvate reductase
HSV-1	Herpes simplex virus type 1
IPP	Isopentenyl pyrophosphate
ISO	International Organization for Standardization
K	Potassium
LAI	Leaf area index
LS	Linalool synthase
LS means	Least-squares means
MDAR	Monodehydroascorbate reductase
MEP	2-C-methyl-D-erythritol 4-phosphate
Mg	Magnesium
N	Nitrogen
n.a.	Not applicable, not available

n.d.	Not determined
ORAC	Oxygen radical absorbance capacity
P	Phosphorus
PAL	Phenylalanine ammonia lyase
PAR	Photosynthetically active radiation
PEP	Phosphoenolpyruvate
Ph. Eur.	European Pharmacopoeia
PPFD	Photosynthetic photon flux density
R:FR	Red:far-red ratio
RA	Rosmarinic acid
RAS	Rosmarinic acid synthase
RH	Rauischholzhausen
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
S	Sulfur
SAS	Shade avoidance syndrome
SOD	Superoxide dismutase
SPAD	Soil & Plant Analyzer Development
TAT	Tyrosine aminotransferase
TE	Trolox equivalents
TEAC	Trolox equivalent antioxidant capacity
TPC	Total phenolic content
TRAP	Total radical trapping antioxidant parameter
VDLUFA	Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten (Association of German Agricultural Analytic and Research Institutes)

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1 Introduction

Lemon balm (*Melissa officinalis*) is, due to its content of secondary metabolites, an important medicinal and aromatic plant in the Lamiaceae family. The typical lemon-like fragrance is caused by its content of essential oil, and makes it an appreciated flavoring ingredient for culinary uses. The dried leaves are also used for the preparation of herbal infusions, but especially important is the wide pharmaceutical use of lemon balm. It is, for instance, used internally for treating tenseness, restlessness and irritability, nervous sleeping disorders, or functional gastrointestinal complaints (Blumenthal et al., 2000, 1998). Externally it is used in the form of extracts, e.g. as the active ingredient in ointments against *Herpes labialis* (cold sores) (ESCOP, 2003; Koytchev et al., 1999). In Germany, it is licensed as a standard medicinal tea for sleep disorders and disorders of the gastrointestinal tract (BfArM, 2015).

The pharmaceutically used parts of the plant are the dried leaves (Melissae folium), and the corresponding requirements, as well as those for the dried extract (Melissae folii extractum siccum), are described in the European Pharmacopoeia (Ph. Eur. 7, 2011).

Two classes of secondary metabolites are of special interest in lemon balm: On the one hand the essential oil, a mixture of several mainly terpenoid lipophilic compounds, and on the other hand the phenolic compounds, such as phenolic acids and flavonoids. The main component among the phenolic substances in lemon balm is rosmarinic acid (Weitzel and Petersen, 2010). For meeting the requirements of the European Pharmacopoeia, dried lemon balm leaves have to contain a minimum of 1%, and lemon balm leaf dry extract a minimum of 2% rosmarinic acid (Ph. Eur. 7, 2011). Phenolic substances, mainly rosmarinic acid and flavonoids, also lead to a high antioxidant capacity of lemon balm.

In the human body, oxidative stress has been related with the development of several diseases (Aksenov et al., 2001; Cai et al., 2011; Reuter et al., 2010; Sayre et al., 2008). In food products, oxidative processes, especially the oxidation of fatty acids, lead to the deterioration of the product quality, and may even produce substances that are harmful for the health of the customers (Choe and Min, 2006). Therefore, synthetic antioxidants, like butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT), are used as food additives (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2012, 2011). However, many consumers prefer natural antioxidants to synthetic antioxidants.

For those reasons, natural antioxidants are of great interest for the food industry, for instance for replacing synthetic antioxidants in meat products (Berasategi et al., 2011;

Falowo et al., 2014; Fernandes et al., 2016; Lara et al., 2011), or even already as a feed supplement to influence the later meat quality (Kasapidou et al., 2014; Marcinčáková et al., 2011).

According to FNR (2014), there is a high demand for lemon balm in Germany. However, most of it is imported from other countries, although it can be cultivated under the given climate conditions. Therefore, increasing the cultivation area and productivity to produce high-quality lemon balm in Germany is of great interest.

Yield and quality of medicinal and aromatic plants can be influenced by genetic, phenological, and environmental factors, as well as by the cultivation management (Azizi et al., 2009; Mortensen, 2014; Mrlianová et al., 2002; Novak et al., 2000; Sellami et al., 2009). Therefore, it is important for quality assurance to better understand the factors that determine yield and quality of lemon balm.

A certain degree of variation between different genotypes in the content of secondary metabolites has been shown in several Lamiaceae plants. In *Ocimum basilicum*, for instance, the total phenolic content varied fivefold, and rosmarinic acid content even a hundredfold between the tested genotypes (Kwee and Niemeyer, 2011). A variation in the content of phenolic substances was also reported for other plants in the Lamiaceae family (Chizzola et al., 2008; Javanmardi et al., 2003; Kiferle et al., 2011; Lamien-Meda et al., 2010; Müller-Waldeck et al., 2010; Yan et al., 2016). Only few studies on the variation between different lemon balm genotypes are available, especially for plants grown under field conditions. Therefore it is of interest to compare different genotypes of lemon balm regarding their content of secondary metabolites and their antioxidant capacity in a field trial.

The development stage of a plant can have an influence on the content of essential oil (Sangwan et al., 2001). However, in investigations on different Lamiaceae plants, differing results regarding the stage with the highest content of essential oil have been reported (Ben Farhat et al., 2016; Mastelić and Jerković, 2003; Mirjalili et al., 2006; Nurzyńska-Wierdak et al., 2017; Tahmasebi et al., 2016). Also for the content of phenolic substances, such differences have been observed (Kiferle et al., 2011; Ozkan et al., 2010; Raudone et al., 2017; Vassão et al., 2006). Thus, further investigations are needed to gather information regarding changes in these quality-determining secondary metabolites in lemon balm.

In the cultivation of a diverse range of plants, especially in horticulture, viticulture and the cultivation of medicinal and aromatic plants, the use of different kinds of nets is getting more and more popular. Among others, they are utilized for the protection of plants from

insects, excessive sunlight, or adverse climatic conditions, as well as for the modification of their morphology, quality, and yield characteristics (Ben-Yakir et al., 2012, 2008; Castellano et al., 2008; Oren-Shamir et al., 2001). For the cultivation of medicinal and aromatic plants, protection nets have been proposed for the prevention of leafhopper infestations (Blum et al., 2011; Meyer et al., 2010). Besides, it has been found that the use of agro-textile coverings for the improvement of the microclimate led to higher essential oil contents in lemon balm cultivated under Swiss conditions (Carron et al., 2008). In Brazil, investigations on the influence of differently colored shading nets reported to some extent higher leaf yields of lemon balm plants cultivated under the nets (Brant et al., 2009; Oliveira et al., 2016). However, it remains an unanswered question how lemon balm plants react on shading nets under the temperate climate conditions in Germany.

As any kind of covering reduces the light intensity, this might impair the photosynthetic processes and thus the energy supply of the plants, and could therefore possibly have an impact on the yield. At the same time, plants receive far more sunlight than they can use for photosynthesis, even under temperate climate conditions, and can therefore experience light stress and photoinhibition (Long et al., 1994; Wilhelm and Selmar, 2011). Plants protect themselves from these harmful conditions by certain mechanisms, like photorespiration (Peterhansel et al., 2010), or the formation of secondary metabolites, such as phenolic compounds (Grace and Logan, 2000). However, these processes also divert precursors from the primary metabolism, are therefore costly for the plants, and might as a result affect the biomass yield (Gershenzon, 1994; Logemann et al., 2000; Walker et al., 2016; Wilhelm and Selmar, 2011). On the other hand, several secondary metabolites, such as phenolic compounds and essential oil, are the valuable components of medicinal and aromatic plants like lemon balm. Too much solar radiation, however, might also increase the loss of the volatile substances that constitute the essential oil. It is therefore of interest to identify suitable cultivation conditions that lead to an improved leaf yield, without impairing the content of valuable secondary metabolites.

Especially the influence of light intensity on phenolic substances, essential oil content and yield parameters in *Melissa officinalis* cultivated in Germany has not been reported. Therefore, it was the aim of the current study to clarify the effect of shading on yield and quality parameters (like the contents of essential oil, total phenolics, and rosmarinic acid) of lemon balm under field conditions in Germany.

Investigations took place in two sites with different soil and climatic conditions, in plant stands observed over a common cultivation period of three years. It was further of interest whether different genotypes react in different ways, and if there are interactions with the development stages of the plants.

Thus, the following questions were addressed in this investigation:

- What is the crop yield potential of different lemon balm genotypes at two locations in Germany with different soil and climate conditions?
- Does the rosmarinic acid content of the tested genotypes meet the requirements of the European Pharmacopoeia under the chosen cultivation conditions?
- Do the tested lemon balm genotypes differ in yield and quality?
- How do yield and quality change at different harvest stages?
- How are yield and quality parameters of lemon balm plants influenced when cultivated under nets with a moderate or a strong light reduction?
- Are there interaction effects between the tested factors genotype, harvest stage, and light reduction?

2 Literature review

2.1 Botanical characterization of lemon balm (*Melissa officinalis* L.)

Lemon balm (*Melissa officinalis* L.) is a perennial plant in the Lamiaceae family, classified in the subfamily Nepetoideae, the tribe Mentheae, and the subtribe Salviinae (Bomme et al., 2013; Moon et al., 2008). Its name is derived from the Greek word μέλι (honey) or μέλισσα (honeybee), as it is said to attract honeybees (Burgett, 1980). It is assumed to be originating from an area between the Mediterranean region and the western Tien Shan (Hanelt and IPK, 2001), but is nowadays widely cultivated not only under subtropical, but also under temperate conditions, as in Germany (Bomme et al., 2013). Wild populations of *Melissa officinalis* can be found in different habitats, ranging from moist temperate forest regions to dry mountain steppe habitats (Abrahamyan et al., 2015). With an Ellenberg value (Hill et al., 2004) / Ellenberg-Pignatti value (Vitasović Kosić et al., 2017) of 6, lemon balm can therefore be described as a plant of a habitat between semi-shade and well lit places.

Three subspecies of *Melissa officinalis* have been described, namely ssp. *officinalis*, ssp. *altissima*, and ssp. *inodora* (Bomme et al., 2013). However, only ssp. *officinalis* is the pharmaceutically used lemon balm (Hänsel et al., 1993).

Lemon balm has been described as a diploid species, with a chromosome number of 2n = 32 (Bomme et al., 2013). However, recent investigations found further ploidy levels among the tested accessions, namely triploid (2n = 3× = 48 chromosomes) as well as tetraploid (2n = 4× = 64 chromosomes) types (Kittler et al., 2015).

Lemon balm is a perennial plant, which is normally cultivated for two to three years, and can typically reach plant heights of about 50–90 cm (Bomme et al., 2013). The stem of the plants is quadrangular, carrying the leaves in decussate position (**Fig. 1**). The leaves can reach a length of about 5–9 cm, and a width of about 3–6 cm (Bomme et al., 2013). They are petiolate, broadly ovate to almost cordate in the vegetative stage, with crenate or serrate margins, and have a distinct venation on the lower surface, giving them an embossed appearance (Wichtl, 2002). During the further development of the plant, the leaf form changes. Flowering shoots show leaves with pointed ends, and a reduced leaf size (Bomme et al., 2013). The surface of the leaves is more or less pubescent, with hairs being visible mainly on the adaxial side of the leaves, and only to a lesser extent on the abaxial side (Wichtl, 2002). Both glandular (essential oil producing) as well as non-glandular trichomes have been described for lemon balm (Chwil et al., 2016; Moon et al., 2009).

The flowers, which are of pale color, have a two-lobed calyx, and are grouped in the axils of the leaves (Wichtl, 2002) (**Fig. 2**). The bicarpellate, superior ovary with a false septum and two ovules per carpel develops into four nutlets of around 1.5–2 mm, with an average thousand seed weight of around 0.6 g (Bomme et al., 2013).

For germination and a fast development of the seedling, a minimum temperature of 18 °C is required, and optimal growth temperature for the plant is 20–30 °C (Bomme et al., 2013).

The aerial parts of the plants die back in winter, and new shoots will regrow from the roots in spring (Bomme et al., 2013). While the root system is quite frost tolerant, late spring frost can severely damage the plants once the regrowth of the new shoots has started (Bomme et al., 2013).

Among the different genotypes, two main growth types can be distinguished: An upright growth type, with erect plants already in the first year, and a procumbent growth type, with the plants growing along the ground in the first year. However, also intermediate types exist. All types will have an upright growth in the second year or after vernalization (Bomme et al., 2013).

Fig. 1: Lemon balm plant in vegetative stage. The typical quadrangular stem carries the leaves in decussate position (own photo).

Fig. 2: Flowering lemon balm plant. The pale-colored flowers are grouped in the axils of the leaves (own photo).

2.2 Cultivation of lemon balm

Lemon balm needs adequate soil conditions, and an appropriate supply with water and nutrients, especially nitrogen, because of the high biomass production (Bomme et al., 2013). An appropriate soil should consist of sandy and loamy fractions, be rich in humus, and warm up easily. It is important that no waterlogging occurs. A pH value of the soil between 5 and 7 is recommended (Bomme et al., 2013).

Because of the origin in the Mediterranean region, lemon balm prefers warmer temperatures, but can also be grown in a temperate climate. However, severe winter losses may occur, especially in the case of a lacking snow cover. Lemon balm plants seem to be especially prone to winter killing in the first winter, and different genotypes seem to exhibit differing degrees of frost tolerance. Thus, especially in regions with low winter temperatures, the choice of proper genotypes is important (Bomme, 2001).

Before the establishment of the crop, the soil needs to be loosened up appropriately. Especially ploughing may also help to reduce weed pressure. Cultivators and harrows may be employed to reach a finer soil texture. A fine-crumbled soil is especially important for direct sowing of lemon balm. The usage of harrows is recommended as a means of preventive weed control (Bomme, 2001). An appropriate weed control is of great importance to reach a good quality of the harvested plant material.

Plant stands of lemon balm are normally cultivated for two to three years (Bomme et al., 2013). Included in a crop rotation, lemon balm can therefore prevent soil erosion and leaching of nutrients. Suitable pre-crops are legumes, potatoes, or cereals. As a subsequent crop, cereals are recommended, which facilitate the control of possible volunteers by herbicides. No plants from the Lamiaceae family should be cultivated before or after lemon balm for four to five years to prevent the spreading of diseases or pests (Bomme, 2001).

A lemon balm plant stand may be established either by direct sowing or by planting of young plantlets. If direct sowing is chosen, a good seedbed preparation is essential. The seeds are quite fine, with a thousand grain weight of around 0.6 g. They must therefore not be placed deeper than 0.5 cm in the ground. To ensure a good germination of the seeds, direct sowing has to be performed when air and especially soil temperature reach at least 18 °C, which means not earlier than May or June in a temperate climate. However, drying out of the germinating seeds has to be prevented. Heavy rainfall in this period can negatively affect the success of direct sowing (Bomme et al., 2013). Because of the difficulties with direct sowing, lemon balm is normally sown under greenhouse conditions. Alternatively to sowing, the use of cuttings may be performed as a means of

plant propagation in the greenhouse. The pre-grown plantlets will later be transferred to the field, with a density of 64.000–80.000 plants/ha (Bomme, 2001).

Although lemon balm plants can withstand a certain degree of drought stress, an appropriate water supply is important to reach good yields (Bomme et al., 2013). Irrigation is of special importance after planting the young plantlets to the field to ensure taking root of the plants, after each cut to help the plants to regrow quickly, as well as generally in the case of longer dry periods (Bomme, 2001).

Due to the high biomass production, an appropriate nutrient supply is essential. The amount of fertilizer should be based on the harvested biomass as well as an analysis of the soil. The nutrient removal for 100 dt fresh matter biomass has been estimated to be 49 kg N, 14 kg P_2O_5, 76 kg K_2O, 9 kg MgO, and 19 kg CaO (Bomme, 2001). Especially the application of N fertilizer should be staggered over the growing season. The first dose should be applied as a basal dressing at the start of the growth phase in spring. After each harvest, an additional dose of N fertilizer is needed. Although an application of organic fertilizer during the cultivation of the pre-crop is regarded as positive for lemon balm, no farmyard manure or liquid manure should be applied on lemon balm due to its bioburden (Bomme, 2001).

During the cultivation of lemon balm, an appropriate crop protection has to be kept in mind. If chemical plant protection agents are used, however, this needs to be cleared with the purchaser of the produced plant material. Weed control should already be started in the pre-crop. Also before sowing or planting, as well as during the growing period, weed should be controlled mechanically or by the use of appropriate, approved herbicides. However, lemon balm may react quite sensitively on the use of herbicides. Among fungal diseases, *Septoria melissae* is quite meaningful, furthermore *Puccinia menthae* and *Neoerysiphe galeopsidis* might occur. A typical insect pest are leafhoppers, mainly *Eupteryx* sp., that damage the leaves by sucking the plant sap and therefore lead to a deterioration of the quality of the harvested plant material (Bomme et al., 2013).

Lemon balm can be harvested several times a year. In the establishing year, up to two harvest cuts may be possible, whereas in the following years two to four cuts might be achieved. Typically, harvest takes place before flowering. If the cutting of the plants is performed too late, the lower leaves start yellowing, therefore decreasing the quality of the product, or might even fall off completely. Additionally, the leaf:stem ratio changes disadvantageously. Cutting height should be around 10 cm above ground, as regrowth might be impaired if cutting is performed too low. If the harvest takes place in a dry period, adequate watering is necessary for a successful regrowth. The last cut must not be

performed too late in the year, as winter hardiness would be reduced. The time frame for harvest is around mid July to end of August as well as September for the two cuts in the first year, and in the following years end of May to beginning of June, mid July, as well as August. Harvesting can be performed with combine harvesters or mowers. Plants are then chopped as fresh plant material after harvest and then undergo a winnowing process to separate the leaves from the stems, and only the leaves are dried. An alternative is to dry the whole harvested plants, and to cut and winnow them after drying. Whichever of the two methods is chosen, a fast but gentle drying (at not more than 40 °C) of the plant material to a target moisture content of 8 to 10% is important to reach a good quality of the final product (Bomme, 2001; Bomme et al., 2013).

2.3 Yield characteristics of lemon balm

For field trials under German climate conditions, biomass yields of 160–350 dt FM (fresh matter)/ha in the first year and 180–450 dt FM/ha for the following years have been described, and leaf yields reached 90–230 dt FM/ha (19–40 dt/ha dried leaves) in the first year and 100–250 dt FM/ha (20–45 dt/ha dried leaves) in the following years (Bomme et al., 2013).

In investigations under Polish climate conditions, biomass yields of 93 dt FM/ha in the first year and 73 dt FM/ha in the second year were described, with a yield of air-dried leaves of 16 dt/ha and 14 dt/ha, respectively (Dzida et al., 2015). In another Polish study, biomass yields at four organic and two conventional farms were investigated over three consecutive years. Averaged annual biomass yields were stated as 44.9 to 241.6 dt FM/ha (9.3 to 55.1 dt/ha dried biomass) at the organic farms, and 81.9 to 149.6 dt FM/ha (29.1 to 13.8 dt/ha dried biomass) at the conventional farms (Seidler-Łożykowska et al., 2015). In a Romanian study with six different lemon balm accessions, biomass yields of 40.4 to 107.7 dt FM/ha in the first year, 216.2 to 288.6 dt FM/ha in the second year, as well as 527.9 to 611.4 dt FM/ha in the third year have been obtained (Marian, 2012). In an investigation under Slovak climate conditions, a biomass yield of 55.6 dt/ha dried plant material as well as a leaf yield of 21.1 dt/ha dried plant material was found in a three-year-old plant stand (Mrlianová et al., 2002). Under the climate conditions of Northern India, biomass yields of 149.0 dt FM/ha (32.8 dt/ha dried plant material) were obtained 180 days after planting (Singh et al., 2014). In a Turkish investigation, yields of 48.2 and 75.8 dt/ha dried biomass as well as 30.0 and 46.0 dt/ha dried leaves were obtained in the first and second year, respectively (Saglam et al., 2004).

Biomass and dry matter (DM) leaf yield of the first cut have been described to be higher than for the second cut (Bomme et al., 2013; Özgüven et al., 1999). In investigations of Özgüven et al. (1999) on the yield parameters of different lemon balm accessions with two to three harvests per year, the highest biomass and leaf yields were almost always found for the first harvest of the year. Under German conditions, up to three cuts within a year have been described, with a tendency of decreasing leaf yields from harvest to harvest (Bomme, 2001).

The percentage of leaves from the harvested biomass has been described as 44–52% (equalling a leaf:stem ratio of 0.79–1.08) for the first cut, and up to 68% (equalling a leaf:stem ratio of 2.13) for the second cut (Bomme et al., 2013).

2.4 Requirements of the European Pharmacopoeia

In the European Pharmacopoeia (Ph. Eur. 7, 2011), the drug Melissae folium (lemon balm leaves) is described as the dried leaves of *Melissa officinalis*. A minimal rosmarinic acid content of 1.0% in the dried drug is requested. The drug needs to have a lemon-like odor. However, no requirements for a minimal content of essential oil are described. Testing for identity has to take place macroscopically, microscopically, and with thin layer chromatography, with citronellal and citral (consisting of neral and geranial) as reference substances. The requirements regarding drug purity are a maximum of 10% stalks with a diameter above 1 mm, and a maximum of 2% impurities. Loss on drying must not exceed 10%, and ash content may reach at most 12%. The determination of rosmarinic acid content needs to be performed with an HPLC (high-performance liquid chromatography) method. In the past, a photometric had been used, with a minimal requirement of 4% hydroxycinnamic acids, calculated as rosmarinic acid (Krüger et al., 2010), which has to be kept in mind when comparing rosmarinic acid contents obtained with these different methods.

2.5 Secondary metabolites of lemon balm

The term "secondary metabolites" covers a wide variety of different substances, among others phenolics, terpenes, glucosinolates, and alkaloids (Bennett and Wallsgrove, 1994). More than 200,000 of these structures have been found (Bresinsky et al., 2013). They play important roles in plants, as they enable them to adapt to adverse environmental conditions (Behnke et al., 2007; Delfine et al., 2000; Loreto et al., 1998; Oh et al., 2009; Ramakrishna and Ravishankar, 2011; Sharkey et al., 2001), protect them from herbivores or pathogens (Bennett and Wallsgrove, 1994; Bleeker et al., 2009; De Moraes et al., 2001; Kang et al., 2010; Kessler and Baldwin, 2001; Ramakrishna and Ravishankar, 2011) as well as from ozone (Jud et al., 2016; S. Li et al., 2018), and attract pollinators (Harborne, 2001) as well as the predators of herbivores attacking the plants (Dicke et al., 1990; Turlings et al., 1995, 1990), thus even exerting an indirect herbivore defense.

The secondary metabolism by which they are synthesized is connected with the primary metabolism, from which it diverts its respective precursors (Logemann et al., 2000). As a result, their production leads to a certain cost for the plants (Gershenzon, 1994; Sharkey and Yeh, 2001). While vascular plants share similarities in their primary metabolism, they differ in the profile of secondary metabolites. Therefore, secondary metabolites are also used as chemotaxonomic markers (Bennett and Wallsgrove, 1994; Bourgaud et al., 2001).

Three groups of secondary metabolites have been described in lemon balm (Awad et al., 2009; Hänsel et al., 1993):

1. Phenolic compounds, like flavonoids and phenolic acids

2. Essential oil, mainly consisting of mono- and sesquiterpenes

3. Pentacyclic triterpenoids (ursolic acid, oleanolic acid)

The first two groups will be further described in the following, whereas the pentacyclic triterpenoids, which have not been analyzed in this project, are mentioned only for the sake of completeness.

2.5.1 Phenolic compounds

Phenolic compounds are different substances that derive their name from phenol (**Fig. 3**), a molecule with an aromatic ring and a hydroxy group, which they share as a strucural element within their molecule. The term "polyphenol" is commonly used for substances with more than one phenolic ring as part of their molecule (Cheynier et al., 2013).

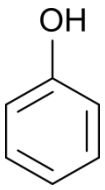

Fig. 3: Structural formula of phenol (according to Schirmeister et al., 2016). Phenolic substances share this structure as a part of their molecules.

Among the phenolic compounds in lemon balm, rosmarinic acid (RA) is accumulated in the highest quantities (Weitzel and Petersen, 2010). The RA content of lemon balm typically reaches several percent in the dried plant material (**Tab. 1**). Besides RA, further phenolic acids have been found in lemon balm, like caffeic, protocatechuic, chlorogenic, *m*-coumaric, *p*-coumaric, gallic, gentisic, ferulic, and *p*-hydroxybenzoic acid (Dastmalchi et al., 2008; Lin et al., 2012; Proestos et al., 2005; Žiaková et al., 2003). Several flavonoids are contained in lemon balm, especially luteolin glycosides (Patora and Klimek, 2002), among which luteolin 3'-O-β-D-glucuronide has been described as the main flavonoid in this plant (Heitz et al., 2000).

RA (**Fig. 4**) is a phenolic substance that is typically found in members of the subfamily Nepetoideae within the Lamiaceae family (Janicsák et al., 1999), and therefore also in lemon balm. It is an ester consisting of caffeic acid and 3,4-dihydroxyphenyllactic acid (Petersen and Simmonds, 2003). It is stored in the vacuoles of the cells (Häusler et al., 1993). RA is regarded an important active constituent of lemon balm. The European Pharmacopoeia therefore requires a minimal RA content of 1.0% in the dried drug (Ph. Eur. 7, 2011).

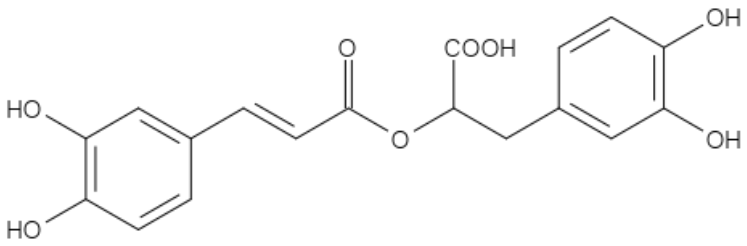

Fig. 4: Structural formula of rosmarinic acid (according to Petersen and Simmonds, 2003). Chemically, it is regarded as an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid.

Tab. 1: Rosmarinic acid (RA) contents of lemon balm (*Melissa officinalis*) from literature.

RA [%] (*)	Treatment	Genotype	Extraction	Experiment type	Literature
4.1%	–	Unknown	Hot water (herbal tea)	Polyphenols in lemon balm tea	(Carnat et al., 1998)
3.24%	–	Unknown	Hot water	Analysis of lemon balm	(Ieri et al., 2017)
0.24 – 2.31%	–	Unknown	Hot water (herbal tea)	Bulgarian commercial samples	(Petkova et al., 2017)
2.74%	–	Unknown	Aqueous ethanol	HPLC method development	(Wang et al., 2004)
3.7% a 5.6% b 5.7% b	Convective drying Vacuum drying Freeze drying	'Citronella'	Aqueous ethanol	Different drying methods (plant material from organic farm)	(Argyropoulos and Müller, 2014)
6.63% a 5.97% b	With arbuscular mycorrhiza Without arbuscular mycorrhiza	'Relax'	Acidified aqueous methanol	Effect of arbuscular mycorrhiza (pot experiment)	(Engel et al., 2016)
3.65%	–	Unknown	Acidified aqueous methanol/ 2-propanol	RA contents in several Iranian Lamiaceae species	(Shekarchi et al., 2012)
3.50% a 3.91% a	Before flowering Full flowering	'Citra'	Methanol	Development stage (field experiment)	(Tóth, et al., 2003)
3.12% a 3.59% a 3.83% a 3.17% a	Control Stepwise water deficit Sharply increased water deficit Permanent water deficit	'Soroksár'	Methanol	Water deficit (pot experiment; growth chamber)	(Radácsi et al., 2016)
2.87% ab 2.43% a 3.01% ab 2.72% b 2.75% b	'Gold Leaf' 'Lemona' 'Lorelei' 'Quedlinburger Niederliegende' 'Soroksár'		Methanol	Genotype comparison (pot experiment)	(Szabó et al., 2016)

(*) Values partly converted into % from differently stated units in the original articles.
Different letters indicate significant differences between the treatments within the original experiment.

RA is known for its antioxidant properties (Adomako-Bonsu et al., 2017; Fadel et al., 2011; Nakamura et al., 1998; Soobrattee et al., 2005). The *ortho*-dihydroxy structure has been suggested to be especially important for the antioxidant activity of RA (Adomako-Bonsu et al., 2017; Woo and Piao, 2004). The antioxidant properties make plant extracts rich in rosmarinic acid interesting for the food industry, as they can inhibit lipid oxidation and therefore extend the shelf life of food products (de Ciriano et al., 2010; Şahin et al., 2017; Sánchez-Escalante et al., 2003). RA is able to be incorporated into lipid membranes, where it can exert its antioxidant potential (Fadel et al., 2011).

Several of the health-related properties of lemon balm can be ascribed to its content of RA. Several effects of RA have been demonstrated, for instance, its **antiviral** activity. In an *in vitro* assay, RA showed a virucidal activity on herpes simplex virus type 1 (HSV-1) (Astani et al., 2012). In a cell model, lemon balm extract and RA inhibited the attachment of HSV-1 to host cells as well as their penetration (Astani et al., 2014, 2012). Human immunodeficiency virus type 1 (HIV-1) integrase, an enzyme that is essential for the replication of the virus, was inhibited *in vitro* by RA (Tewtrakul et al., 2003). Antiviral and anti-inflammatory effects of RA were also shown in an experimental murine model of Japanese encephalitis, where a reduced viral replication was observed (Swarup et al., 2007). The antiviral effects of a methanolic lemon balm extract on enterovirus 71 *in vitro* and in a mouse model were also attributed to rosmarinic acid (Chen et al., 2017). As a practical application, the antiviral activity of lemon balm extracts is used in the form of a cream against *Herpes labialis* (Koytchev et al., 1999).

Neuroprotective effects of RA were shown in *in vitro* models of neuronal death (Fallarini et al., 2009). Human dopaminergic neuronal cells were protected by RA under oxidative stress conditions (H. J. Lee et al., 2008). In an *in vitro* study, RA protected cells from amyloid-β peptide-induced neurotoxicity (Iuvone et al., 2006). In a transgenic mouse model for Alzheimer's disease, amyloid-β deposition in the brain was significantly decreased by RA (Hamaguchi et al., 2009).

Antiinflammatory activities of RA were shown in several studies. In an *in vitro* investigation with rat platelets and polymorphonuclear leukocytes, RA inhibited the activities of 12-lipoxygenase and 5-lipoxygenase (Yamamoto et al., 1998). The release of HMGB1 was inhibited in primary human umbilical vein endothelial cells, and the inflammatory responses dependent on HMGB1 were down-regulated (Yang et al., 2013). In the mouse ear edema model, antiinflammatory activities of RA, such as inhibition of adhesion molecule, chemokine and eicosanoid synthesis, were observed (Osakabe et al., 2004). Antiinflammatory effects were also shown in rat models of local and systemic inflammation (Rocha et al., 2015), as well as in a mouse model infected with Japanese

encephalitis virus (Swarup et al., 2007). In a mouse cecal ligation and puncture model, the release of HMGB1 was markedly decreased, as was the sepsis-related mortality (Yang et al., 2013). An antiinflammatory effect was also observed in lipopolysaccharide-induced mastitis in mice (Jiang et al., 2017). Also in a human study, antiinflammatory effects of RA were observed. In persons suffering from seasonal allergic rhinoconjunctivitis, several symptoms were improved after oral supplementation with RA, and the numbers of neutrophils and eosinophils in nasal lavage fluid were significantly decreased (Osakabe et al., 2004). Because of its antiinflammatory properties, RA has also been proposed for the treatment of atopic dermatitis (J. Lee et al., 2008).

Antiangiogenic properties of RA were shown in an *in vitro* model with human umbilical vein endothelial cells, where RA inhibited several steps that are important for angiogenesis (Huang and Zheng, 2006), as well as in a human retinal endothelial cell model, and in a mouse model of retinopathy (Kim et al., 2009). In studies with mouse models, **antimutagenic** effects of RA were shown (De Oliveira et al., 2012; Furtado et al., 2008). **Nephroprotective** properties have been attributed to RA according to investigations in cell and mouse models (Domitrović et al., 2014; Makino et al., 2000). Studies on cell, mouse, and rat models showed **hepatoprotective** properties of RA (Domitrović et al., 2013; Li et al., 2010; Osakabe et al., 2002). RA may also exert a **photoprotective** activity, as it increased the melanin content and tyrosinase expression in a murine cell model (Lee et al., 2007), and protected human keratinocytes from the harmful effects of UV-A radiation (Psotova et al., 2006).

The biosynthesis of phenolic compounds is based on precursors from the primary metabolism. In the shikimate pathway, the primary metabolites phosphoenolpyruvate (PEP) and erythrose-4-phosphate are used for the synthesis of chorismate (Herrmann, 1995), which is further metabolized to arogenate, the precursor for the formation of the aromatic amino acids L-phenylalanine and L-tyrosine (Schmid and Amrhein, 1995). These two aromatic amino acids are needed for the formation of rosmarinic acid (**Fig. 5**), as well as for other phenolic substances (Weitzel and Petersen, 2010).

The first step of the phenylpropanoid pathway starts with the transformation of L-phenylalanine by the enzyme phenylalanine ammonia lyase (**PAL**; EC 4.3.1.24) into *t*-cinnamic acid, which is then further hydroxylated to 4-coumaric acid by the enzyme *t*-cinnamic acid 4-hydroxylase (**C4H**; EC 1.14.13.11). In the following step, an activation is accomplished by the enzyme 4-coumarate-CoA ligase (**4CL**; EC 6.2.1.12) to form 4-coumaroyl-CoA, an intermediate that is a precursor not only for rosmarinic acid, but also for other phenolic substances, like flavonoids. For the biosynthesis of rosmarinic acid, in a parallel biosynthetic route L-tyrosine is converted to 4-hydroxyphenylpyruvic acid by

tyrosine aminotransferase (**TAT**; EC 2.6.1.5), and then to 4-hydroxyphenyllactic acid by hydroxyphenylpyruvate reductase (**HPPR**; EC 1.1.1.237). By the enzyme rosmarinic acid synthase (**RAS**; EC 2.3.1.140), an ester is formed between the two precursors 4-coumaroyl-CoA and 4-hydroxyphenyllactic acid, resulting in 4-coumaroyl-4'-hydroxyphenyllactic acid. In the following enzymatically catalyzed steps, hydroxylation at positions 3 and 3' occurs to ultimately form rosmarinic acid (**Fig. 5**) (Weitzel and Petersen, 2010).

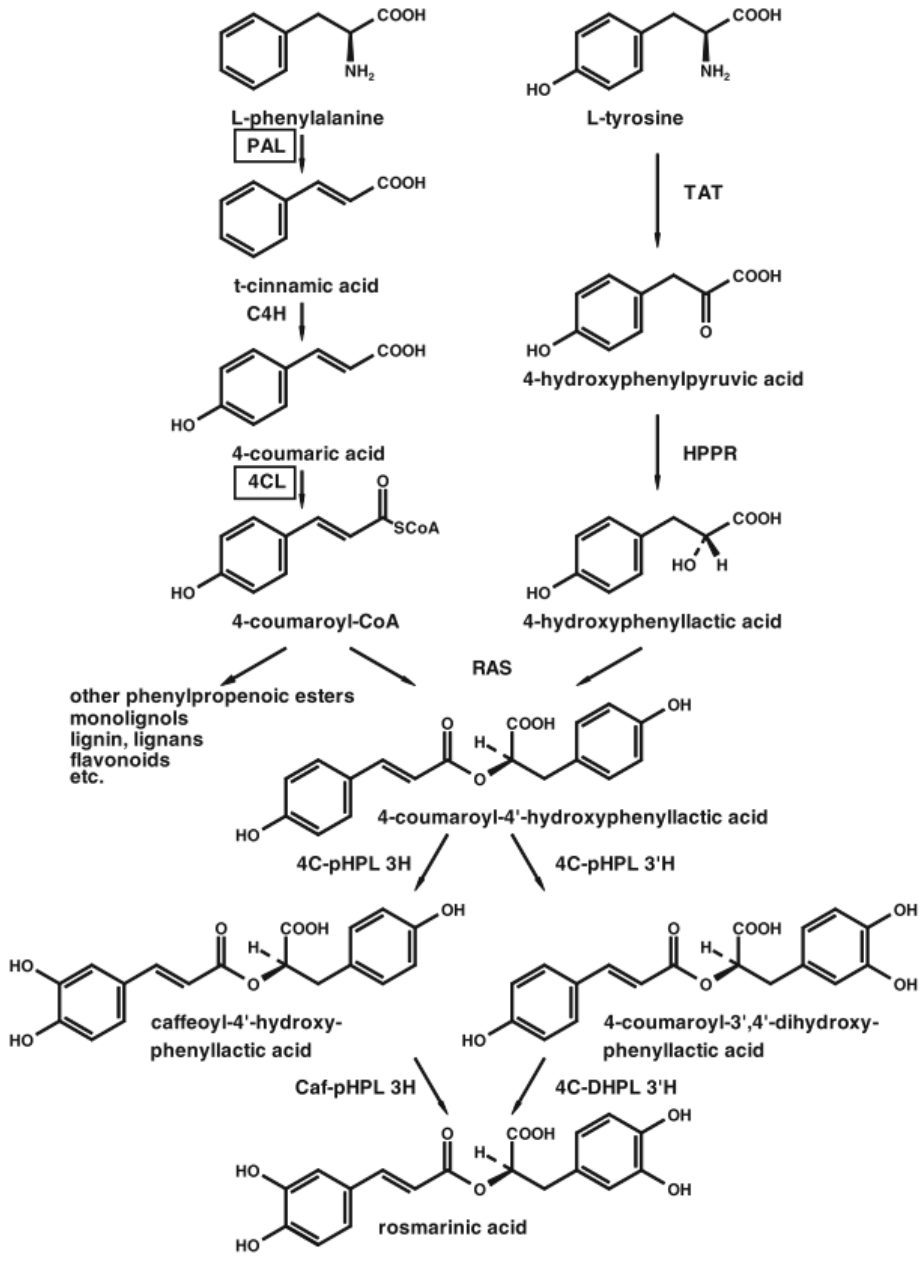

Fig. 5: Biosynthetic pathway of rosmarinic acid in *Melissa officinalis* (Weitzel and Petersen, 2010). **PAL**, phenylalanine ammonia-lyase; **C4H**, cinnamic acid 4-hydroxylase; **4CL**, 4-coumarate:coenzyme A ligase; **TAT**, tyrosine aminotransferase; **HPPR**, hydroxyphenylpyruvate reductase; **RAS**, rosmarinic acid synthase, hydroxycinnamoyl-CoA:hydroxyphenyllactate hydroxycinnamoyltransferase; **4C-pHPL 3H**, **4C-pHPL 3'H**, 4-coumaroyl-4'-hydroxyphenyllactate 3/3'-hydroxylases; **Caf-pHPL 3'H**, caffeoyl-4'-hydroxyphenyllactate 3'-hydroxylase; **4C-DHPL 3H**, 4-coumaroyl-3', 4'-dihydroxyphenyllactate 3-hydroxylase.

2.5.2 Essential oil

The International Organization for Standardization (ISO) describes essential oils (EO) as a "product obtained from a natural raw material (...) of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase - if any - by physical processes" (ISO, 2013).

Essential oils are therefore not single substances, but rather complex mixtures of volatile lipophilic compounds, consisting mainly of terpenoid substances (especially mono- and sesquiterpenes), but may also contain phenylpropanoids, alkanes, alcohols, ketones, or even sulfur- or nitrogen-containing substances (Grassmann and Elstner, 2003). Because of their volatility, they exert a characteristic odor.

Several plants are appreciated by humans because of their typical aroma which is caused by their content of essential oil. Some of them are used mainly as herbs and spices, like rosemary, oregano, or thyme, and some are also consumed in the form of herbal teas, like peppermint or lemon balm.

Despite its distinct lemon-like fragrance, lemon balm contains quite low levels of essential oil. In Polish investigations, EO contents of 0.12 and 0.19% (Kowalska et al., 2014), 0.20% and 0.21% (Dzida et al., 2015), 0.22–0.28% (Politycka and Seidler-Łożykowska, 2009), 0.08–0.22% EO (Patora et al., 2003), 0.115–0.15% (Seidler-Łożykowska et al., 2015) as well as 0.3% (Nurzyńska-Wierdak et al., 2014) were presented. The screening of 22 accessions cultivated under Polish climate conditions resulted in EO contents of 0.05% to 0.44% (Seidler-Łożykowska et al., 2013). An investigation in Slovakia presented EO contents of 0.14% in lemon balm leaves, being higher in the upper leaves of the plant, with 0.39% (Mrlianová et al., 2002). For lemon balm cultivated in Iran, 0.10% to 0.26% EO, depending on the nitrogen fertilization (Abbaszadeh et al., 2009b), 0.2% (Mohamadpoor et al., 2018), as well as 0.13% to 0.35% in a collection of different accessions (Pouyanfar et al., 2018) were found. In Algerian lemon balm leaves, EO contents of 0.17% (Feknous et al., 2014) and 0.34% (Abdellatif et al., 2014) were determined. 0.3–0.4% EO have been found in Tajikistan (Sharopov et al., 2013). EO contents of lemon balm in Spain were investigated depending on the leaf position, with 0.21% for middle-basal leaves and 0.32% for terminal leaves for a harvest in August, and 0.23% as well as 0.33% for a harvest in November, respectively (Adzet et al., 1992a). In a breeding program under the climatic conditions of the Ebro Delta in Spain, EO contents of 0.28–0.31% were found in the beginning, which were then increased to 0.68–0.80% in the fifth year (Adzet et al., 1992b). The EO content of lemon balm cultivated under Turkish climate conditions varied from 0.23 to 0.45%, depending on development stage and year (Avci and Giachino, 2016), and different accessions gave EO yields varying in a range

from 0.03% to 0.47% in another Turkish investigation (Özgüven et al., 1999). In northwestern Turkey, EO contents of 0.20–0.28% were determined at the beginning of flowering (Saglam et al., 2004). In a field experiment in Brazil, an EO content of 0.11% was found for lemon balm cultivated under monocropping conditions, which was increased to 0.22% by intercropping with yarrow (Silva et al., 2018). In a commercial lemon balm sample from France, harvested in the stage just before flowering, an EO content of 0.32% was found in the dried leaves (Carnat et al., 1998). In Egypt, EO contents of 0.18%–0.42% were found, depending on row spacing, which was further increased by the application of active dry yeast to up to 0.64% (Rashed, 2012).

A number of essential oil containing plants are used as medicinal plants, and at least parts of their medical properties are connected to their content of essential oil. Also in the case of lemon balm, several health related effects have been ascribed to the essential oil or its components. For instance, the essential oil of lemon balm has been shown to exert an antiviral action *in vitro* studies, where it inhibited the replication and infectivity of *Herpes simplex* virus type 1 and type 2 (Allahverdiyev et al., 2004; Schnitzler et al., 2008), or the replication of avian influenza virus subtype H9N2 (Pourghanbari et al., 2016). In *in vitro* investigations, the essential oil of lemon balm showed an acetylcholinesterase inhibitory activitiy (Ferreira et al., 2006; Perry et al., 1996). A spasmolytic activity of lemon balm essential oil as well as its main component citral was observed in an investigation on rat ileum (Sadraei et al., 2003). Antiparasitic (Mikus et al., 2000), or anti-diabetic effects (Chung et al., 2010) have been described as well. In a study with patients suffering from severe dementia, positive effects of aromatherapy with lemon balm essential oil could be seen regarding agitation of the patients (Ballard et al., 2002).

The essential oil of lemon balm has been described to contain as the major compounds (cf. **Fig. 6**) the monoterpene aldehydes geranial (= citral a) and neral (= citral b), often summed up and stated together as "citral", as well as citronellal, furthermore the sesquiterpene β-caryophyllene and its oxidized product caryophyllen oxide, and finally several components mostly found in minor concentrations, like citronellol, linalool, geraniol, germacrene D, geranyl acetate, β-ocimene, and others (Carnat et al., 1998; Hollá et al., 1997; Kitzler, 2008; Sari and Ceylan, 2002; Seidler-Łożykowska et al., 2013; Tittel et al., 1982; Weitzel, 2009).

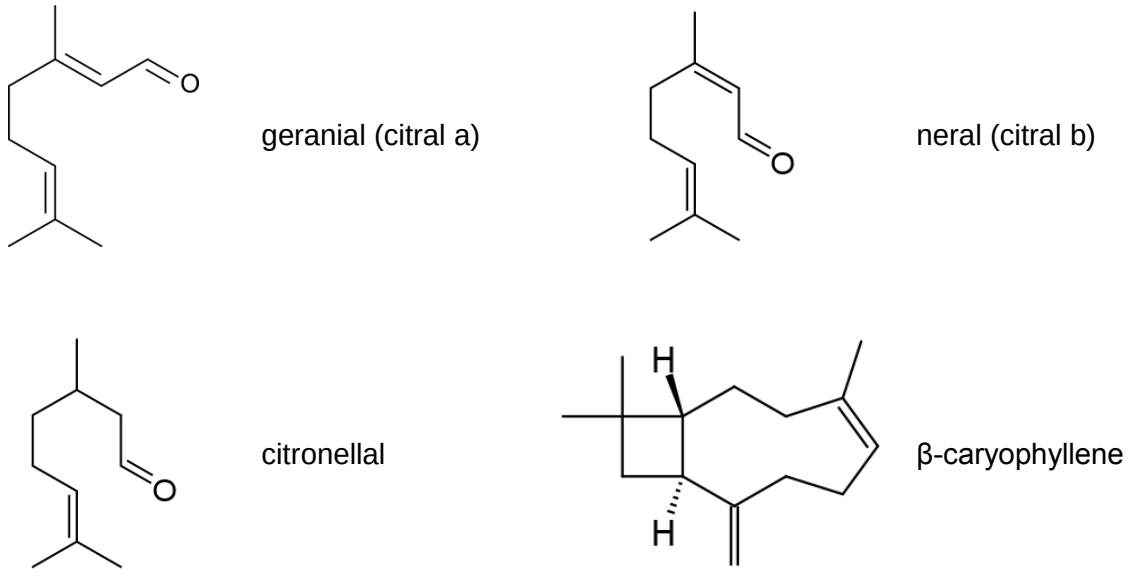

Fig. 6: Structural formulas of major compounds in lemon balm essential oil (according to Adams, 2007). Geranial, neral, and citronellal are monoterpenes, β-caryophyllene is a sesquiterpene.

Structurally, terpenes consist of isoprene units, a molecule with five carbon atoms (C_5). Biochemically, however, they are biosynthesized from the C_5 units isopentenyl pyrophosphate (IPP), sometimes called "activated isoprene", and its isomer dimethylallyl pyrophosphate (DMAPP). Two biosynthetic pathways for IPP are known in plants (Bresinsky et al., 2013; Vranová et al., 2013):

1. The classical, cytoplasmatic mevalonate pathway (also called the acetate-mevalonate pathway)
2. The relatively recently discovered non-mevalonate pathway, also called MEP pathway (MEP = 2-C-**m**ethyl-D-**e**rythritol 4-**p**hosphate), or 1-**d**e**o**xy-D-**x**ylulose 5-**p**hosphate pathway (= DOXP pathway), taking place in the plastids

The biosynthesis of IPP in either of the two pathways is closely related to the primary metabolism, from which its precursors are taken. The mevalonate pathway (**Fig. 7**, left-hand side) starts with three molecules of acetyl-CoA, a key metabolite of the primary metabolism. Two molecules of acetyl-CoA are joined to form acetoacetyl-CoA, out of which 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) is produced by addition of another acetyl-CoA molecule. In the next step, HMG-CoA is enzymatically reduced to form mevalonate, the molecule that gave this pathway its name. Mevalonate is then phosphorylated in two steps to mevalonate-5-phosphate. After an ATP-dependent decarboxylation, IPP is formed.

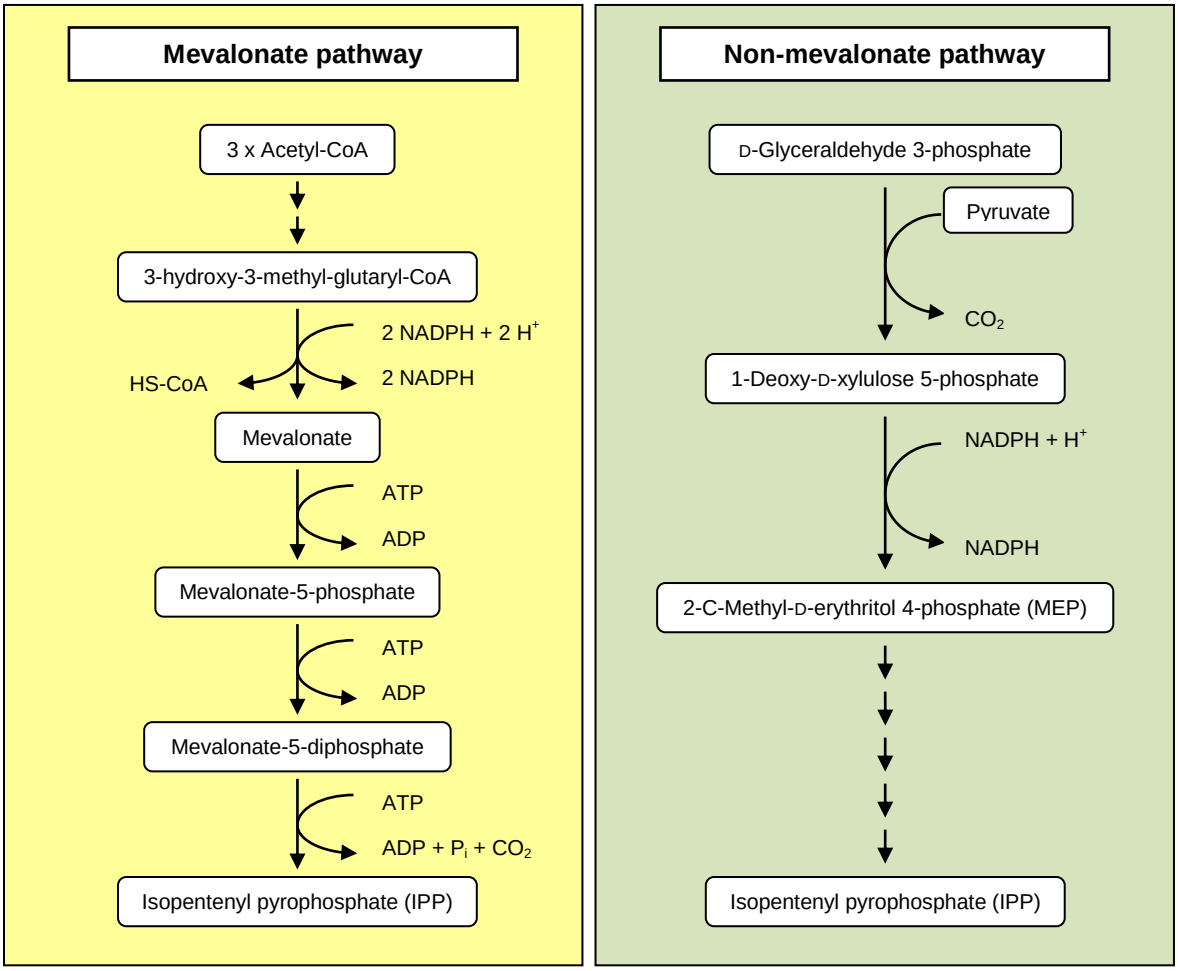

Fig. 7: Schematic, simplified illustration of isopentenyl pyrophosphate (IPP) biosynthesis in plants (modified from Vranová et al., 2013). Left-hand side: Cytoplasmic mevalonate pathway, starting with three Acetyl-CoA molecules from primary metabolism. Right-hand side: Plastidial non mevalonate pathway, starting with D-glyceraldehyde 3-phosphate and pyruvate from primary metabolism.

Also the non-mevalonate pathway (**Fig. 7**, right-hand side) starts with precursors from the primary metabolism, namely the C_3 units D-glyceraldehyde 3-phosphate and pyruvate. In a condensation reaction and after the release of CO_2, the C_5 unit 1-deoxy-D-xylulose 5-phosphate (DOXP) is produced, which is then reduced and rearranged to form 2-C-Methyl-D-erythritol 4-phosphate (MEP). After several additional enzymatically catalyzed steps, IPP is formed (Vranová et al., 2013).

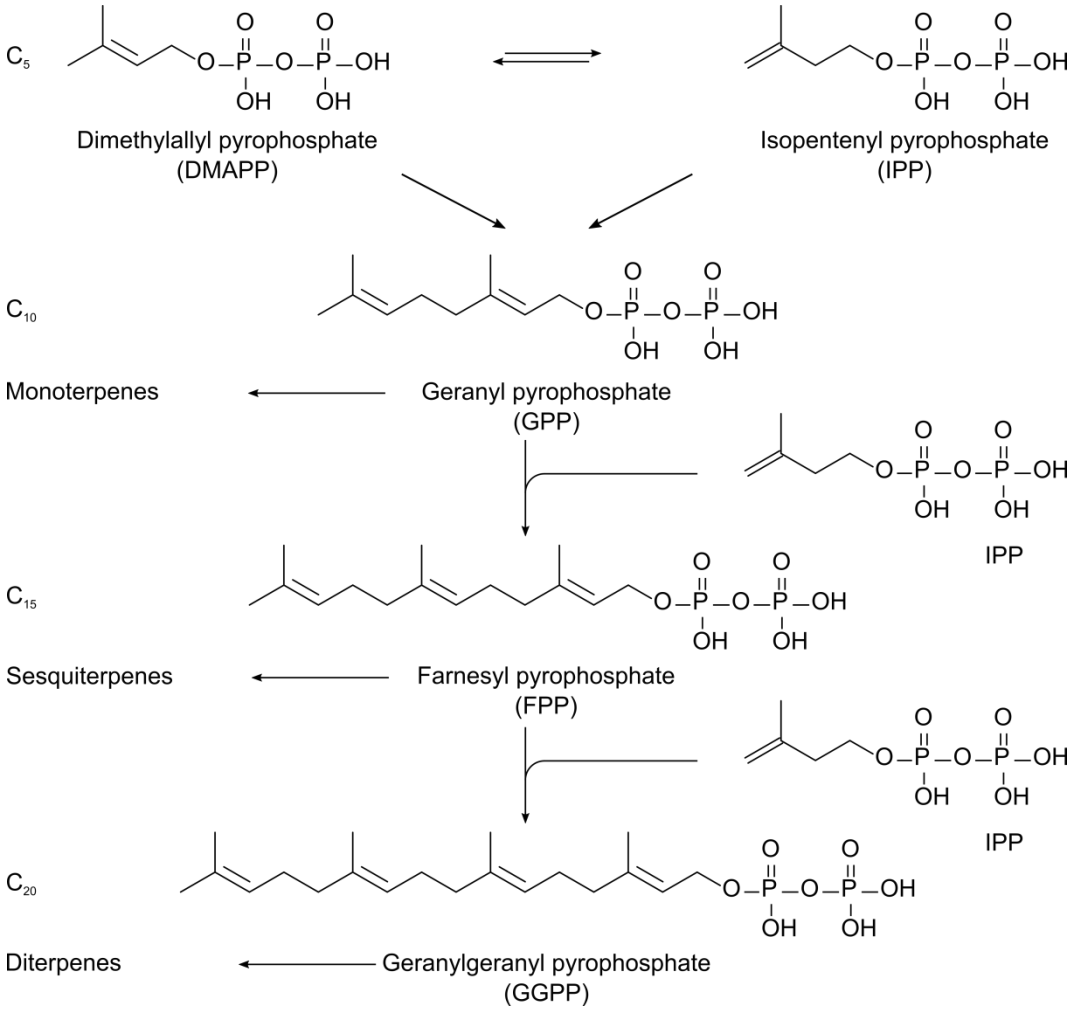

Fig. 8: Biosynthesis of terpenoids, starting from dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) (modified from Bresinsky et al., 2013). The precursors for mono-, sesqui- and diterpenes are formed stepwise.

IPP can enzymatically be converted into its isomer DMAPP by isopentenyl diphosphate isomerase, also called IPP isomerase (EC 5.3.3.2) (Berthelot et al., 2012). Both IPP and DMAPP are needed as precursors for the formation of mono-, sesqui-, and diterpenes (**Fig. 8**), as well as for terpenes of higher order, like tri- or tetraterpenes (not shown). In a first biosynthetic step, DMAPP and IPP undergo a condensation reaction, to form geranyl pyrophosphate (GPP), a C_{10} unit. GPP is the precursor for the formation of monoterpenes (C_{10}). After the addition of another IPP unit, farnesyl pyrophosphate (FPP) is produced, the precursor for the formation of sesquiterpenes (C_{15}). By the addition of another C_5 unit in the form of IPP, geranylgeranyl pyrophosphate (GGPP) is synthesized, the precursor for the formation of several C_{20} molecules, the diterpenes (Bresinsky et al., 2013).

2.6 Antioxidants

Reactive oxygen species (ROS) are oxygen containing chemical species that are more reactive than O_2 (Halliwell, 2015). Among others, these include superoxide anions ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), peroxyl radicals ($ROO^{\bullet}$), hydroxyl radicals ($HO^{\bullet}$), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$) (Huang et al., 2005). Some ROS, like $O_2^{\bullet-}$, $ROO^{\bullet}$, and $HO^{\bullet}$, are free radicals, meaning that they contain one or more unpaired electrons, contributing to their reactive behaviour (Halliwell, 2015). Similar to ROS, also reactive nitrogen species (RNS) exist, like the free radical nitric oxide ($NO^{\bullet}$) (Valko et al., 2007).

Antioxidants act as antagonists of ROS. Plants use enzymatic and non-enzymatic ways to protect themselves from oxidative stress, and enzymatic and non-enzymatic antioxidants partially work together synergistically (Gill and Tuteja, 2010).

Antioxidant enzymes include, for instance, superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), monodehydroascorbate reductase (MDAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1), glutathione S-transferase (GST, EC 2.5.1.18), as well as glutathione reductase (GR, EC 1.8.1.7), whereas glutathione (GSH), ascorbic acid (vitamin C), carotenoids, tocopherols (vitamin E), flavonoids and other phenolic substances are examples for non-enzymatic antioxidants (Gill and Tuteja, 2010; Verma and Dubey, 2003).

Phenolic substances can act as antioxidants in different ways. One mechanism is the chelation of prooxidant ions of transition metals like iron and copper, and thus the inhibition of the Fenton reaction in which the aggressive radical $HO^{\bullet}$ is generated from $O_2^{\bullet-}$ and H_2O_2 (Quideau et al., 2011). The catechol or galloyl groups (two hydroxy-groups in *ortho*-position or three hydroxy groups in neighbouring positions, respectively) are responsible for the chelating activity of phenolic substances (Andjelković et al., 2006). Other mechanisms involve the inhibition of superoxide radical producing enzymes, like xanthine oxidase (Cos et al., 1998), or the regeneration of other important antioxidants, like α-tocopherol (Pazos et al., 2007). The most investigated effect, however, is the direct scavenging of ROS or free radicals (Leopoldini et al., 2011; Quideau et al., 2011; Wright et al., 2001). Generally, it has been stated that signs for a good radical scavenging activity of phenolic substances are multiple hydroxy groups at the aromatic ring, an *ortho*-dihydroxy position of these hydroxy groups, the planar structure allowing conjugation, electron delocalization, and resonance effects, as well as further functional groups, like carbonyl groups or carbon-carbon double bonds (Leopoldini et al., 2011).

Two mechanisms by which phenolic substances exert their antioxidant activity are hydrogen atom transfer (HAT) and single electron transfer (ET) (Leopoldini et al., 2011; Wright et al., 2001).

HAT involves the donation of a hydrogen atom from the phenolic group (ArOH) to a free radical $R^{\bullet}$ (Leopoldini et al., 2011):

$$ArOH + R^{\bullet} \rightarrow ArOH^{\bullet} + RH$$

The phenolic substance thus becomes itself a radical ($ArOH^{\bullet}$, phenoxy radical; **Fig. 9**). However, depending on the number and positions of hydroxy groups, these phenoxy radicals can be quite stable due to a delocalization of electrons within their molecular structure (Leopoldini et al., 2011, 2004; Rice-Evans et al., 1996).

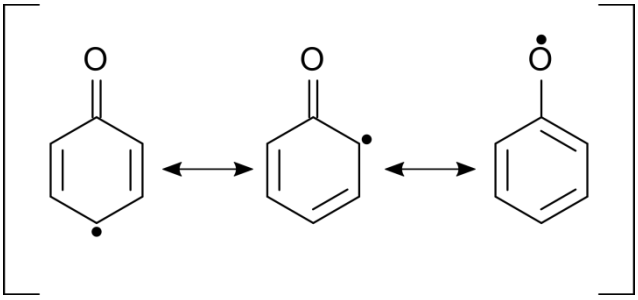

Fig. 9: Phenoxy radical, resonance-stabilized (modified from Quideau et al., 2011).

The HAT mechanism plays an important role for the inhibition of lipid peroxidation. The phenolic function is able to donate a hydrogen atom to a lipid peroxy radical $LOO^{\bullet}$, and thus to inhibit the chain reaction of lipid peroxidation (chain-breaking antioxidant) (Quideau et al., 2011). It has been suggested that RA exerts its antioxidant activity through a H-abstraction reaction, and the forming free radical to be stabilized by a semiquinone or quinone structure (Cao et al., 2005; Fujimoto and Masuda, 2012).

The ET mechanism involves the donation of an electron to a radical (Leopoldini et al., 2011):

$$ArOH + R^{\bullet} \rightarrow ArOH^{+\bullet} + R^{-}$$

The anion R^{-} is energetically more stable than the former free radical, as it now contains an even number of electrons. Also the radical cation formed from the antioxidant is relatively stable (Leopoldini et al., 2011; Quideau et al., 2011).

In the human organism, several metabolic pathways lead to the production of free radicals, like ROS and RNS. These processes are not per se detrimental, as ROS and

RNS play significant physiological roles, for instance in signal transduction or pathogen defense (Valko et al., 2007).

Because of possibly harmful effects, however, a balance between oxidants and antioxidants in the human body is important. In an unbalanced state, with too little antioxidant capacity, oxidative stress occurs (Reuter et al., 2010).

This oxidative stress can lead to the oxidation of macromolecules (like proteins, lipids, or DNA) (Gill and Tuteja, 2010), damage of cell structures, inflammatory processes, and has been linked to the development of several diseases, like cardiovascular diseases, cancer, diabetes, or even Alzheimer's disease (Aksenov et al., 2001; Cai et al., 2011; Reuter et al., 2010; Sayre et al., 2008).

Additionally, oxidative processes can lead to the oxidation of fats or other components in foods. In the case of a free radical reacting with another, non-radical molecule (e.g. a lipid), another free radical is formed (Halliwell, 2015). This can lead to a chain reaction, like the one occurring in the process of lipid peroxidation (Gill and Tuteja, 2010; Halliwell and Chirico, 1993). This does not only negatively influence the organoleptic properties, but can also reduce the content of nutrients, or even lead to the formation of oxidized substances that are harmful to the human health (Choe and Min, 2006; Marnett, 1999).

Therefore, antioxidants, like the synthetic substances butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT), are used as food additives (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2012, 2011). However, some concern regarding the use of these synthetic antioxidants exists, and many consumers prefer products without them.

For those reasons, natural antioxidants, with a high acceptance by customers, are of great interest for the food industry for the replacement of synthetic antioxidants (Berasategi et al., 2011; Fernandes et al., 2016; Lara et al., 2011).

Antioxidant capacity assays

The antioxidant capacity of samples can be investigated by several assays. These assays can roughly be divided into two groups: Assays with a reaction based on a single electron transfer (ET), and those based on a hydrogen atom transfer (HAT) reaction (Huang et al., 2005). In an ET-based assay, the reducing capacity of an antioxidant is measured, while the HAT-based assays measure the hydrogen donating capacity of the antioxidant (Huang et al., 2005).

HAT-based assays include ORAC (oxygen radical absorbance capacity), TRAP (total radical trapping antioxidant parameter), and crocin bleaching assays. The group of ET-based assays includes TEAC (trolox equivalent antioxidant capacity), FRAP (ferric ion reducing antioxidant parameter), and DPPH (diphenyl-1-picrylhydrazyl) assays, as well as the common determination of the total phenolic content (TPC) by the Folin-Ciocalteu (FC) assay (Huang et al., 2005).

Total phenolic content - Folin-Ciocalteu assay

The reagent used in the Folin-Ciocalteu assay was originally developed for the determination of the aromatic amino acid tyrosine in proteins (Folin and Ciocalteu, 1927). Later it was employed on wine, food and plant extracts for the determination of phenolic substances (Singleton and Rossi, 1965), which share the phenolic ring as a common structural element with the amino acid tyrosine.

The Folin-Ciocalteu reagent contains, among others, sodium tungstate, sodium molybdate, as well as lithium sulfate, and is characterized by an intense yellow color. Under basic conditions, phenolic compounds can reduce this reagent via dissociation of a phenolic proton and formation of a phenolate anion. The reduction of the Folin-Ciocalteu reagent leads to a color change from yellow to blue, probably by the formation of $(PMoW_{11}O_{40})^{4-}$, which can be measured photometrically to determine what is commonly called the total phenolic content (TPC) (Huang et al., 2005). Strictly speaking, the Folin-Ciocalteu assay measures the reducing capacity of the sample, including ascorbic acid as the most prominent contributor, and not only the content of phenolic substances (Everette et al., 2010; Huang et al., 2005). For plant extracts with a much higher content of phenolic compounds compared to e.g. ascorbic acid, however, the assay gives a rough approximation of the content of phenolic substances (Everette et al., 2010).

ORAC

The oxyen radical absorbance capacity (ORAC) assay was originally developed by Cao et al. (1993). The original protocol used the fluorescent protein β-phycoerythrin as an indicator, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) as the peroxyl radical generator, and the water soluble vitamin E analogue trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a reference standard substance. Because of some drawbacks, like high cost, a lot-to-lot inconsistency, lacking photostability over time (photobleaching), as well as nonspecific protein binding to polyphenols, β-phycoerythrin was later replaced by fluorescein as a fluorescent probe (Naguib, 2000; Ou et al., 2001). For a higher sample throughput, the ORAC protocol has later been modified for the use of 96-well microplates in combination with a microplate fluorescence reader (Huang et al., 2002).

The procedure of performing the ORAC assay begins with the samples, blank controls, and trolox standards to be mixed with fluorescein. After an incubation period at 37 °C, a solution of the radical generator AAPH is added, and fluorescence intensity is measured in short time intervals for a certain time period with two different wavelengths for excitation and emission. By the reaction with AAPH, fluorescence intensity of fluorescein decreases, a process that is delayed by antioxidants. The measurements result in kinetic curves (cf. **Fig. 10**), of which the area under the curve (AUC) is calculated, followed by the calculation of the net AUC as the difference between the AUC of the sample and the AUC of the blank. With the net AUC of the trolox standard, a calibration curve is obtained, which is then used to calculate the antioxidant capacity of the samples, normally presented as trolox equivalents (TE). Because of the AUC approach, this method can be equally applied to antioxidants showing a distinct lag phase, and those that do not (Huang et al., 2005).

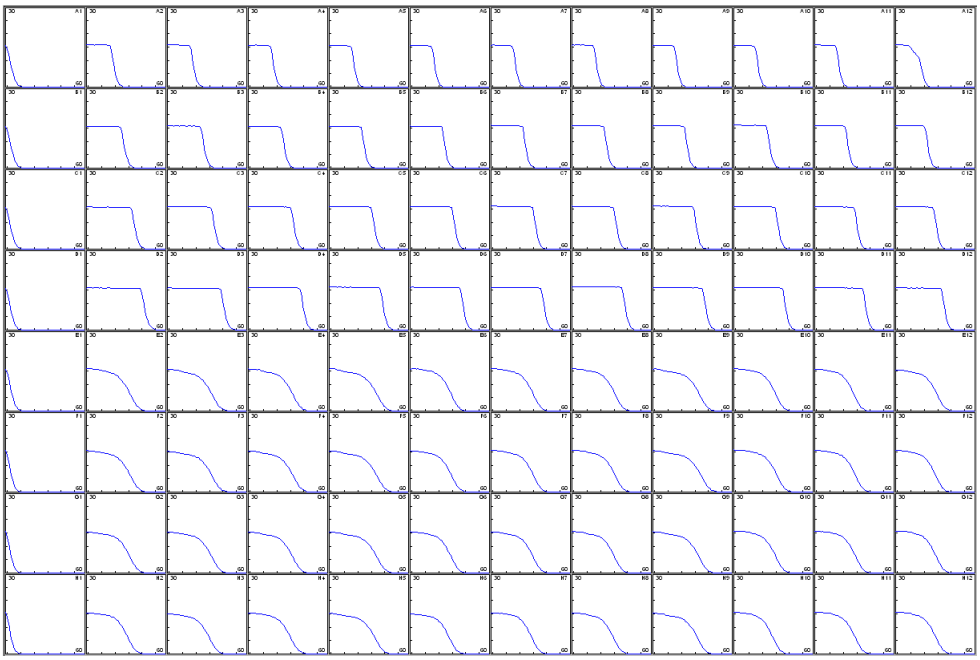

Fig. 10: Example curves of ORAC measurements on a 96-well microplate. The left column shows the blank, where the kinetic curve of fluorescence intensity drops very quickly due to the lack of antioxidants. The following columns show the curves for trolox solutions in four concentrations in the upper four rows (increasing concentrations from the first to the fourth row). In the lower four rows, the kinetic curves of sample extracts are plotted (four replicates of the same sample within the same column). Own measurements.

2.7 Light and its influence on plant growth and secondary metabolites

Light is a part of the electromagnetic spectrum. Typically, it is defined as the radiation that is visible to the human eye, covering the range between about 380 and 720 nm. However, sometimes the neighbouring regions of far-red (about 700 to 800 nm), infrared (800 to 3000 nm) as well as ultraviolet (about 200 to 400 nm) radiation are partly included, especially because of their effects on plants (Bresinsky et al., 2013; Sager and McFarlane, 1997; Schopfer and Brennicke, 2010). For photosynthesis, plants use radiation in the spectral region between 400 and 700 nm, which is termed as photosynthetically active radiation (PAR) (McCree, 1971).

Measurements of light intensity (or radiation) are either reported as the number of photons reaching a certain area (typically µmol $m^{-2} s^{-1}$), or as the energy per area, measured in W m^{-2} (Sager and McFarlane, 1997). Global radiation, comprising direct and diffuse components, is typically measured in W m^{-2} (Bresinsky et al., 2013). The radiation coming from the sun measured above the atmosphere is called the solar constant, reaching around 1367 W m^{-2} (Gueymard, 2004). Because of reflection and absorption in the atmosphere, only a part of this radiation reaches the earth's surface, being about a third less than the solar constant (Bresinsky et al., 2013). PAR readings are typically given in µmol $m^{-2} s^{-1}$ to report the photosynthetic photon flux density (PPFD) (Bresinsky et al., 2013).

2.7.1 Light and plant growth

Light is not only important as an energy source for photosynthesis, but also as a signal for different processes regarding plant growth, partly mediated via phytohormones. These processes include, among others, plant elongation, formation of structural elements, induction of flowering, dormancy, growth rate, leaf drop, or branching of the plants (Bresinsky et al., 2013).

Photosynthesis is a process in which carbon is fixed from CO_2. However, plants do not only fix CO_2, they also release CO_2 through respiration. Plants therefore need a minimum of light for their survival, to compensate the loss of CO_2. The point at which the photosynthesis rate equals the respiration rate (or CO_2 uptake equals CO_2 release) is called the light compensation point. This point is not equal for all plants, there are quite big differences between different plant species. Plants that are adapted to conditions of lower light intensities (shade plants) show a lower light compensation point than plants that typically grow under higher light intensities (sun plants). Ranges for light compensation points of shade plants have been stated as 1–10 µmol $m^{-2} s^{-1}$ (Bresinsky et al., 2013) or 5–20 µmol $m^{-2} s^{-1}$ (Schopfer and Brennicke, 2010), and for sun plants as

10–50 µmol m^{-2} s^{-1} (Bresinsky et al., 2013) or 20–100 µmol m^{-2} s^{-1} (Schopfer and Brennicke, 2010). Little information can be found in literature regarding the light compensation point of lemon balm. In the work of a Brazilian group, a light compensation point of lemon balm of less than 30 µmol photons m^{-2} s^{-1} was documented (Oliveira et al., 2016). This would substantiate the classification of lemon balm as a plant growing preferably between semi-shade and sunny habitats, as indicated by an Ellenberg value (Hill et al., 2004) / Ellenberg-Pignatti value (Vitasović Kosić et al., 2017) of 6. However, a Chinese group found a light compensation point of almost 50 µmol photons m^{-2} s^{-1} under a CO_2 concentration of 400 µmol mol^{-1} (Jia et al., 2012).

While plants need a minimum of light for their survival, their ability to use light for photosynthesis is saturated at a certain point. An excess of light above this level does not increase the photosynthetic activity of the plant, but can rather lead to light stress (Wilhelm and Selmar, 2011). The so-called light saturation point of sun plants has been described to be in the range of about 500–1,500 µmol m^{-2} s^{-1}, and for shade plants at about 100–500 µmol m^{-2} s^{-1} (Bresinsky et al., 2013). With a light saturation point of around 600 µmol m^{-2} s^{-1} (Oliveira et al., 2016) lemon balm lies at the lower end of the range for sun plants. However, a Chinese group found a light saturation point of more than 1000 µmol photons m^{-2} s^{-1} under a CO_2 concentration of 400 µmol mol^{-1} (Jia et al., 2012).

The need of light for photosynthesis and therefore for the production of biomass is an important point regarding the yield parameters of cultivated plants. This might explain the findings of an investigation with *Ocimum basilicum*, where a strong shading significantly reduced plant height, plant weight, leaf area, as well as the number of shoots. A moderate light reduction, on the other hand, did not significantly reduce leaf area (Chang et al., 2008).

However, plants usually face the situation of receiving much more sunlight than they can use for photosynthesis, even in temperate regions (Wilhelm and Selmar, 2011). Therefore, shading can be regarded as beneficial under certain circumstances. In an investigation on *Myrica rubra*, for instance, Zeng et al. (2017) found that leaf dry mass was increased under shade. They stated that less photoinhibition occurred under the shaded condition (Zeng et al., 2017).

Generally, it must be stated that different agricultural crops may react differently on a light reduction. It has been shown, for instance, that the yield of eggplant, soybean, peanut, and sweet potato decreased under reduced light intensity, while the yield of semihead lettuce was increased (Wolff and Coltman, 1990).

Besides its importance for photosynthesis and, in excess, for light stress, light acts also as a signal for several processes in plants, and can thus also influence plant morphology. Different photoreceptors enable the plants to perceive the light quality, like phytochromes, cryptochromes, and phototropins. Phytochromes, homodimeric chromoproteins, enable the plant to sense red and far-red light (Bresinsky et al., 2013). Phytochromes can exist in two states which can reversibly be changed. The inactive form is sensitive to red light, therefore called P_r. On illumination with red light, it is converted into the active, far-red sensitive form P_{fr}. With the phytochrome system, the plant perceives changes in the red:far-red ratio (R:FR) and can respond accordingly. R:FR is especially lowered under a plant canopy. Therefore, the phytochrome system enables a plant to perceive other plants with which it competes for light. As a consequence, it can react with the so-called shade avoidance syndrome (SAS) in its gain to get more PAR. Also the cryptochromes, with which the plant senses changes in blue light, play an important role in the SAS of plants (Pedmale et al., 2016). SAS involves, for instance, an elongation of the plant to reach a position where it gets more light, a reduced branching, or changes in leaf position or leaf morphology (Casal, 2013). To compensate for a loss of PAR, plants tend to increase chlorophyll content under shade (Niinemets, 1999; Zhang et al., 2016). Another compensation mechanism is increased specific leaf area (Miralles et al., 2011; Zhang et al., 2016)

Shading increased plant height and reduced number of lateral shoots in sweet pepper (Rylski and Spigelman, 1986). Plant height of *Myrica rubra* was increased under 50% and 75% shade, but not under 25% shade (Zeng et al., 2017). In an investigation on three chrysanthemum cultivars, a reduction of far-red light under a photoselective film reduced the elongation of the plants (Li et al., 2003). In wheat (*Triticum aestivum*), however, a lower R:FR did not increase plant height (Ugarte et al., 2010). The influence of a reduced light intensity on plant morphology seems thus to be dependent on plant species and the level of the light reduction.

2.7.2 Light and selected secondary metabolites

Light can generally affect the accumulation of several secondary metabolites in plants, like phenolic substances, terpenes, or carotenoids. Because of the importance of phenolic substances and terpenes (as components of EO) for the quality of lemon balm, only these two classes will be focused on in this chapter.

As an excess of light in general and especially UV light can be harmful for the plants, different mechanisms exist in plants to protect themselves from harmful radiation (Munné-Bosch and Alegre, 2000a). Certain secondary metabolites, such as flavonoids, are able to act as sunscreen. Generally, the production of secondary metabolites is costly for the plants, as precursors are diverted from the primary metabolism (Gershenzon, 1994; Logemann et al., 2000). Secondary metabolites are produced constitutively for general defense, and can be induced by different stressors, like herbivore or fungal attack, or UV light. UV light increases the production of flavonoids by induction of the enzymes of their biosynthetic pathways, while also enzymes of the corresponding supply pathways are induced, leading to a diversion of metabolites from the primary to the secondary metabolism (Logemann et al., 2000).

Light and phenolic substances

The phenylpropanoid pathway leads to the formation of different phenolic compounds, like phenolic acids and flavonoids. PAL, a key enzyme of the phenylpropanoid pathway, is responsible for the transformation of L-phenylalanine into *t*-cinnamic acid, and therefore also important for the biosynthesis of rosmarinic acid in *Melissa officinalis* (Weitzel and Petersen, 2010). As shown in *Arabidopsis*, UV-B radiation induces the expression of the PAL gene (Li et al., 1993). Under 80% light reduction, PAL expression was decreased 4.45-fold in *Camellia sinensis* plants (Wang et al., 2012). Even in hairy root cultures of *Echinacea purpurea*, PAL activity and the biosynthesis of some caffeic acid derivatives, as cichoric acid, caftaric acid and chlorogenic acid, was increased under continuous light (Abbasi et al., 2007).

Other enzymes in the biosynthesis of phenolic substances are cinnamic acid 4-hydroxylase (C4H), which catalyzes the transformation of *t*-cinnamic acid into 4-coumaric acid, and 4-coumarate:CoA-ligase (4CL), leading in a next step to the formation of 4-coumaroyl-CoA (Weitzel and Petersen, 2010). The expression of C4H mRNA has been shown to be light-dependent in *Arabidopsis* (Bell-Lelong et al., 1997). In cell cultures of *Onosma paniculatum*, however, the isoform C4H2 was expressed constantly and not in a light-dependent manner, whereas the expression of PAL1, 4CL1, and CYP98A6 (coding for an enzyme involved in the final steps of rosmarinic acid

synthesis) was induced by red, blue, and white light (Liu et al., 2006). In leaves and cell suspension cultures of parsley (*Petroselinum crispum*), UV containing white light induced the accumulation of PAL1/2, PAL3, C4H as well as 4CL mRNAs, but not of the PAL isoform PAL4 (Logemann et al., 1995).

Irradiation of lemon balm plants with UV-B has been shown to influence several primary and secondary metabolites. Especially the levels of sugars, such as fructose or sucrose, decreased significantly, whereas metabolites relevant for the phenylpropanoid biosynthesis, like phenylalanine, quinic acid, shikimic acid or caffeic acid, were significantly increased (Kim et al., 2012).

Although higher contents of phenolic substances in plants have been linked to higher irradiation, there are also examples where the opposite is true. In *Labisia pumila* var. *alata*, the content of total flavonoids, phenolics and anthocyanin decreased steadily with increasing irradiation (Ibrahim and Jaafar, 2012). In tea plants (*Camellia sinensis*), different reactions of several groups of phenolic compounds on a light reduction were observed. While flavonoid and lignin content decreased, some phenolic acids, like gallic acid, increased (Wang et al., 2012). In white 'Riesling' grape berries (*Vitis vinifera*), an increased illumination led to lower contents of several amino acids (primary metabolites), whereas the content of several phenolic substances, as well as total flavonoids and total phenolics (secondary metabolites), was increased. Interestingly, the content of phenylalanine, a precursor for the biosynthesis of phenolic substances, was significantly increased under the shade treatment (Friedel et al., 2015). The strong negative correlation between the contents of amino acids and phenolic substances indicates the connection between primary and secondary metabolism and the role of light intensity for the balance between these metabolic pathways.

Light and terpenoid substances

Besides an influence on the accumulation of phenolic substances, light might also influence the content of terpenoid substances, and therefore also the EO content in plants. Investigations of Sasaki et al. (2016) on the influence of different light intensities and light qualities in *Vitis vinifera* revealed a significant influence on linalool content as well as on enzymes of the monoterpene biosynthesis. Under a UV blocking film, as well as under a strong shading reducing both UV radiation as well as PAR, the content of the monoterpene linalool was significantly reduced. The relative gene expression levels of the enzyme responsible for the formation of 1-deoxy-D-xylulose 5-phosphate (DXP), DXP synthase (DXS), as well as DXP reductoisomerase (DXR) – both enzymes of the MEP pathway – were significantly decreased. The same was true for geranyl pyrophosphate

synthase (GPPS), linalool synthase (LS), as well as farnesyl pyrophosphate synthase (FPPS). HMG-CoA reductase (HMGR), as an enzyme of the mevalonate pathway, however, showed only a non-significant tendency of a decreased relative expression. An increase in the light intensity investigated in the same work by placing reflective sheets on the ground led to significant increases in linalool content, as well as increased relative expression levels of DXS, DXR, GPPS, and LS (Sasaki et al., 2016). A light-dependent accumulation of free and glycosylated monoterpenes in grape berries has also been shown by Friedel et al. (2016). In this case, the expression of some genes of monoterpene biosynthesis, like the linalool/nerolidol synthase genes VvTPS54 and VvTPS56, was reduced under a shade treatment. However, the expression of VvHDR, an enzyme in the MEP pathway for the formation of IPP and DMAPP, was not significantly influenced by the different light treatments (Friedel et al., 2016). An increased accumulation of some mono- and sesquiterpenes in *Vitis vinifera* under high UV-B radiation was also shown by Gil et al. (2012).

In etiolated thyme seedlings, the importance of light was shown for monoterpene accumulation, as well as for the development of peltate glandular trichomes (Yamaura et al., 1989). The essential oil content of *Ocimum basilicum* was significantly reduced in strongly shaded plants (Chang et al., 2008). The authors suggested that a higher synthesis of photosynthates as precursors is essential for the accumulation of secondary metabolites. However, EO content in sage was found to be higher under shade in Egypt (Abd El Azim and Badawy, 2015).

There might be even interaction effects between the light intensity and other factors. Emission of volatiles induced by herbivore oral secretion in *Zea mays* plants was increased with increasing light intensity, whereas non-induced plants did not show a light-dependent reaction on the amount of emitted volatiles (Gouinguené and Turlings, 2002). Also a genotype specific reaction might be possible, as was seen in the Lamiaceae plant *Mentha spicata*, regarding an increased UV-B radiation on EO content (Karousou et al., 1998).

It can be seen that light has an important function on plant growth, which can be both beneficial and detrimental for the plants, and has also an important influence on the formation of several secondary metabolites in different plants. However, despite its importance as a medicinal and aromatic plant, this influence has not been investigated extensively in lemon balm.

3 Material and Methods

3.1 Soil and climate conditions

3.1.1 Experimental site Gross-Gerau

The experimental site in Gross-Gerau (49° 56' 27" N, 8° 30' 01" E), Hesse, Germany, is situated on a sandy soil (soil type: arenosol; humus content: 1.1–1.5%; soil value: 20–25 points; pH: 6.5). The climate conditions at the experimental station are characterized by a long-term air temperature of 9.9 °C, and an annual precipitation of 606 mm (**Tab. 2**). During the experimental period 2013–2015, higher air temperatures compared to long-term average were observed in all three years. Regarding the annual precipitation, the experimental years were quite different, with a high value in 2013 (739 mm), followed by lower precipitation in 2014 (662 mm) and 2015 (491 mm). The year 2013 was characterized by a high precipitation in April and especially in May, followed by an exceptionally dry July. In contrast, the year 2014 showed an exceptionally high precipitation in the summer, especially in August (**Tab. 2**).

Tab. 2: Air temperature and precipitation at the experimental station Gross-Gerau in 2013–2015.

	Temperature - Monthly average [°C]				Precipitation - Monthly sum [mm]			
Month	**2013**	**2014**	**2015**	**Lt-av.[a]**	**2013**	**2014**	**2015**	**Lt-av.[a]**
January	1.8	4.3	2.9	1.0	34.6	40.2	69.9	37.0
February	0.8	5.2	2.0	1.9	40.2	40.4	26.1	35.1
March	2.6	8.4	6.4	5.7	32.8	19.2	23.7	40.6
April	10.0	13.3	10.3	9.7	75.3	30.2	24.5	39.9
May	12.8	14.2	14.4	14.1	138.0	61.9	20.1	58.7
June	17.7	18.5	18.1	17.3	59.6	27.5	65.4	66.6
July	21.9	21.0	22.2	19.2	13.1	85.5	36.3	66.4
August	19.0	17.5	21.5	18.3	72.3	128.1	43.1	66.9
September	14.8	16.1	14.3	14.5	60.6	36.1	62.3	47.2
October	11.5	12.6	9.3	9.6	111.1	76.9	18.5	50.6
November	5.5	7.2	8.0	5.0	70.4	58.1	68.8	48.5
December	4.0	3.7	6.7	2.1	30.8	58.3	32.5	48.7
Sum					738.8	662.4	491.2	606.2
Average	10.2	11.8	11.3	9.9				

[a] Lt-av. = long-term average (1954–2015).

3.1.2 Experimental site Rauischholzhausen

The experimental site in Rauischholzhausen (50° 45' 53" N, 8° 51' 55" E; 237 m above sea level), Hesse, Germany, is situated on a loess soil classified as L4 Lö 71/70 (soil type: luvisol, humus content: 2.0%, soil value: 71 points; pH: 5.5).

The climate conditions at the experimental station are characterized by a long-term air temperature of 8.5 °C, and an annual precipitation of 610 mm (**Tab. 3**). During the experimental period 2013–2015, the observed air temperatures were higher compared to the long-term average. The annual precipitation was quite different in the investigated years, with a high value in 2013 (687 mm), followed by a slightly lower, but still above-average, precipitation in 2014 (654 mm), and a distinctively lower precipitation in 2015 (537 mm). The year 2013 was characterized by a high precipitation in May, followed by a dry summer. However, in the year 2014, an exceptionally high precipitation occurred in the summer, especially in July (**Tab. 3**).

Tab. 3: Air temperature and precipitation at the experimental station Rauischholzhausen in 2013–2015.

	Temperature - Monthly average [°C]				Precipitation - Monthly sum [mm]			
Month	**2013**	**2014**	**2015**	**Lt-av.[a]**	**2013**	**2014**	**2015**	**Lt-av.[a]**
January	1.2	4.2	2.7	-0.2	47.8	43.3	55.2	40.6
February	0.2	5.2	1.9	-0.3	41.1	43.1	16.0	38.2
March	0.9	7.3	5.3	4.0	30.6	6.3	40.8	36.2
April	9.1	11.8	9.1	8.0	45.9	19.6	37.3	40.3
May	12.1	12.9	13.2	12.9	104.1	95.6	19.4	58.1
June	16.4	16.4	16.7	15.7	43.9	22.2	36.3	63.4
July	19.8	19.7	20.3	17.0	22.6	139.9	56.6	64.3
August	19.8	16.3	20.5	17.1	55.8	93.4	67.3	73.2
September	12.4	15.1	13.3	13.2	58.9	36.5	56.3	47.6
October	10.9	11.6	8.9	8.8	117.3	67.5	36.0	50.2
November	4.8	6.6	7.7	4.2	67.5	40.5	82.9	48.9
December	4.4	3.4	7.1	1.1	52.0	46.5	33.2	49.3
Sum					687.2	654.4	537.0	610.2
Average	9.3	10.9	10.6	8.5				

[a] Lt-av. = long-term average (1954–2015).

3.2 Design and experimental procedure

3.2.1 Experimental design

A block design was established, including the factors light, genotype, and harvest time (**Tab. 4**). Due to the perennial growth of lemon balm plants, the same plant stand was used during the three years of the investigation. Each plant was harvested two times a year, referred to as cut 1 and cut 2 in the following.

The light treatment consisted of two levels (natural light and shading). The plots for the factor genotype were randomly distributed within the light treatments, and subplots for the harvest time were placed within each genotype plot. Three lemon balm genotypes were investigated in the field trial in Gross-Gerau, namely 'Aufrechter Typ', 'Lemona' (both procumbent growth types), and 'NLC' (upright growth type), whereas in Rauischholzhausen, only the first two genotypes were investigated (see below).

Within each cut, plants from different plots were harvested at different harvest stages The first harvest stage (early harvest stage) was scheduled to be at the vegetative stage, before flowering, and the following harvest stages (medium and late harvest stage) for further developed plants (beginning or full flowering). The factor harvest stage was only investigated in 2013 and 2014. The treatments "natural light" and "medium harvest stage" are regarded as the reference variants.

Tab. 4: Investigated factors and levels for the field experiments in Gross-Gerau (GG) and Rauischholzhausen (RH).

Factor	Levels	Notes
Light	**Natural light**	sunlight
	Shading	GG: moderate shading RH: strong shading
Genotype	**'Aufrechter Typ'**	Procumbent growth type
	'Lemona'	Procumbent growth type
	'NLC'	Upright growth type; only field experiment GG
Harvest stage	**Early**	Only in 2013 and 2014
	Medium	
	Late	

The seeds of the genotypes 'Aufrechter Typ' and 'Lemona' (procumbent growth types) were obtained from Pharmasaat (Artern, Germany), and those of 'NLC' (upright growth type) from N.L. Chrestensen (Erfurt, Germany). Propagation of the plants by seeds was started under greenhouse conditions in February by the use of nutrient-poor substrate (Fruhstorfer Aussaat- und Stecklingserde, Archut, Lauterbach, Germany). Approximately three weeks after germination, the seedlings were transplanted into 104-cell trays, and later to 54-cell trays (nutrients-containing substrate: Fruhstorfer Erde Typ N, Archut, Lauterbach, Germany) to improve the growth space. To reach a more compact growth and a stronger development of shoots, plants were pinched. About a month before planting to the field, the plantlets were gradually exposed to outdoor conditions to harden them off. Planting to the field (beginning of June) was performed at a plant height of approximately 10 cm.

The year 2012 was the establishing year for the plant stands, and the harvests took place in the years 2013, 2014, and 2015, with two cuts in each year.

3.2.1.1 Field experiment Gross-Gerau

The field trial at the experimental station Gross-Gerau was established in 2012 after the pre-crop summer barley, by transferring the young plantlets to the field on June 5, 2012. A block design was established as shown in **Fig. 11**, with four blocks within each of the two light treatments (natural sunlight vs. moderate shading).

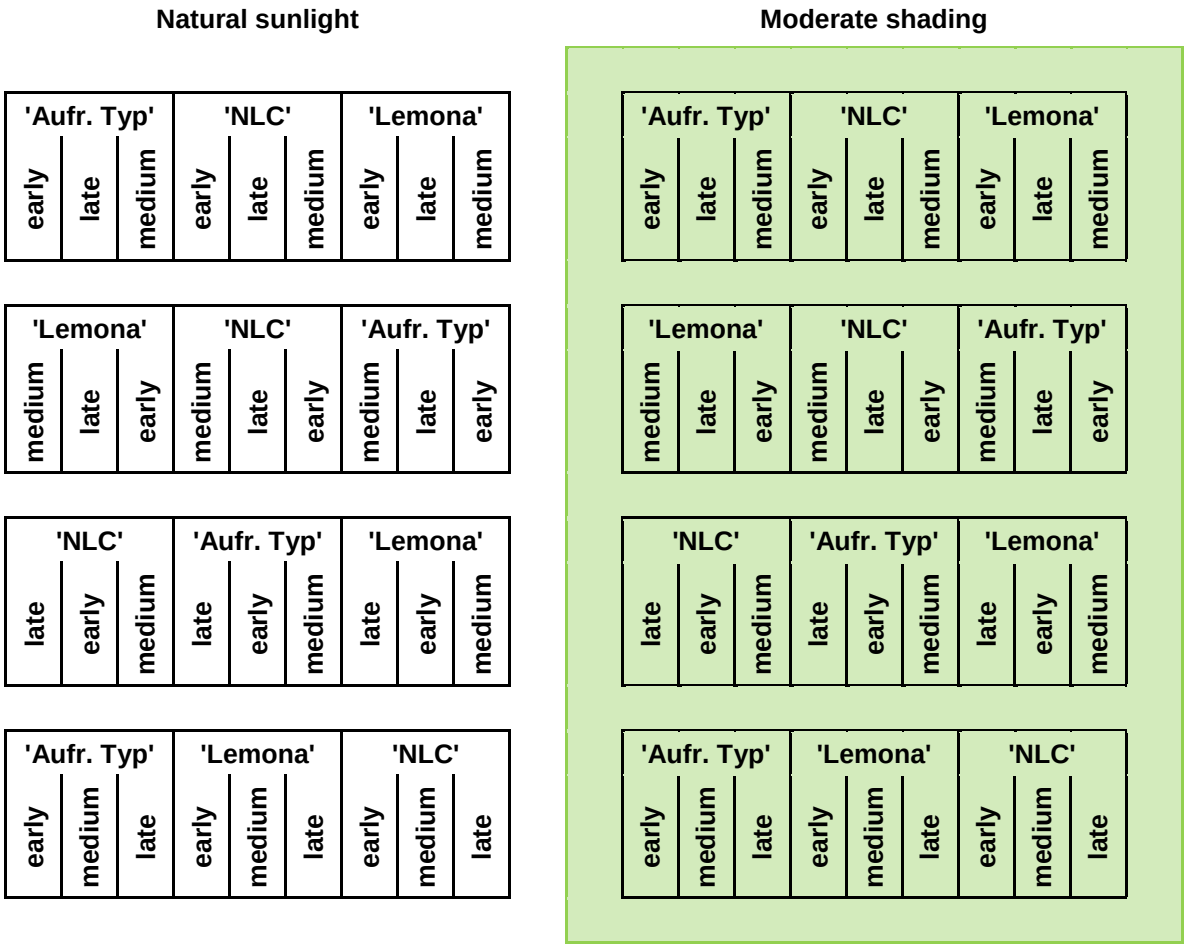

Fig. 11: Design of the field experiment in Gross-Gerau 2013–2015. Two light treatments (natural sunlight and moderate shading) with four blocks each. Every block consisted of three genotype plots, and three subplots for the three harvest stages.

All blocks within each of the light treatments consisted of three plots for the three genotypes, and three subplots within each genotype plot representing the three harvest stages. Each subplot, measuring 7.0 x 1.5 m (10.5 m^2) was planted with four rows of 19 lemon balm plants, summing up to 76 plants per subplot (**Fig. 12**). For the exclusion of a possible border effect, only the inner parts were regarded as harvest plots. Due to severe winter losses in the winter 2012/2013 for the genotypes 'Aufrechter Typ' and 'Lemona', the size of the harvest plots had to be reduced. To reach a closed plant stand within each harvest plot, surviving plants were planted together. Harvest plot size was thus reduced for 'Aufrechter Typ' to 1.66 m^2 (12 plants) and for 'Lemona' to 2.21 m^2 (16 plants), while 'NLC' kept the original harvest plot size of 4.70 m^2 (34 plants).

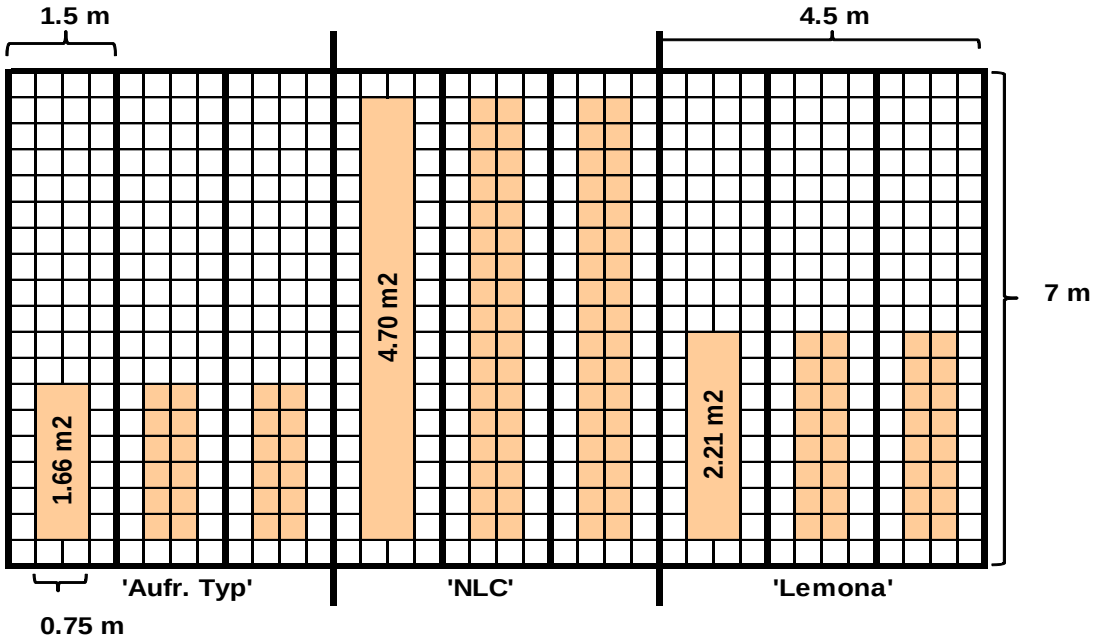

Fig. 12: Plot dimensions of the field experiment Gross-Gerau. Each little square represents one plant. The colored parts are the harvest plots. Three subplots within each genotype plot for investigation of the three harvest stages.

To reach a homogenous plant development and growth in the first harvest year, the lemon balm plants of all plots were topped in spring after the first winter (May 6, 2013). Harvest dates for the years 2013, 2014, and 2015 are presented in **Tab. 5**.

Plants were supplied with P, K, Mg, and S in the form of a basal dressing in spring according to the recommendations of VDLUFA (Association of German Agricultural Analytic and Research Institutes) for an optimal nutrient content in the soil (VDLUFA, 1991). Nitrogen fertilization was given in the form of calcium ammonium nitrate. In the establishing year 2012, plants received 50 kg N/ha in June, and at the end of August an additional 40 kg N/ha were applied.

According to the mineral N content of the soil, plants were supplied with N in the harvest years in spring (2013: 90 kg N/ha; 2014: 60 kg N/ha; 2015: 50 kg N/ha), and received another 50 kg N/ha after each cut.

Due to the sandy soil and weather conditions (cf. **Tab. 2**), irrigation was given as needed, with 20–30 mm per irrigation time (in total 2012: 60 mm, 2013: 25 mm, 2014: 80 mm and 2015: 140 mm). The irrigation received for each cut and harvest stage is presented in **Tab. 5**.

Tab. 5: Harvest dates, vegetation days, air temperature, precipitation and irrigation of the field experiment in Gross-Gerau.

Year	Cut	Harvest stage	Harvest date	Vegetation days [(a)]	Air temperature [° C]		Precipitation [mm] [(a)]	Irrigation [mm] [(a)]
					Average temperature [(a)]	Cumulative temperature [(a) (b)]		
2013	Cut 1	Early	24.06.2013	49	15.4	511.0	178.0	0.0
		Medium	01.07.2013	56	15.3	575.7	196.8	0.0
		Late	08.07.2013	63	15.8	681.2	203.3	0.0
	Cut 2	Early	02.09.2013	70	19.8	1032.5	104.2	25.0
		Medium	09.09.2013	70	20.3	1070.6	91.6	25.0
		Late	16.09.2013	70	19.7	1030.7	113.3	25.0
2014	Cut 1	Early	26.05.2014	86	11.7	589.3	95.0	30.0
		Medium	10.06.2014	101	12.6	776.5	113.4	60.0
		Late	24.06.2014	115	13.3	968.1	116.2	60.0
	Cut 2	Early [(c)]	05.08.2014	56	20.0	838.4	120.8	20.0
		Medium [(c)]	29.07.2014	64	19.3	913.4	123.7	50.0
		Late	10.09.2014	78	18.9	1081.5	237.7	20.0
2015	Cut 1	Medium	10.06.2015	101	11.2	632.2	82.0	55.0
	Cut 2	Medium	19.08.2015	70	21.0	1116.6	111.2	85.0

[(a)] Starting from March 1 until harvest date for cut 1, and for cut 2 from the preceding harvest date. For cut 1 in 2013, starting from topping the plants on May 6.

[(b)] Cumulative temperature calculated as the sum of daily temperatures above the basal temperature of 5 °C, starting from March 1.

[(c)] Plants harvested at the earlier date (29.07.2014) were already further developed than plants harvested at the next harvest date (05.08.2014). Therefore, 05.08.2014 is regarded as the early harvest stage, and 29.07.2014 as the medium harvest stage.

Weeds were controlled manually, as well as with Fusilade Max (1 l/ha, a.i. fluazifop-P-butyl) or Basagran (2 l/ha, a.i. bentazon). For pest control, Karate Zeon (1 l/ha, a.i. lambda-cyhalothrin) and Calypso (0.25 l/ha, a.i. thiacloprid) were applied against leafhoppers (*Eupteryx* sp.), and Askon (75 ml/ha, a.i. azoxystrobin and difenoconazole) against *Septoria* sp.

Light reduction was realized with a light green polyethylene anti bird net with a mesh size of 18 mm (Novatec, Germany), installed at about 1.90 m height above the shaded plots. In 2015, the net was used in a double layer because of a decreased shading capacity of the net due to wear. Setup of the nets took place on May 16, 2013, April 4, 2014, as well as April 4, 2015, and nets were left above the plant stand until the last harvest.

Light intensity was continuously registered within each of the two light treatments. Light sensors were placed within the plant stand, at a height of about 1.20 m, to ensure a position not being overgrown by the plants during their development.

Two types of light sensors were used: A Silicon Pyranometer Smart Sensor for global radiation, and a Photosynthetically Active Radiation (PAR) Smart Sensor. Both were connected to a HOBO Micro Station (Onset Computer Corporation, Bourne, USA).

Registration of light intensity took place in the periods August 2 to September 15, 2013, April 4 to September 9, 2014, as well as May 7 to June 6, 2015, and July 7 to August 18, 2015 (**Fig. 13** and **Fig. 14**). Mean light reduction, as calculated from the light measurements from 8:00 till 18:00 h on the shaded and non-shaded plots, was 13.8%, 7.4%, and 15.4% on the basis of PAR values, as well as 10.9%, 7.4%, and 8.5% on the basis of total radiation, in the years 2013, 2014, and 2015, respectively. The light reduction established in Gross-Gerau will be termed as "moderate shading" in this work.

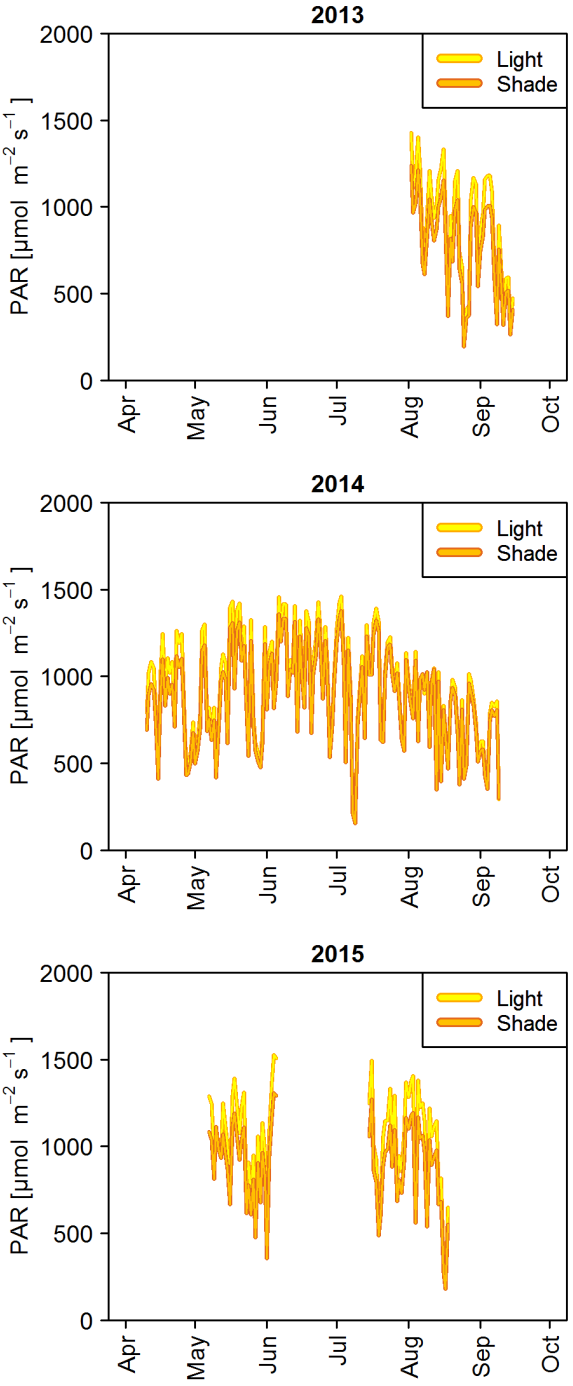

Fig. 13: Photosynthetically active radiation (PAR) under full sunlight and moderate shading. Field experiment Gross-Gerau, 2013–2015. Daily averages between 08:00 and 18:00 h.

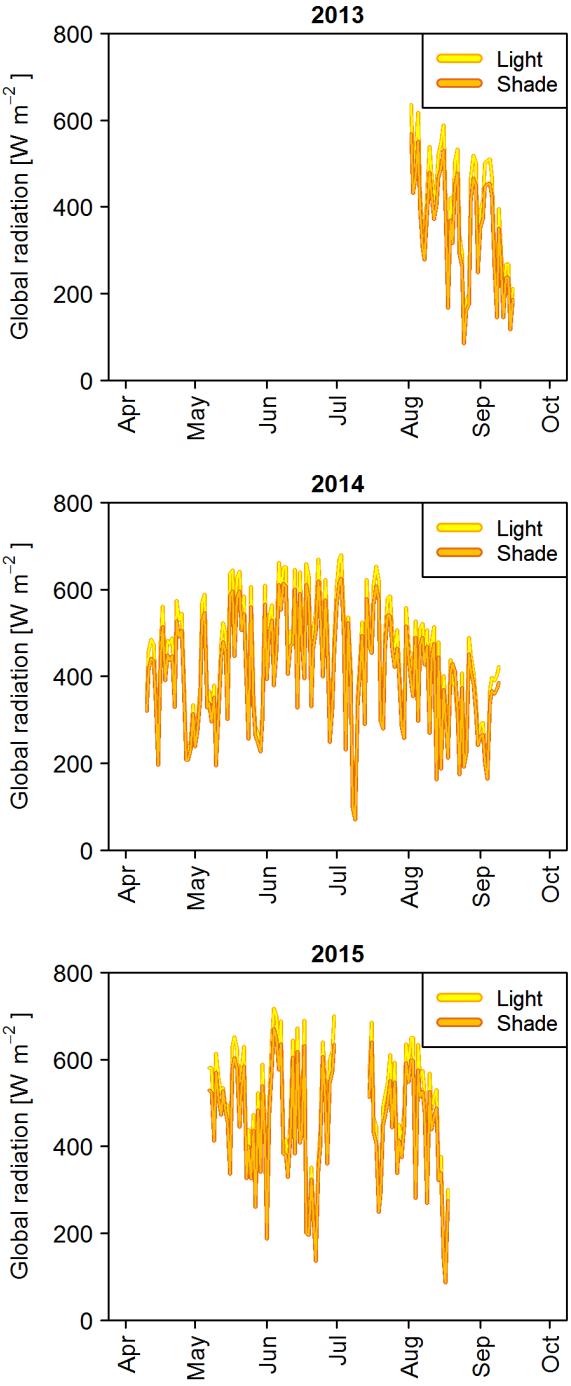

Fig. 14: Global radiation under full sunlight and moderate shading. Field experiment Gross Gerau, 2013–2015. Daily averages between 08:00 and 18:00 h.

The effect of the used shading net on temperature (**Fig. 15**) and relative humidity (**Fig. 16**) was negligible, as monitored in both light treatments during the vegetation period. Temperature reduction under the net was on average less than 0.05 °C, and the difference in relative humidity between shaded and non-shaded plots was in the range of ± 1%.

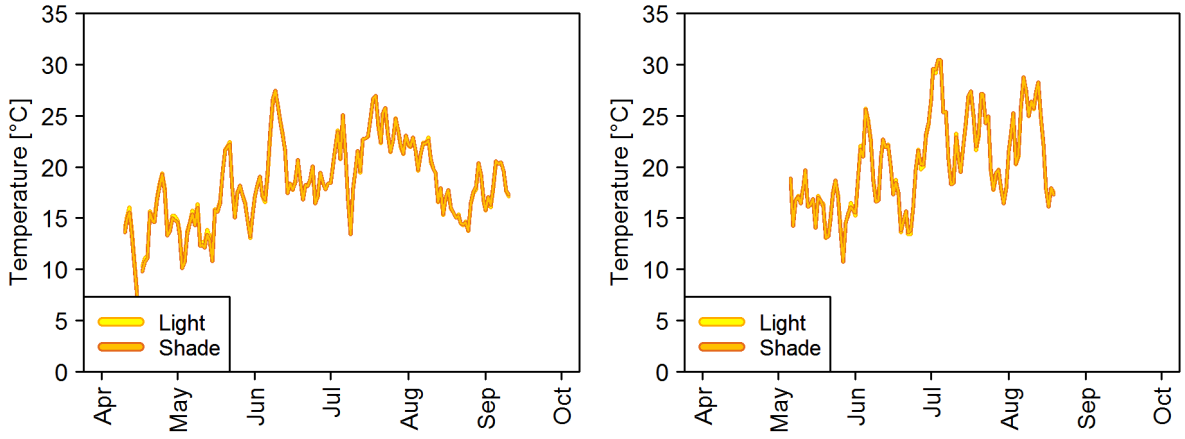

Fig. 15: Differences in temperature between light and shade treatment during the vegetation periods 2014 and 2015. Field experiment Gross-Gerau. Left-hand side 2014, right-hand side 2015.

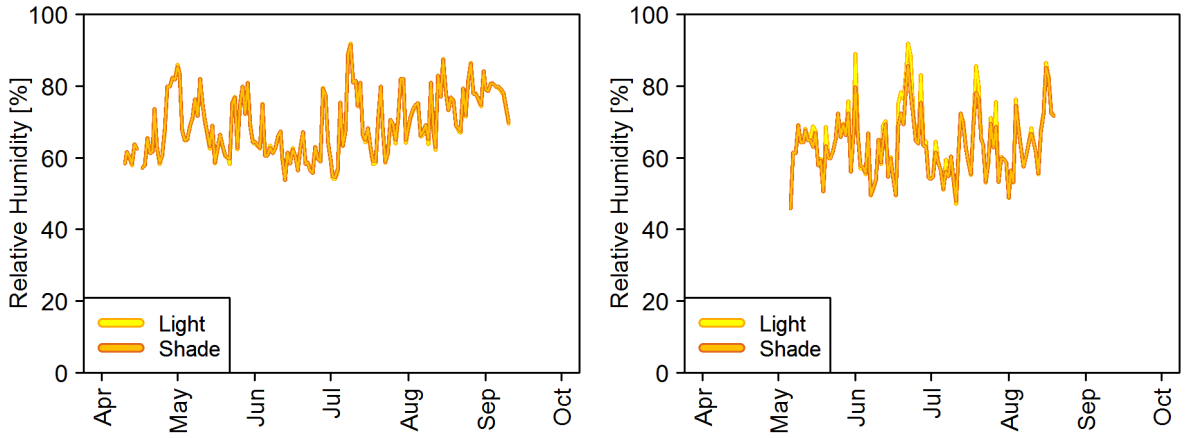

Fig. 16: Differences in relative humidity between light and shade treatment during the vegetation periods 2014 and 2015. Field experiment Gross-Gerau. Left-hand side 2014, right-hand side 2015.

3.2.1.2 Field experiment Rauischholzhausen

The field trial at the experimental station Rauischholzhausen was established in 2012 after the pre-crop grass clover. The pre-grown plantlets were transferred to the field on June 6, 2012. A block design was established as shown in **Fig. 17**, with four blocks within each of the two light treatments (natural sunlight vs. strong shading). In 2015, a modified design was established, as shown in **Fig. 18**, due to substantial plant losses on two blocks, as well as to the investigation of only the medium harvest stage.

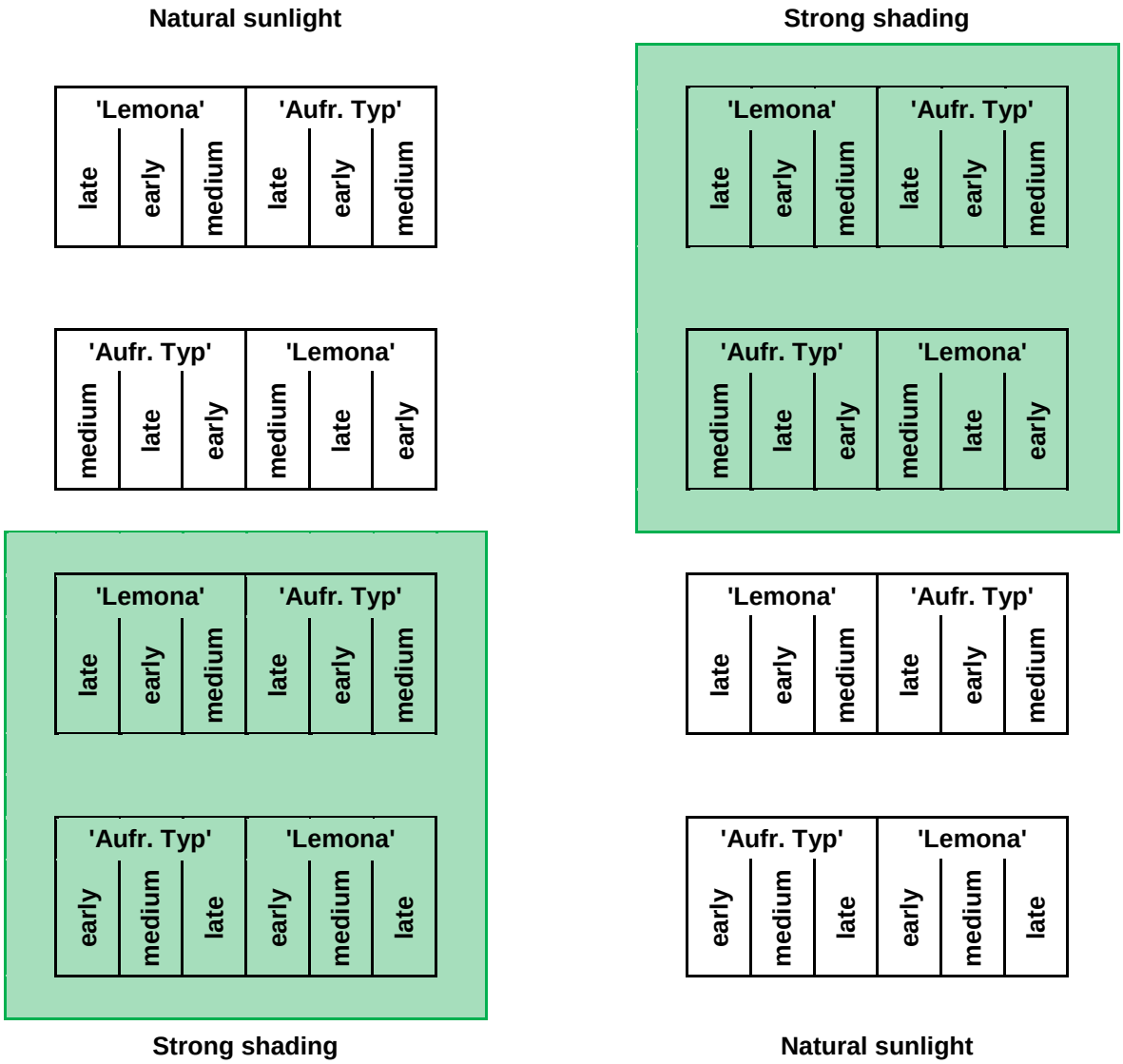

Fig. 17: Design of the field experiment in Rauischholzhausen 2013–2014. Two light treatments (natural sunlight and strong shading) with four blocks each. Every block consisted of two genotype plots, and three subplots for the three harvest stages.

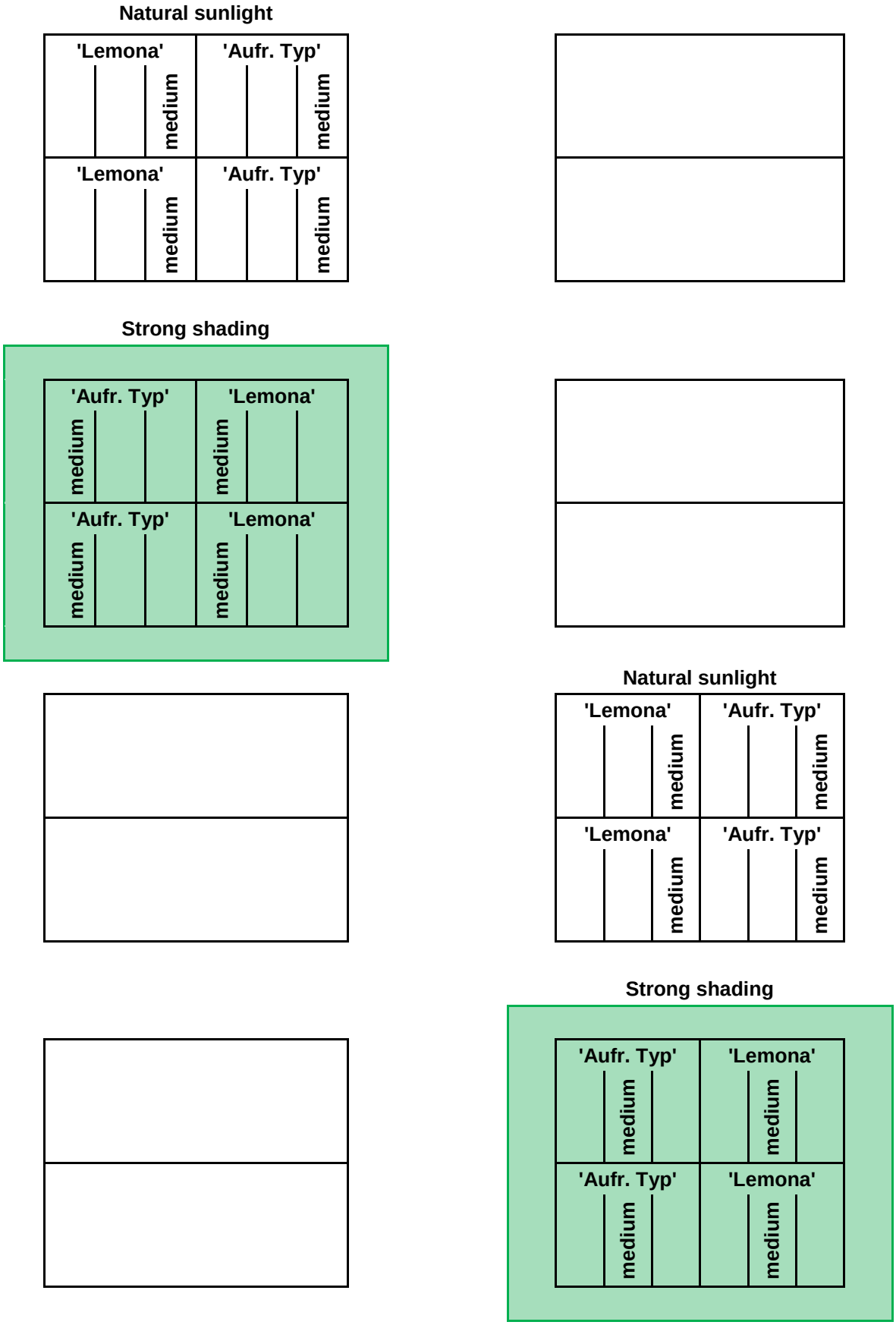

Fig. 18: Design of the field experiment in Rauischholzhausen 2015. Two light treatments (natural sunlight and strong shading) with four blocks each. Every block consisted of two genotype plots. Only the subplots at medium harvest stage were investigated in 2015. Empty plots were not harvested.

All blocks within each of the two light treatments consisted of two plots for the two genotypes, and three subplots within each genotype plot representing the three harvest stages (**Fig. 19**). Each subplot, measuring 5.0 x 1.5 m (7.5 m^2) comprised four rows of 14 lemon balm plants, giving 56 plants per subplot. Only the inner parts were regarded as harvest plots to exclude a possible border effect, resulting in a harvest plot size of 3.21 m^2, from which 24 plants were harvested for the investigations (2013 and 2014). Harvest plot size in 2015 was 1.61 m^2 (12 plants).

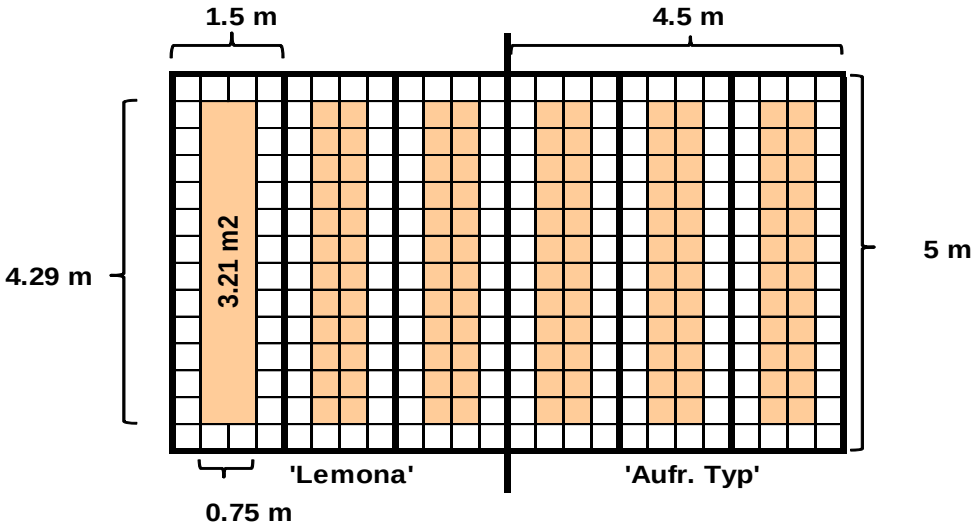

Fig. 19: Plot dimensions of the field experiment Rauischholzhausen 2013–2014. Each little square represents one plant. The colored parts are the harvest plots. Three subplots within each genotype plot for the investigation of the three harvest stages.

To reach a homogenous plant development and growth in the first harvest year, the lemon balm plants of all plots were topped in spring after the first winter (May 21, 2013). Harvest dates for the years 2013, 2014, and 2015 are presented in **Tab. 6**.

Plants received nitrogen fertilization in the form of calcium ammonium nitrate, with 50 kg N/ha every spring, and an additional 30 kg N/ha after the first cut in 2013.

Light reduction in 2013 and 2014 was realized with a dark green silage protection cover (Tec240, Zill GmbH & Co. KG, Lauingen, Germany). Due to technical reasons, the shading net was set up not until after the first cut in 2013. In 2015, the shading of the plants was accomplished by using a dark green shading net (50 g/m^2, shading value approx. 50%, Accura NTV KG, Ulm, Germany). Light intensity was registered with the same type of sensors as described above for the field experiment in Gross-Gerau. Registration of light intensity (PAR and global radiation) took place in the periods July 31 to September 23, 2013, May 3 to September 28, 2014, as well as June 4 to August 24, 2015 (**Fig. 20** and **Fig. 21**).

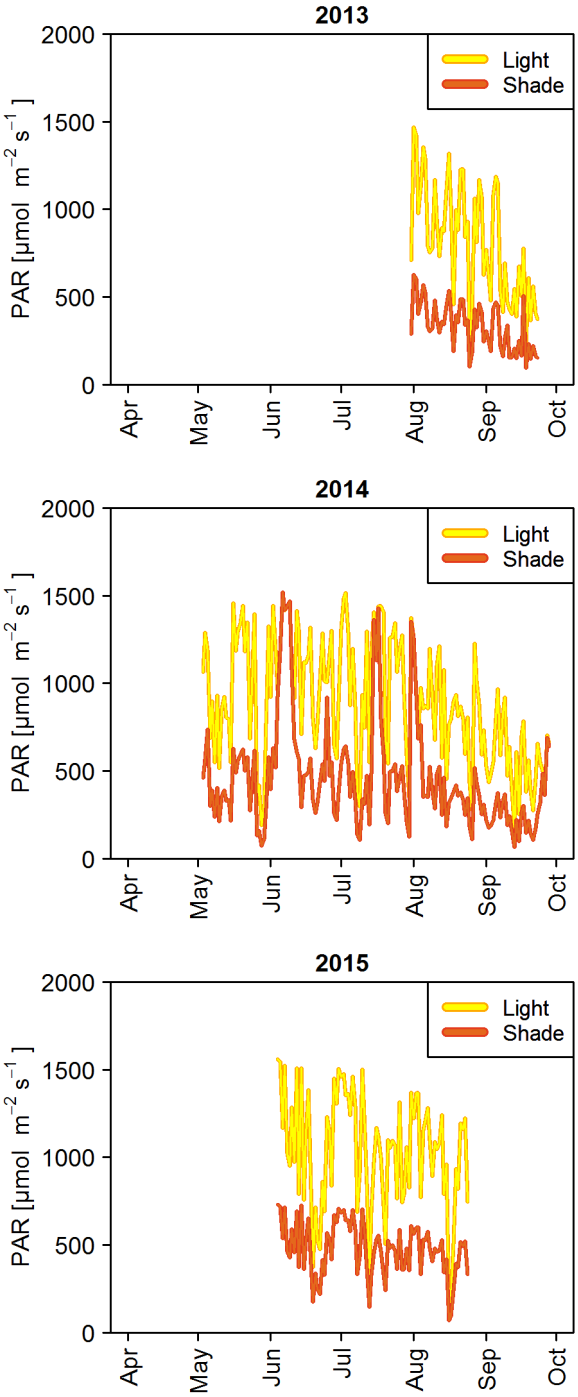

Fig. 20: Photosynthetically active radiation (PAR) under full sunlight and strong shading. Field experiment Rauischholzhausen, 2013–2015. Daily averages between 08:00 and 18:00 h.

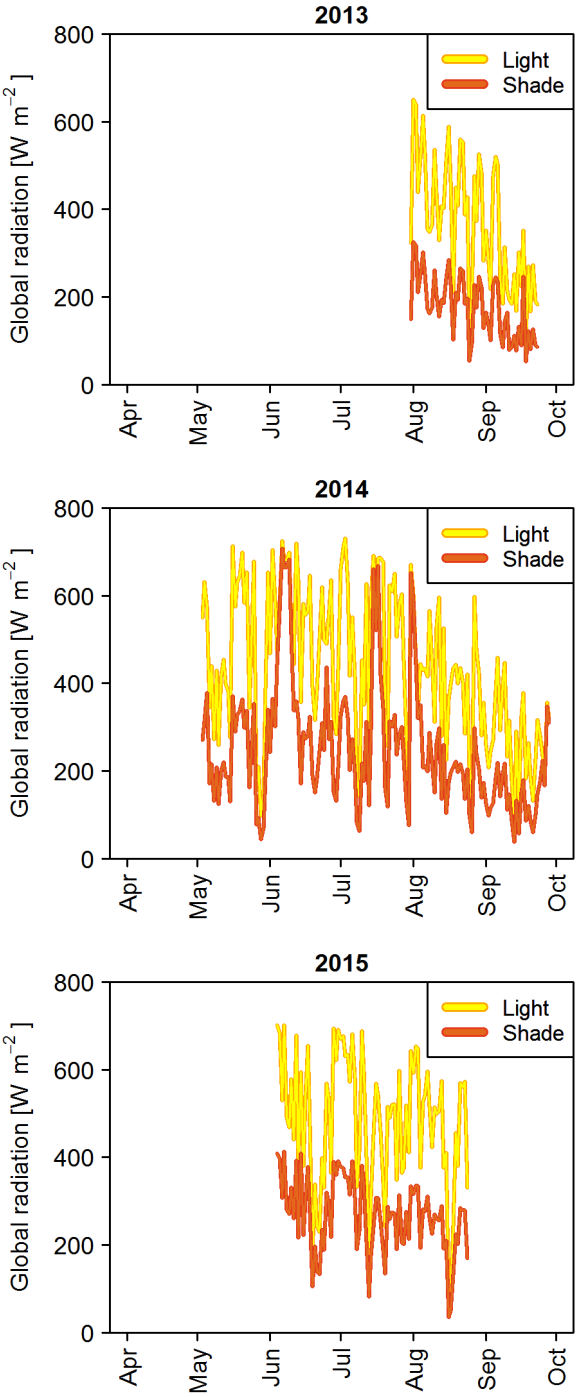

Fig. 21: Global radiation under full sunlight and strong shading. Field experiment Rauischholzhausen, 2013–2015. Daily averages between 08:00 and 18:00 h.

Light reduction, calculated from the differences between the light measurements in each of the two light variants from 8:00 till 18:00 h, was calculated for the years 2013, 2014, and 2015 as 59.0%, 51.3%, as well as 54.5% on the basis of PAR values, and 52.1%, 45.3%, as well as 45.2% on the basis of total radiation. The light reduction established in Rauischholzhausen will be termed as "strong shading" in this work.

The influence of the shading net on temperature (**Fig. 22**), as monitored by data loggers in both light treatments during the vegetation period, was negligible. On average over the vegetation period, temperature under the shading net was reduced by less than 0.5 °C.

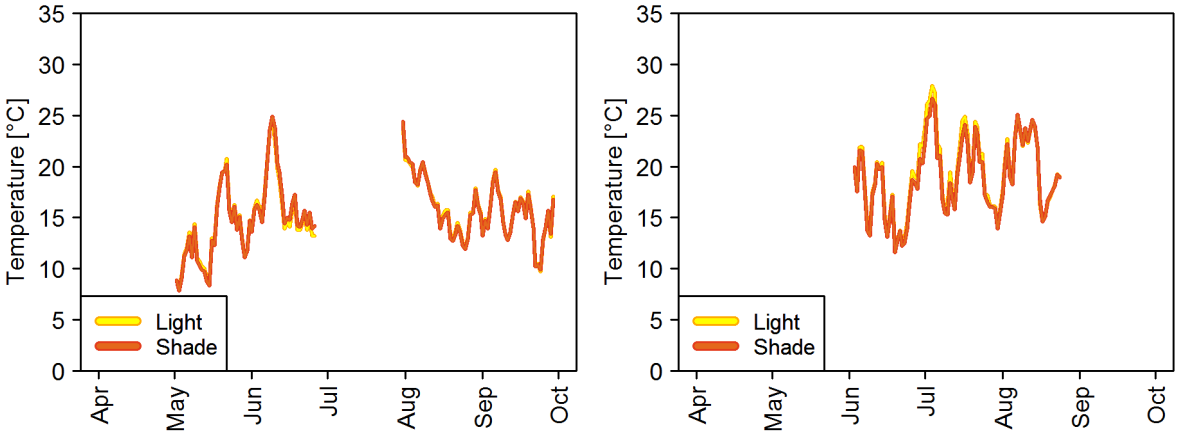

Fig. 22: Differences in temperature between light and shade treatment during the vegetation periods 2014 and 2015. Field experiment Rauischholzhausen. Left-hand side 2014, right-hand side 2015. Missing values are caused by a broken data logger.

An increase of relative humidity (**Fig. 23**), averaged over the registration period, was less than 0.1% in 2014, and around 2.1% in 2015. However, in 2015, a period of around four weeks from end of June to end of July with an increased relative humidity of around 5% under the shade net was observed. Missing values for temperature and relative humidity in 2014 have been caused by a damaged data logger.

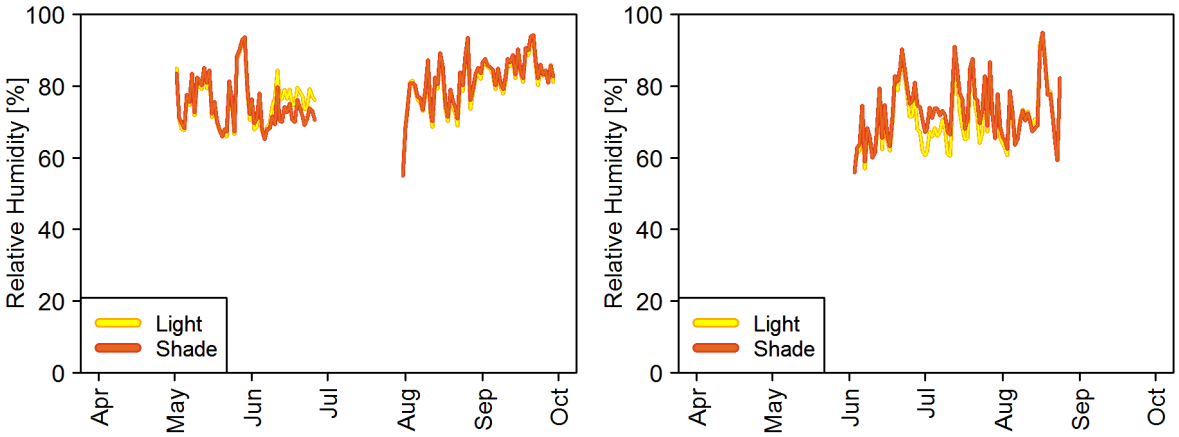

Fig. 23: Differences in relative humidity between light and shade treatment during the vegetation periods 2014 and 2015. Field experiment Rauischholzhausen. Left-hand side 2014, right-hand side 2015. Missing values are caused by a broken data logger.

Tab. 6: Harvest dates, vegetation days, air temperature, precipitation and irrigation of the field experiment in Rauischholzhausen.

Year	Cut	Harvest stage	Harvest date	Vegetation days [(a)]	Air temperature [° C]		Precipitation [mm] [(a)]	Irrigation [mm] [(a)]
					Average temperature [(a)]	Cumulative temperature [(a) (b)]		
2013	Cut 1	Early	04.07.2013	44	15.0	439.8	114.5	n.a.
		Medium	11.07.2013	51	15.6	541.5	114.7	n.a.
		Late	18.07.2013	58	15.9	630.8	114.7	n.a.
	Cut 2	Early	10.09.2013	68	19.4	977.1	83.2	n.a.
		Medium	17.09.2013	68	18.6	927.7	117.0	n.a.
		Late	24.09.2013	68	17.9	877.7	125.3	n.a.
2014	Cut 1	Early	04.06.2014	95	10.8	557.3	121.5	n.a.
		Medium	25.06.2014	116	11.9	810.5	135.5	n.a.
		Late	15.07.2014	136	12.7	1052.8	252.9	n.a.
	Cut 2	Early	31.07.2014	57	18.2	754.4	162.1	n.a.
		Medium	25.09.2014	92	17.1	1110.4	275.7	n.a.
		Late	29.09.2014	76	16.9	904.4	158.5	n.a.
2015	Cut 1	Medium	16.06.2015	107	10.4	601.1	104.8	n.a.
	Cut 2	Medium	24.08.2015	69	19.5	1000.5	131.4	n.a.

[(a)] Starting from March 1 until harvest date for cut 1, and for cut 2 starting from the preceding harvest date. For cut 1 in 2013, starting from topping the plants on May 21.

[(b)] Cumulative temperature calculated as the sum of daily temperatures above the basal temperature of 5 °C, starting from March 1.

3.2.2 Plant parameters

The plant parameters plant height, leaf area index (LAI), SPAD values (Soil & Plant Analyzer Development), and number of shoots per plant were determined on the day of each harvest.

For the determination of the LAI, a SunScan Canopy Analysis System (Delta-T Devices Ltd, Cambridge, UK) was utilized. In every harvest plot, two measurements were taken at two different positions within the plant stand, and the average of these two values was used for further calculations. SPAD values, as a representation of the relative chlorophyll content of the plants, were determined with a Chlorophyll Meter SPAD-502Plus (Konica Minolta, Japan). Six measurements were taken per harvest plot, from the first fully developed leaves on six randomly chosen representative plants, and averaged. Plant height of the harvest plots is the mean value of ten measurements taken at different positions of the plots. The number of shoots in a randomly mixed 400 g sample of the harvested plant material was counted, and taking into account the amount of harvested biomass per plot and number of plants per plot, the average number of shoots per plant was calculated.

3.2.3 Biomass and leaf yield

The harvest of the plants was performed manually. The harvested plant material of each plot was weighed to give the fresh matter biomass yield of the plots. On a representative, randomly mixed 300 g sample, a manual separation into leaves and stems was performed. The percentage of fresh leaves from this sample was used to determine the fresh matter leaf yield of each plot. Leaves and stems of this sample were separately dried at 105 °C for 24 h, and their weight determined for the further calculation of dry matter biomass yield, dry matter leaf yield, as well as the leaf:stem ratio (calculated on a dry matter basis). The remaining major part of the harvested plant material was air-dried at less than 40 °C until dryness (approx. 10% final humidity content) for the determination of the laboratory parameters. Biomass and leaf yield were calculated as dt/ha.

3.3 Laboratory analysis

3.3.1 Plant material

From the air-dried plant material (dried at less than 40 °C until dryness), a representative sample was taken, and the leaves were manually separated from the stems. For methanolic and ethanolic extraction (see below), samples of dried lemon balm leaves were ground to a fine powder in a coffee grinder, and, if extraction could not follow immediately, stored at -20 °C until extraction. For further calculations, DM content of the dried leaves was determined in duplicate by drying about 5 g of the dried leaves at 105 °C for 3 h until weight consistency, and calculating DM content according to the weight loss.

3.3.2 Chemicals and reagents

Acetonitrile, methanol and phosphoric acid as well as rosmarinic acid were purchased from Carl Roth (Germany). HPLC water was obtained from Fisher Scientific (UK). Folin-Ciocalteu's phenol reagent, potassium dihydrogen phosphate, di-potassium hydrogen phosphate, sodium carbonate, as well as ethanol were purchased from Merck (Germany). Gallic acid monohydrate, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), fluorescein sodium salt, and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) were purchased from Sigma Aldrich (Germany). Solvents used for HPLC analysis were of gradient grade.

3.3.3 Essential oil content

Before the distillation, the air-dried plant material was separated into leaves and stems. 30 g of the dried leaves were then hydro-distilled in duplicate in a clevenger-type apparatus for 2 h (Ph. Eur. 7, 2011). The essential oil content of the dried leaves was determined gravimetrically. The average of the duplicates was used for statistical analysis.

3.3.4 Methanolic extraction

An amount of 50 mg of the powdered leaves was extracted with 12.5 ml of 80% methanol for 30 min in an ultrasonic bath. Afterwards, the extract was diluted with 12.5 ml H_2O, filtered through 615 ¼ filter papers (Macherey & Nagel), and stored until analysis at -20 °C. The obtained extract was used for both Folin-Ciocalteu assay (total phenolic content) and ORAC assay (antioxidant capacity).

3.3.5 Total phenolic content

For the determination of the total phenolic content (TPC), 40 µl of the methanolic extracts were mixed with 3.16 ml H_2O and 200 µl Folin-Ciocalteu's phenol reagent. After incubation at room temperature for 5 min, 600 µl saturated sodium carbonate solution were added. The mixture was then incubated for 30 min at 40 °C in a water bath, and cooled down in cold water for 5 min before the measurements. The extinction was measured photometrically at 765 nm (SPECORD 205, Analytic Jena). Gallic acid served as a standard, in a concentration range from 50 to 500 µg/ml. The results are presented as milligram gallic acid equivalents (GAE) per gram DM.

3.3.6 Antioxidant capacity

For the determination of the antioxidant capacity, the ORAC (Oxygen Radical Absorbance Capacity) assay was used. The water-soluble vitamin E analogue trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) served as a standard, in a concentration range from 50 to 125 µmol/ml. As a fluorescence dye, fluorescein (6-Hydroxy-9-(2-carboxyphenyl)-(3*H*)-xanthen-3-on) was utilized. AAPH served as the free-radical generator. All reagents were dissolved in potassium phosphate buffer (75 mM, pH 7.4), and samples were diluted 1:40 in the same buffer.

25 µl of standard solution or sample were pipetted on a 96 well plate according to the pipetting scheme in **Fig. 24**, mixed with 150 µl of a fluorescein solution (0.02 µM), and preincubated at 37 °C for 30 min. After addition of 25 µl of a freshly prepared AAPH solution (153 mM) to each well, the fluorescence signal (excitation wavelength 485 nm, emission wavelength 538 nm) was detected every 90 s for 90 min using a fluorescence photometer (Fluoroskan Ascent FL, Thermo SCIENTIFIC). Results are presented as µmol trolox equivalents (TE) per gram DM.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	125	125	125	125	125	125	125	125	125	125	125
B	0	100	100	100	100	100	100	100	100	100	100	100
C	0	75	75	75	75	75	75	75	75	75	75	75
D	0	50	50	50	50	50	50	50	50	50	50	50
E	0	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a
F	0	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a
G	0	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a
H	0	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a

Fig. 24: Pipetting scheme for ORAC assay on a 96-well plate. In column 1, the blank is pipetted. In the remaining columns, sample extracts are pipetted in rows E–H, with four wells for each extract, and two extracts (a and b) of the same sample in the adjacent columns. Standards are pipetted in rows A–D, leading to separate calibration curves for each sample.

3.3.7 Rosmarinic acid content

Rosmarinic acid (RA) content was determined with an HPLC method based on the European Pharmacopeia (Ph. Eur. 7, 2011). 50 mg of dried powdered leaves were extracted under reflux with 45 ml aqueous ethanolic solution (50% ethanol) for 30 min, the extract filtered through 615 ¼ filter papers (Macherey & Nagel) into a volumetric flask (50 ml), the filter washed with the aqueous ethanolic solution, and the extract made up to volume. An aliquot of the extract was filtered through a 0.45 µm syringe filter into HPLC vials, and stored until analysis at -20 °C, if HPLC analysis could not be performed directly after the extraction. HPLC Analysis was performed with a Smartline HPLC System (Knauer, Germany) on a Knauer Eurospher II 100-5 C18 column (length × inner diameter: 150 × 4.0 mm) at 30 °C, with detection at 330 nm by a photodiode array detector (UV Detector 2600; Knauer, Germany). Eluent A consisted of phosphoric acid 85%, acetonitrile, and water (1:19:80, v/v/v), and eluent B of phosphoric acid 85%, methanol, and acetonitrile (1:40:59, v/v/v). The following gradient was used for the separation, at a flow rate of 1 ml/min: 0 min, 0% B; 10.9 min, 45% B; 13.6 min, 100% B; 16.3 min, 0% B. The gradient was held with the initial conditions for another 15.7 min after the run before the next injection. Identification of RA was based on a comparison with the retention time and absorption spectrum of a reference standard. RA quantification was performed with a calibration curve of the RA standard of known concentrations (20 to 80 µg/ml).

3.4 Statistical analysis

Statistical analysis was performed with R (Version 3.3.1) (R Core Team, 2015), with the additional packages lmerTest, lsmeans, lme4, and multcomp. Analysis of variance (ANOVA) of a mixed linear model was calculated with the fixed factor light. The results have been analyzed statistically in two steps: In a first step, calculation of a three-way ANOVA was performed to determine the effect of light, genotype, and harvest time, as well as possible interactions. In a second step, a two-way ANOVA followed, with the factors light and harvest time calculated for each of the tested genotypes separately. Means of the different treatments were calculated as least-squares means (LS means). For the pairwise comparisons, contrasts were calculated with Tukey adjustment. Pearson's test was used for the calculation of correlations.

The tables with the results of the three-factorial analysis, which will be referred to in the text, can be found in the appendix. The results of the two-factorial analysis, split up by the different genotypes, will be presented in the following chapter in the form of box plots. Due to the high number of results, the box plots of each investigated parameter are separately illustrated for the two cuts (except for LAI values, where less data were available). Within the figure of each cut, the tested genotypes are presented one below the other, whereas the investigated years are shown next to one another. The p-values for the investigated factors light, harvest time, and the interaction light*harvest time are presented below the single diagrams.

4 Results

4.1 Field experiment Gross-Gerau

4.1.1 Plant parameters

4.1.1.1 Plant height

The lemon balm plants were reaching heights of less than 20 cm to about 80 cm, depending on genotype, harvest stage, cut, and year. Generally, the plants of the first cuts were higher than those of the second cuts.

The three-factorial analysis (**Tab. A 1**) revealed significant differences between the genotypes in all years and cuts, except for the first cut in 2015. For the second cut in 2013, however, this genotype effect occurred in combination with the two-way interactions light*genotype and genotype*harvest stage. In all the cases where a genotype effect was significant, 'Lemona' was found in the groups with the lowest plant height. For the first cut in 2013, 'Lemona' reached a plant height of 37.3 cm, which was significantly lower than 'NLC' (48.1 cm), but not significantly different from 'Aufrechter Typ' (38.4 cm). For the first cut in 2014, 'Lemona' exhibited a plant height of 61.7 cm, which was significantly lower than 'Aufrechter Typ' (68.4 cm), while 'NLC' (63.9 cm) was not significantly different from the other two genotypes. For the second cut in 2013, where an interaction effect was found, 'Lemona' still had significantly lower plant heights than 'NLC' at all three harvest stages. Also for the second cuts in 2014 and 2015, 'Lemona' (18.1 cm and 18.9 cm) remained significantly lower than 'NLC' (38.5 cm and 44.6 cm). With a plant height of 27.2 cm, 'Aufrechter Typ' was, significantly higher than 'Lemona', but significantly lower than 'NLC' for the second cut in 2014, and with 24.5 cm not significantly different from 'Lemona' for the second cut in 2015.

To further elucidate the different reactions of the tested genotypes, a two-factorial analysis followed, whose results are shown in **Fig. 25** (first cut) and **Fig. 26** (second cut). For the first cut (**Fig. 25**), only a tendency of increasing plant heights over time was observed in 2013 for the investigated genotypes, albeit not reaching significance. In 2014, however, the harvest time effect was clearly significant for all three genotypes, with significantly higher plant heights at medium and at late harvest stage, compared to early harvest stage. No significant effect of the moderate shading on plant height was observed for the first cut in all three years and all tested genotypes (**Fig. 25**).

For the second cut (**Fig. 26**), no significant differences between the harvest stages were observed in the year 2013 for 'Aufrechter Typ' (**Fig. 26 a**) and 'Lemona' (**Fig. 26 b**). However, 'Lemona' showed a significant influence of the moderate shading on the plant

height in this case, with the shaded plants being higher than the non-shaded plants (**Fig. 26 b**). For 'NLC', a harvest time effect was observed in 2013, with the plants harvested at the late stage being significantly higher than the plants harvested at the early stage, and additionally the shaded plants were significantly higher than the non-shaded plants (**Fig. 26 c**). However, for the second cut in 2014, no significant differences were observed for this genotype. 'Aufrechter Typ', on the contrary, showed a significant increase in plant height in this case from early to medium harvest stage, and a significant decrease to late harvest stage (**Fig. 26 a**), whereas for the genotype 'Lemona', an interaction effect light*harvest time was observed (**Fig. 26 b**). No effect of the moderate light reduction on plant height was observed in all three genotypes for the second cut in 2015 (**Fig. 26**).

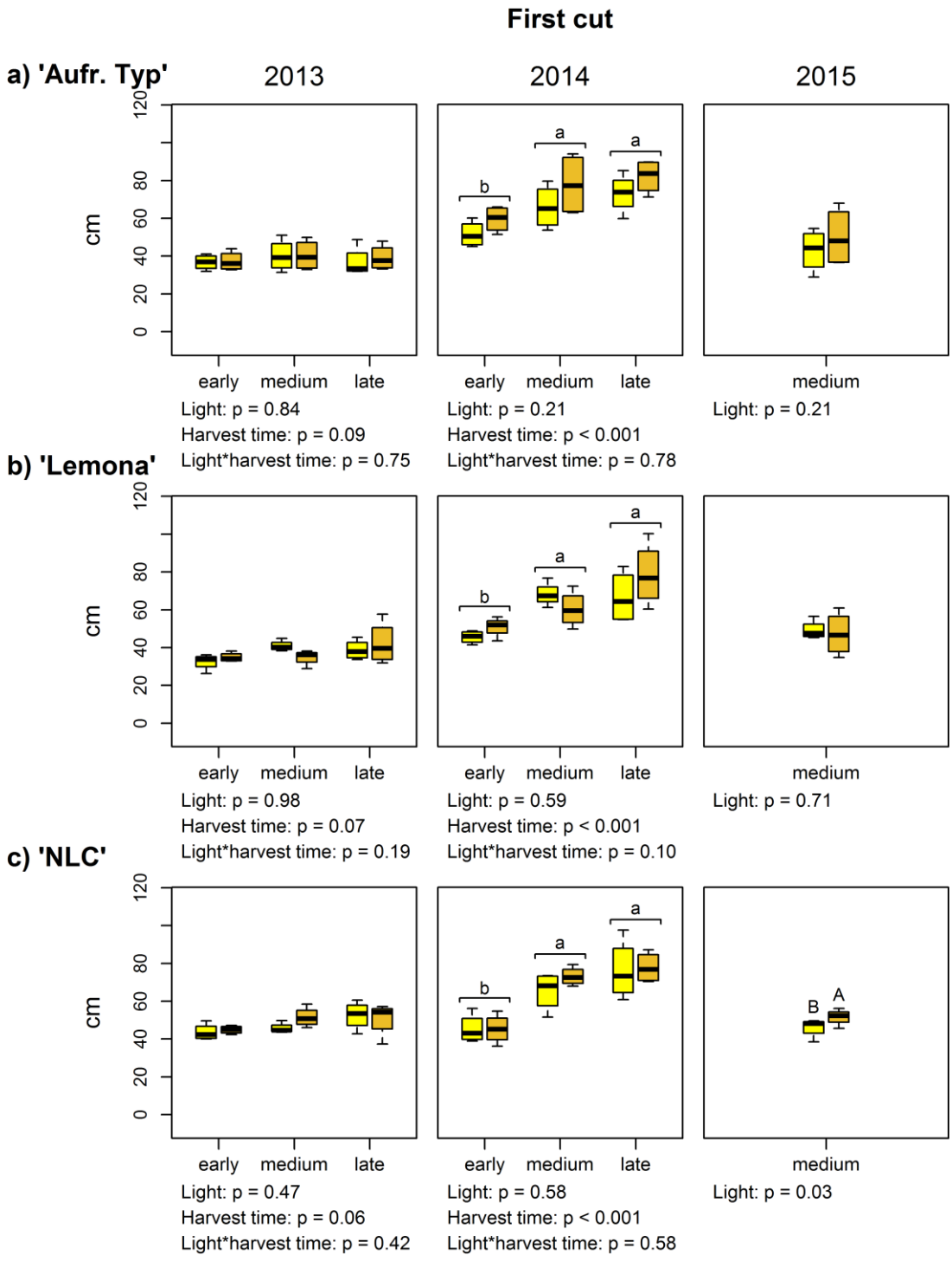

Fig. 25: Plant height [cm] of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

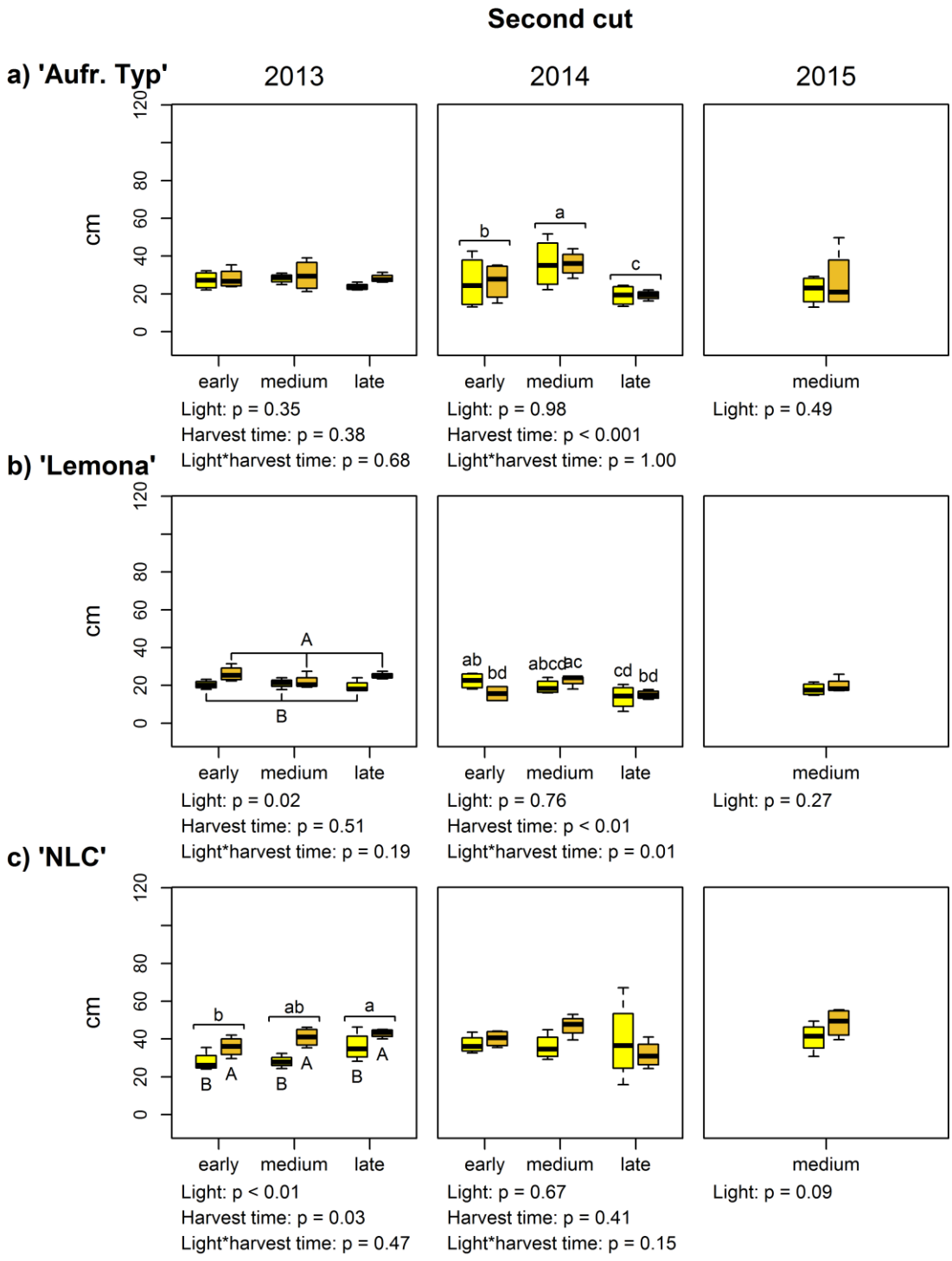

Fig. 26: Plant height [cm] of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; ■ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.1.1.2 Leaf area index (LAI)

The Leaf Area Index (LAI) at harvest time has only been determined in the years 2014 and 2015. The dimensionless LAI values were covering a range from about 3.1 to more than 11, depending on genotype, harvest stage, year, and cut within a year.

The three-factorial analysis (**Tab. A 2**) showed a significant genotype effect for both cuts in both years, albeit in combination with an interaction effect genotype*harvest stage for the first cut in 2013, which will be further elucidated below. 'NLC' had significantly lower LAI values than 'Aufrechter Typ' for early and medium harvest stage for the first cut in 2013. Also for the first cut in 2014, 'NLC', with an LAI of 8.9, was found in the group with the lower values, which was significantly less than 'Lemona' (10.6), but not significantly different from 'Aufrechter Typ' (10.0). However, for the second cuts of the years 2014 and 2015, 'NLC' had significantly higher LAI values (7.3 and 9.6) than 'Lemona' (5.7 and 6.4), whereas 'Aufrechter Typ' (6.7 and 6.9) was not significantly different from the other two genotypes.

The results of the two-factorial analysis of both cuts are presented in **Fig. 27**. For the first cut in 2014, a significant effect of the harvest stage was seen for 'Aufrechter Typ' and 'NLC', and an interaction light*harvest time occurred for 'Lemona'. For 'Aufrechter Typ', LAI of the late harvest stage was significantly decreased, compared to early and medium harvest stage (**Fig. 27 a**, left-hand side). 'NLC' showed a significantly decreased LAI already at medium harvest stage, from which the late harvest stage was not significantly different (**Fig. 27 c**, left-hand side). For 'Lemona', a significantly decreased LAI at late harvest stage, compared to early harvest stage, could be seen in both light treatments. However, at medium harvest stage, LAI of the non-shaded plants was not significantly different from the early harvest stage, while the opposite was true for the LAI of the shaded plants (**Fig. 27 b**, left-hand side). Otherwise, no effect of the moderate shading on LAI could be seen in the first cut of 2015, nor in the second cut in both years. For the second cut in 2014, no significant differences of the LAI between the different harvest stages were observed either (**Fig. 27**, right-hand side).

There was only a weak, but significant correlation between LAI and plant height ($r = 0.19$, $p < 0.01$) (**Fig. A 1**).

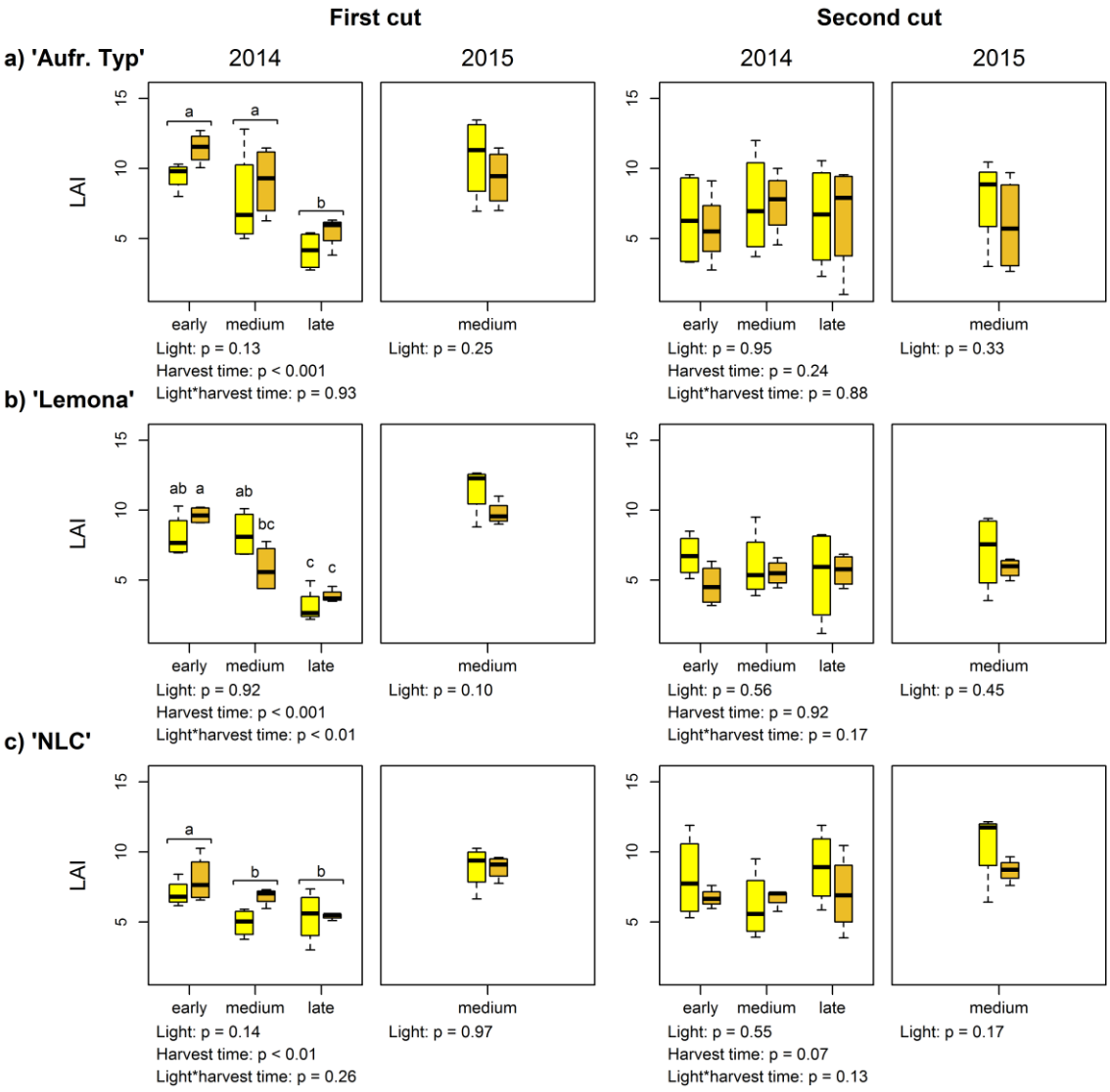

Fig. 27: Leaf Area Index (LAI) of lemon balm. First and second cut in Gross-Gerau, 2014 and 2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late) in two cuts (left-hand side: first cut; right-hand side: second cut). Boxes with different letters are significantly different ($p < 0.05$).

4.1.1.3 SPAD values

SPAD values, measured at the day of harvest as a representation of the chlorophyll content of the plants, were found in a range from about 20 until about 30 SPAD units. The three-factorial analysis (**Tab. A 3**) revealed significant differences between the investigated genotypes for the second cut in 2013, for both cuts in 2015, as well as (but only in combination with an interaction effect genotype*harvest time) for the second cut in 2014. For the second cut in 2013, 'Lemona' showed significantly higher SPAD values (29.8 SPAD units) than the other two genotypes, and 'Aufrechter Typ' (27.8 SPAD units) still was significantly higher than 'NLC' (25.0 SPAD units). For the second cut in 2014, 'Lemona' still exhibited significantly higher SPAD values than 'NLC' for the early harvest stage. For cut 1 and cut 2 in 2015, Lemona (23.8 and 25.1 SPAD units) showed significantly higher values than 'Aufrechter Typ' (20.8 and 21.4 SPAD units). In the first cut of 2015, 'NLC' reached 22.3 SPAD units, which was not significantly different from the other two genotypes, and in the second cut 20.2 SPAD units, which was not significantly different from 'Aufrechter Typ'.

The results of the two-factorial analysis are presented in **Fig. 28** (first cut) and **Fig. 29** (second cut). For the first cut, a clear decrease of the SPAD values from early to medium harvest stage could be observed in 'Aufrechter Typ' (**Fig. 28 a**) and 'NLC' (**Fig. 28 c**) in 2013 and 2014, with the late harvest stage not being significantly different from the medium harvest stage. The same was true for 'Lemona' in 2013, whereas the tendency of decreasing SPAD values over time was only almost significant ($p = 0.06$) in 2014 (**Fig. 28 b**). No significant influence of the moderate shading on SPAD values could be observed at all for the first cut (**Fig. 28**).

For the second cut in 2013 (**Fig. 29**), where the SPAD values for the early harvest stage were missing due to technical problems, no significant differences between medium and late harvest stage were observed. For the second cut in 2014, however, a significant increase of the SPAD values from early to late harvest stage could be seen in 'Aufrechter Typ' (**Fig. 29 a**) and 'NCL' (**Fig. 29 c**). Just like for the first cut, no significant effect of the moderate shading on the SPAD values was seen for the second cut in all investigated genotypes and years (**Fig. 29**).

There was no correlation between SPAD values and DM leaf yield ($r = 0.06$, $p = 0.26$; **Fig. A 2**), and a weak, but significant correlation between SPAD values and essential oil content ($r = 0.40$, $p < 0.001$; **Fig. A 3**).

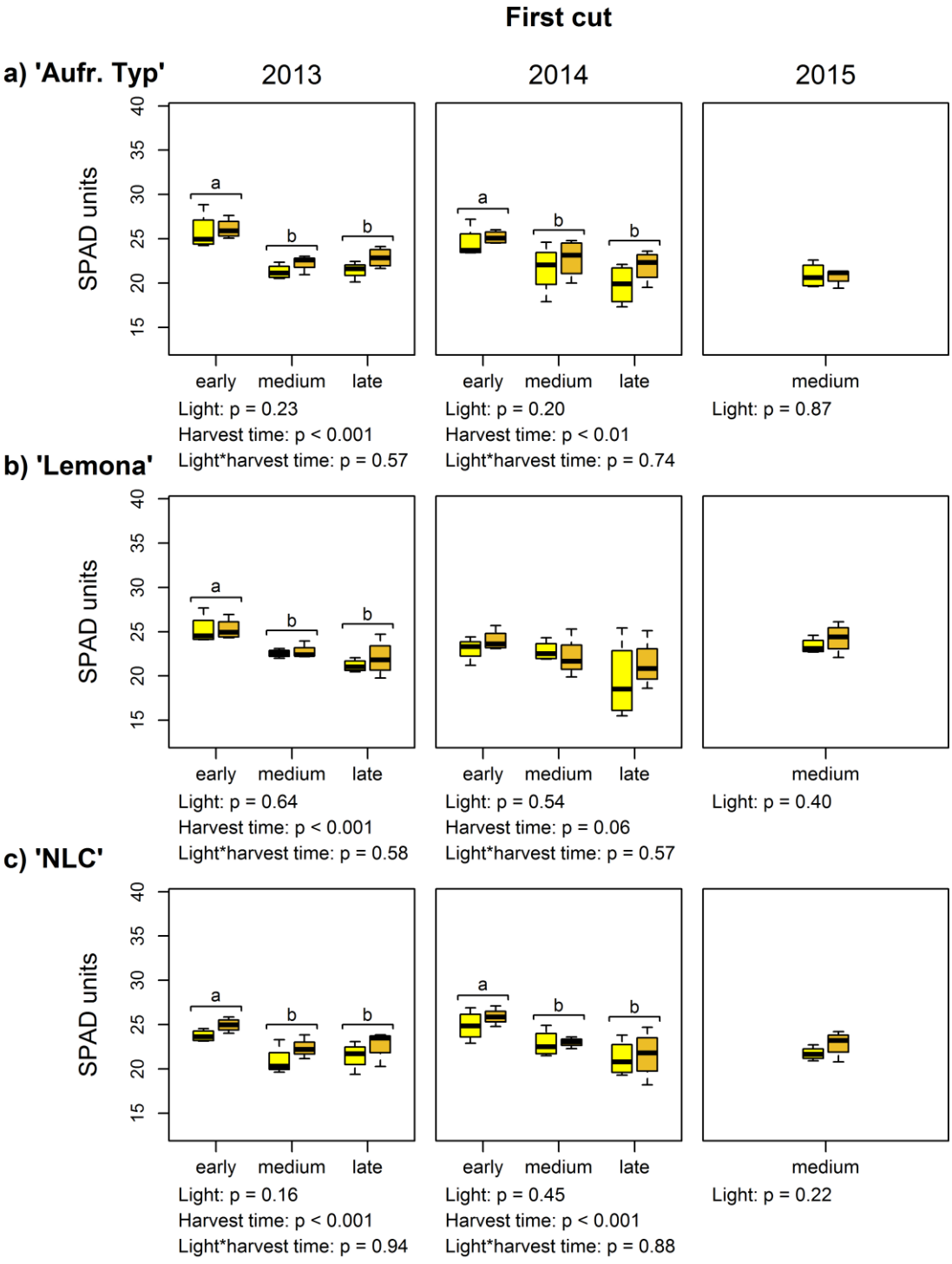

Fig. 28: SPAD values of lemon balm (first fully developed leaf). First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

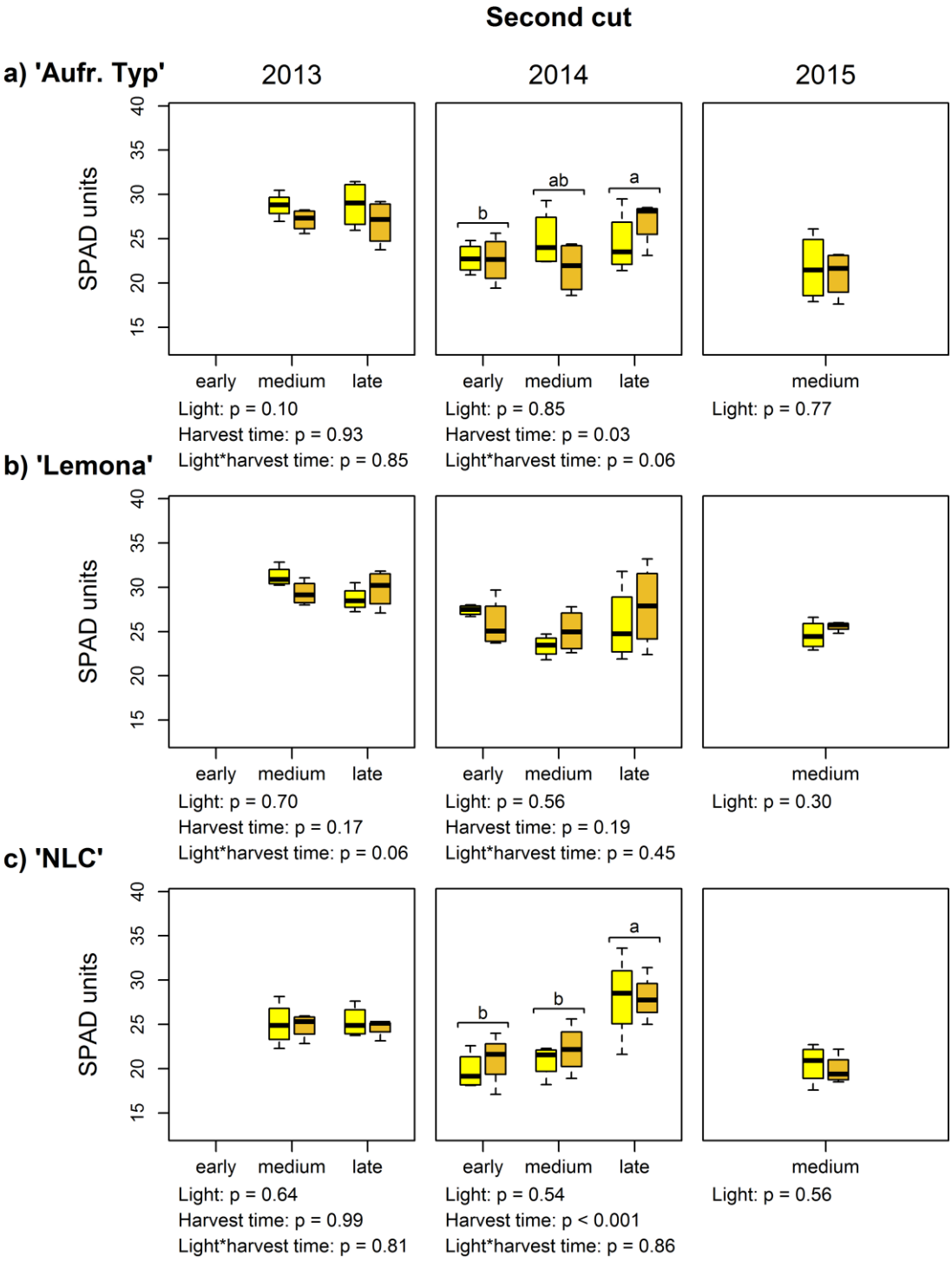

Fig. 29: SPAD values of lemon balm (first fully developed leaf). Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

4.1.1.4 Number of shoots per plant

The three-factorial analysis of the number of shoots per plant (**Tab. A 4**) showed a significant genotype effect for both cuts in the years 2013 and 2014, albeit in combination with an interaction effect genotype*harvest time for cut 2 in 2014. In 2013, 'Lemona' reached numbers of 35 and 41 shoots per plant for first and second cut, which was significantly more than 'Aufrechter Typ' (30 and 35 shoots per plant). 'NLC' did not differ significantly from the other two genotypes for cut 1 (with 34 shoots), and with 41 shoots per plant for cut 2, it was significantly higher than 'Aufrechter Typ', and not statistically different from 'Lemona'. For the first cut in 2014, 'Lemona' was once again found in the group with the highest number, reaching 69 shoots per plant, which was not significantly different from 'Aufrechter Typ' (61 shoots per plant), but significantly more than 'NLC' (40 shoots per plant). For the second cut in 2014, where an interaction effect genotype*harvest time was found, 'Lemona' still had significantly more shoots per plant at medium harvest stage, compared to 'NLC'.

The results of the two-factorial analysis are presented in **Fig. 30** and **Fig. 31**. For the first cut, no significant differences in the number of shoots per plant could be seen for the different harvest stages and light treatments in all three genotypes (**Fig. 30**). For the second cut, however, differences between the treatments could be observed (**Fig. 31**). In 2013, 'Aufrechter Typ' showed an increased number of shoots per plant from early to medium harvest stage, whereas the late harvest stage did not differ significantly from the medium harvest stage (**Fig. 31 a**). 'Lemona' also exhibited an increase from early to medium harvest stage, but then another significant increase for the late harvest stage (**Fig. 31 b**). Although the two-factorial ANOVA calculated a significant interaction effect light*harvest time for 'NLC', the following pairwise comparisons resulted in no different grouping of the different light treatments. However, a significant increase from early to medium harvest stage was observed in shaded and in non-shaded plants (**Fig. 31 c**).

For the second cut in 2014, the number of shoots per plant at the late harvest stage was significantly lower than at the early harvest stage for 'Aufrechter Typ' (**Fig. 31 a**). 'Lemona', however, exhibited a significantly higher number of shoots per plant at medium harvest stage, compared to early and late harvest stage, but only in the non-shaded plants (**Fig. 31 b**). No significant differences between the harvest stages were observed in 'NLC' (**Fig. 31 c**), neither was an influence of the moderate shading significant for the second cut in 2015 for any of the investigated genotypes (**Fig. 31**).

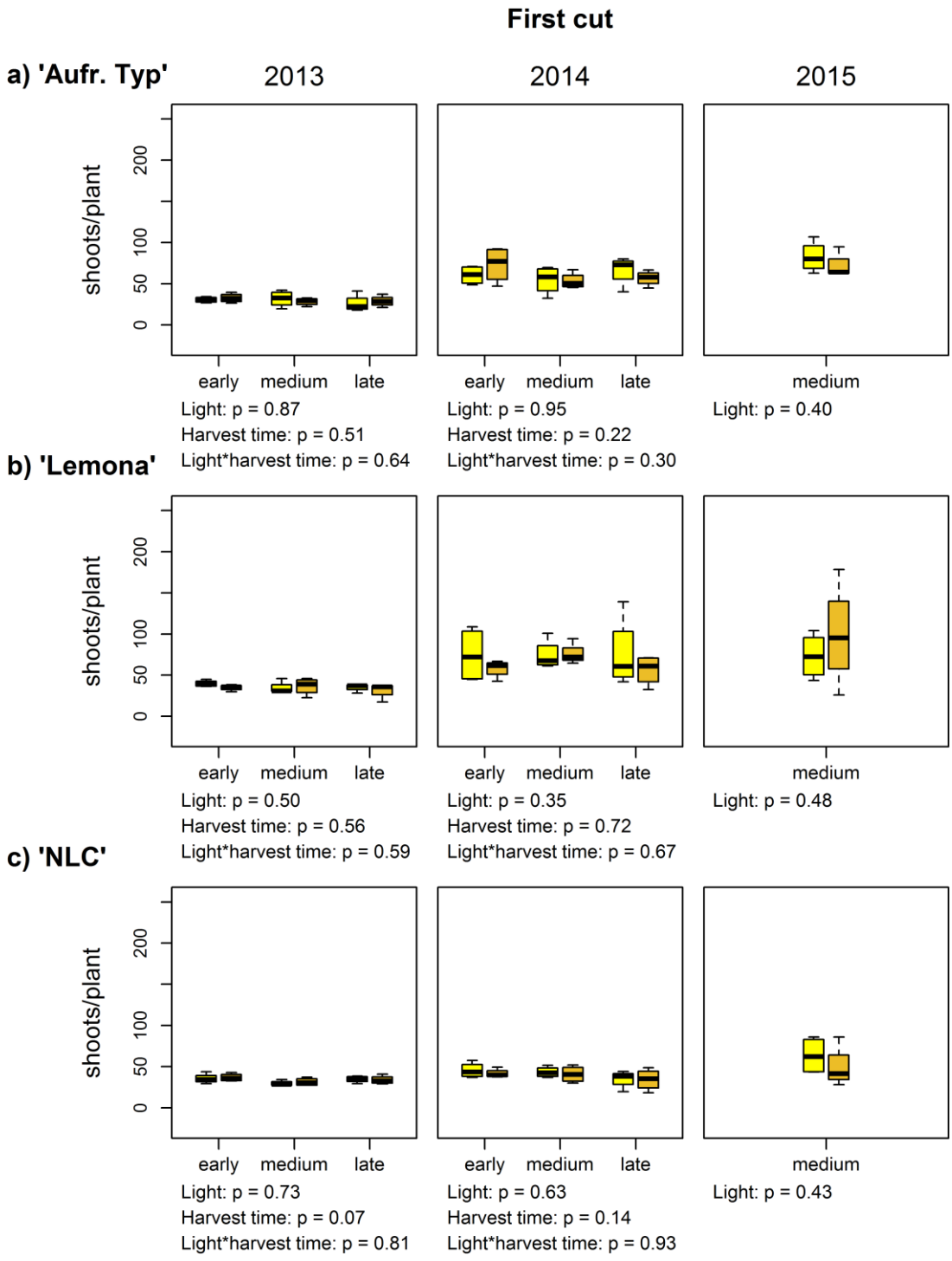

Fig. 30: Shoots per plant of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

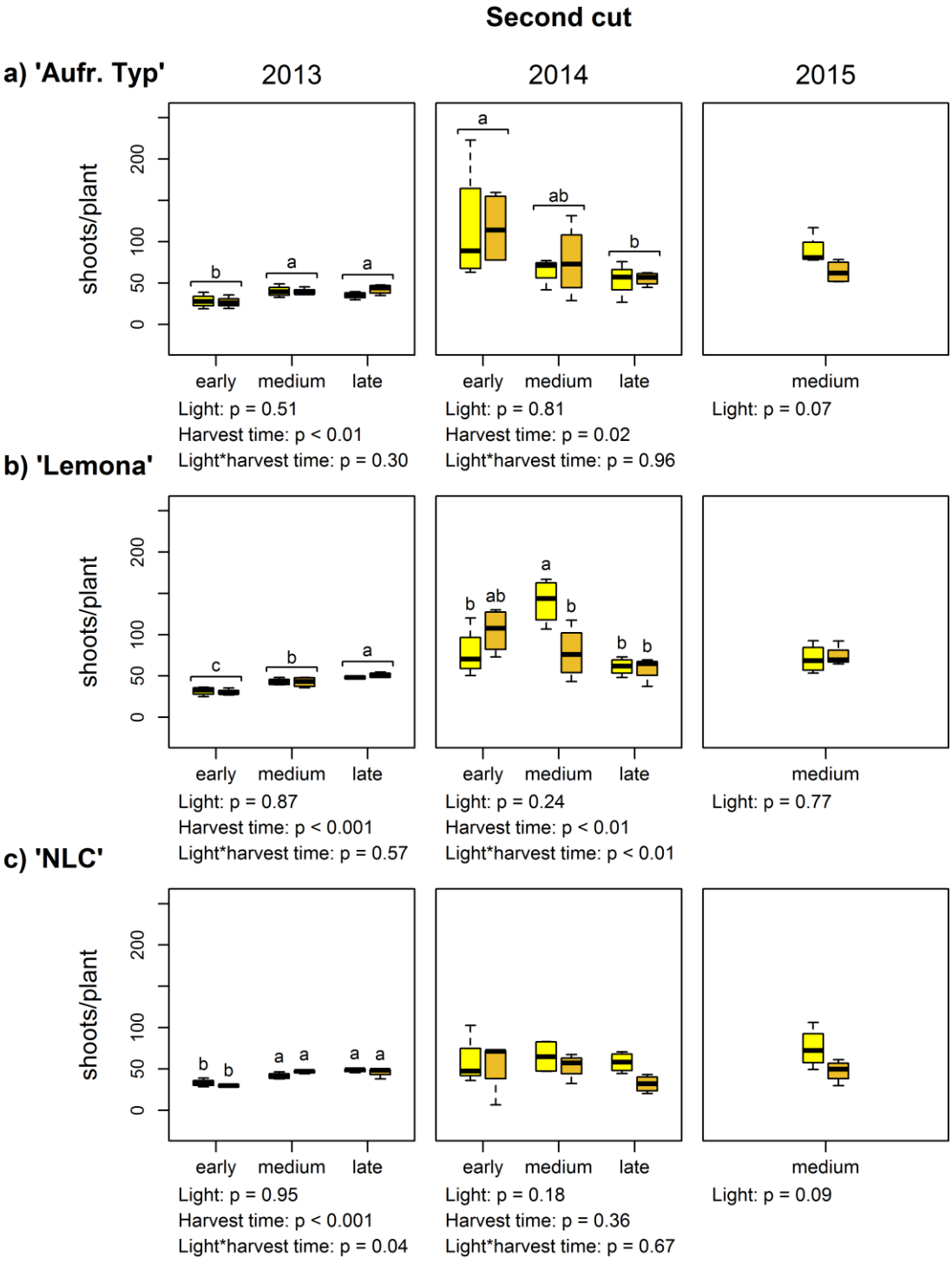

Fig. 31: Shoots per plant of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; ■ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

4.1.2 Yield parameters

4.1.2.1 Biomass yield (FM)

The biomass yield (FM) was found in a range from 60.7 to 243.6 dt FM/ha, depending on genotype, light intensity, harvest stage, year, and cut within the year (**Tab. A 5**). For the variant "natural light" and "medium harvest stage", in the following regarded as the reference variant, annual sums of 190.4, 293.9, as well as 249.3 dt FM/ha were harvested for 'Aufrechter Typ', 190.7, 228.6, as well as 297.2 dt FM/ha for 'Lemona', and 187.6, 211.2, as well as 247.2 dt FM/ha for 'NLC' in the years 2013, 2014, and 2015, respectively. The yields of the second cuts were around a third lower than those of the first cuts in 2014 and 2015, whereas both cuts reached a similar level in 2013.

No negative effect of the moderate shading could be observed on the biomass yield (FM). However, the three-factorial analysis (**Tab. A 5**) revealed a statistically significant genotype effect in 2013 and 2014, where 'Lemona' was always in the group with the lowest values. During these years, the biomass yield of 'NLC' was generally at a high level, being significantly higher than 'Lemona', except for the first cut in 2014, where the genotype 'Aufrechter Typ' reached a significantly higher biomass yield than the other two tested genotypes. In 2015, there were no significant differences between the genotypes for cut 1, whereas a possible genotype effect was only marginally above the significance level for cut 2, with a tendency of higher values for 'NLC' compared to 'Aufrechter Typ', and 'Lemona' being in between.

An expected significant increase of the biomass yield (FM) from early to late harvest stage could not be observed, except for cut 2 in 2013 for 'NLC' (**Fig. 33 c**), and in 'Lemona' from medium to late harvest only for the shaded plants (**Fig. 33 b**). In 2014, 'Aufrechter Typ' even showed a significant decrease from early to late harvest stage in the first cut (**Fig. 32 a**), and from medium to late harvest stage in the second cut (**Fig. 33 a**).

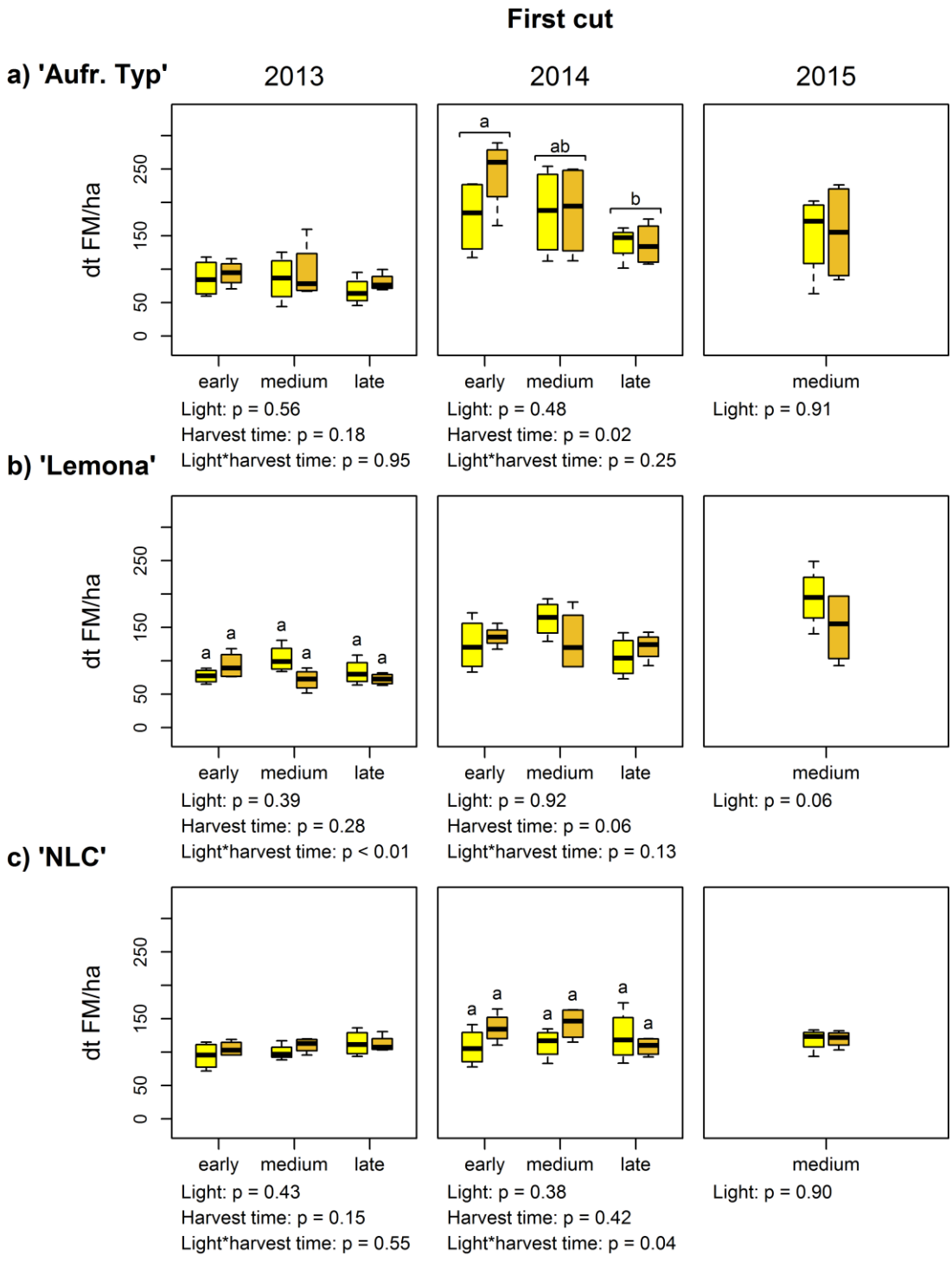

Fig. 32: Fresh matter biomass yield [dt FM/ha] of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

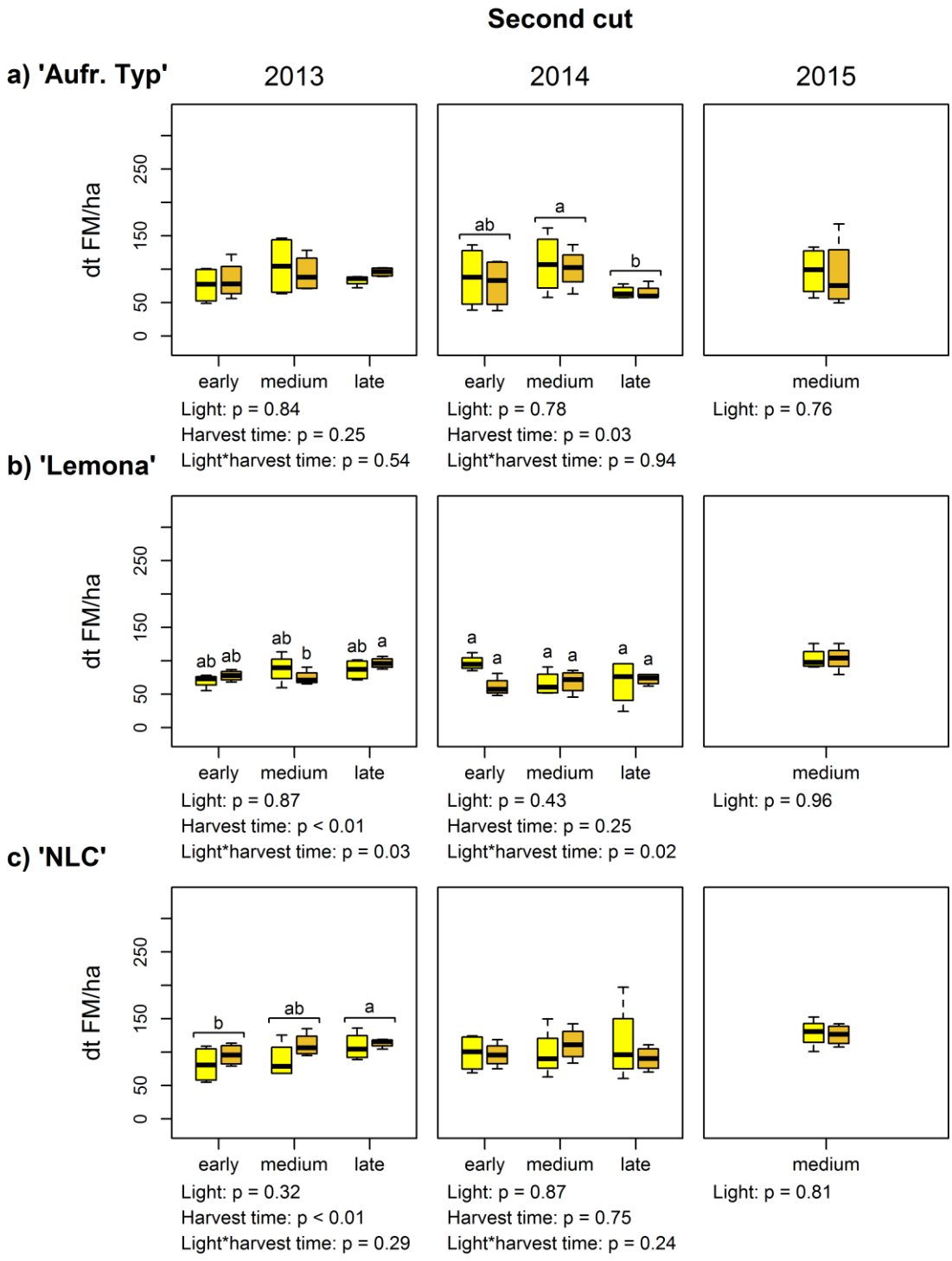

Fig. 33: Fresh matter biomass yield [dt FM/ha] of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

4.1.2.2 Biomass yield (DM)

The biomass yield (DM) was found in a range from 12.1 to 43.1 dt FM/ha, depending on genotype, light intensity, harvest stage, year, and cut within the year (**Tab. A 6**). For the reference variant (natural light, medium harvest stage), annual sums of 49.6, 65.2, as well as 50.6 dt DM/ha were harvested for 'Aufrechter Typ', 46.6, 48.7, as well as 57.3 dt DM/ha for 'Lemona', and 46.4, 47.0, as well as 58.1 dt DM/ha for 'NLC' in the years 2013, 2014, and 2015, respectively. Similar to the fresh matter biomass yield, the dry matter biomass yield of the second cuts were around a third lower than those of the first cuts in 2014 and 2015, whereas both cuts were on a similar level in 2013.

As for the biomass yield (FM), no negative effect of the moderate shading on the biomass yield (DM) could be observed, and the pattern of 'NLC' having significantly higher values than 'Lemona' in both cuts in 2013, as well as in cut 2 in 2014, could be observed again. Equal to the biomass yield (FM), in 2015, there were no significant differences between the genotypes for cut 1, whereas once again the biomass yield (DM) of 'NLC' was on a high level, being significantly higher than 'Aufrechter Typ', and 'Lemona' did not differ significantly from the other genotypes (**Tab. A 6**).

As for the biomass yield (FM), an unexpected decrease of biomass yield (DM) of the genotype 'Aufrechter Typ' from medium to late harvest stage could be seen for cut 2 in 2014 (**Fig. 35 a**). On the other hand, and in contrast to the biomass yield (FM), an increase of the biomass yield (DM) from early to late harvest stage was observed in the genotypes 'Lemona' (first cut 2014, **Fig. 34 b**; second cut 2013, **Fig. 35 b**) and 'NLC' (first cut in 2013 and 2014, **Fig. 34 c**).

As expected, there was a strong and highly significant correlation between FM biomass and DM biomass ($r = 0.86$, $p < 0.001$) (**Fig. A 4**).

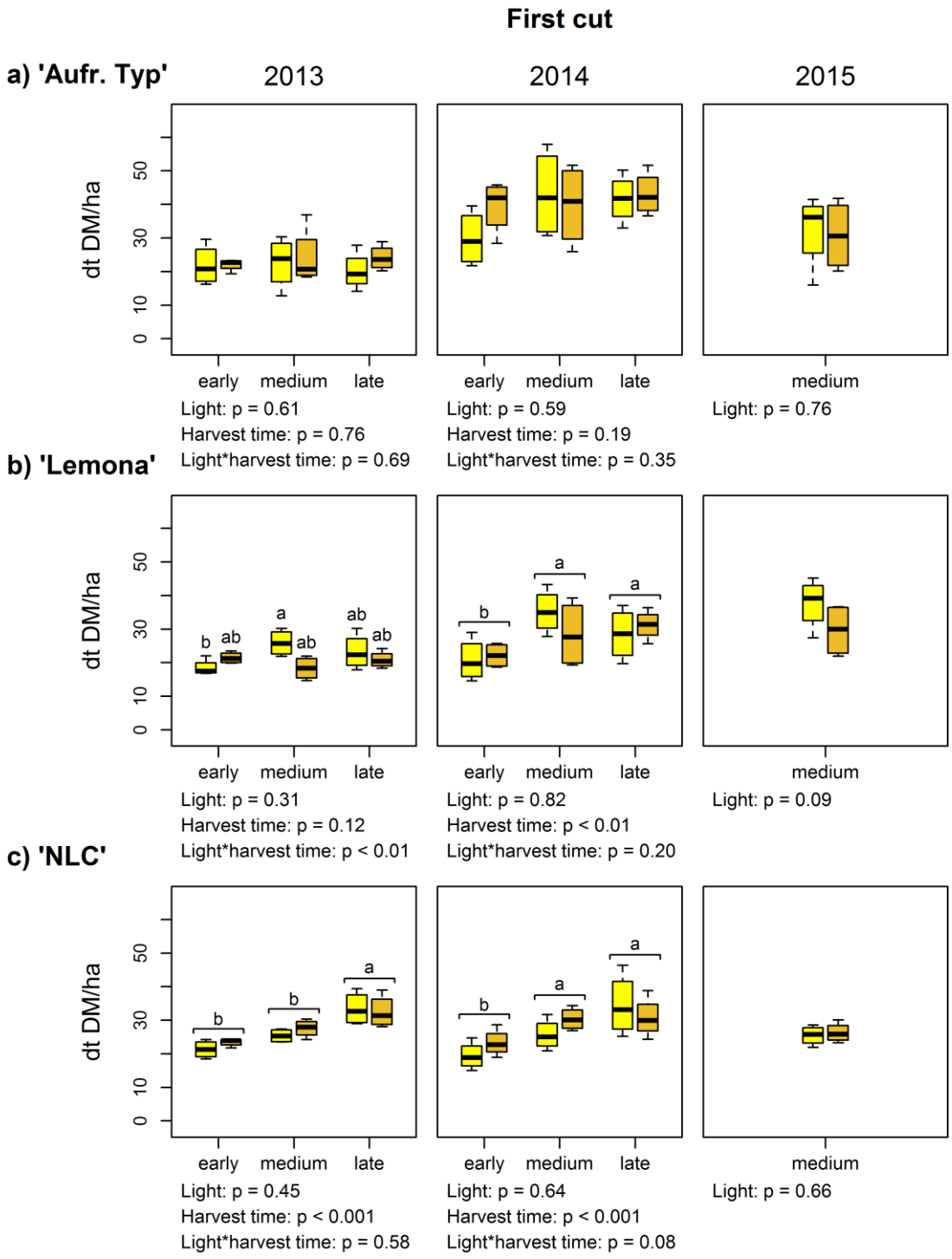

Fig. 34: Dry matter biomass yield [dt DM/ha] of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (■ = natural light; ■ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

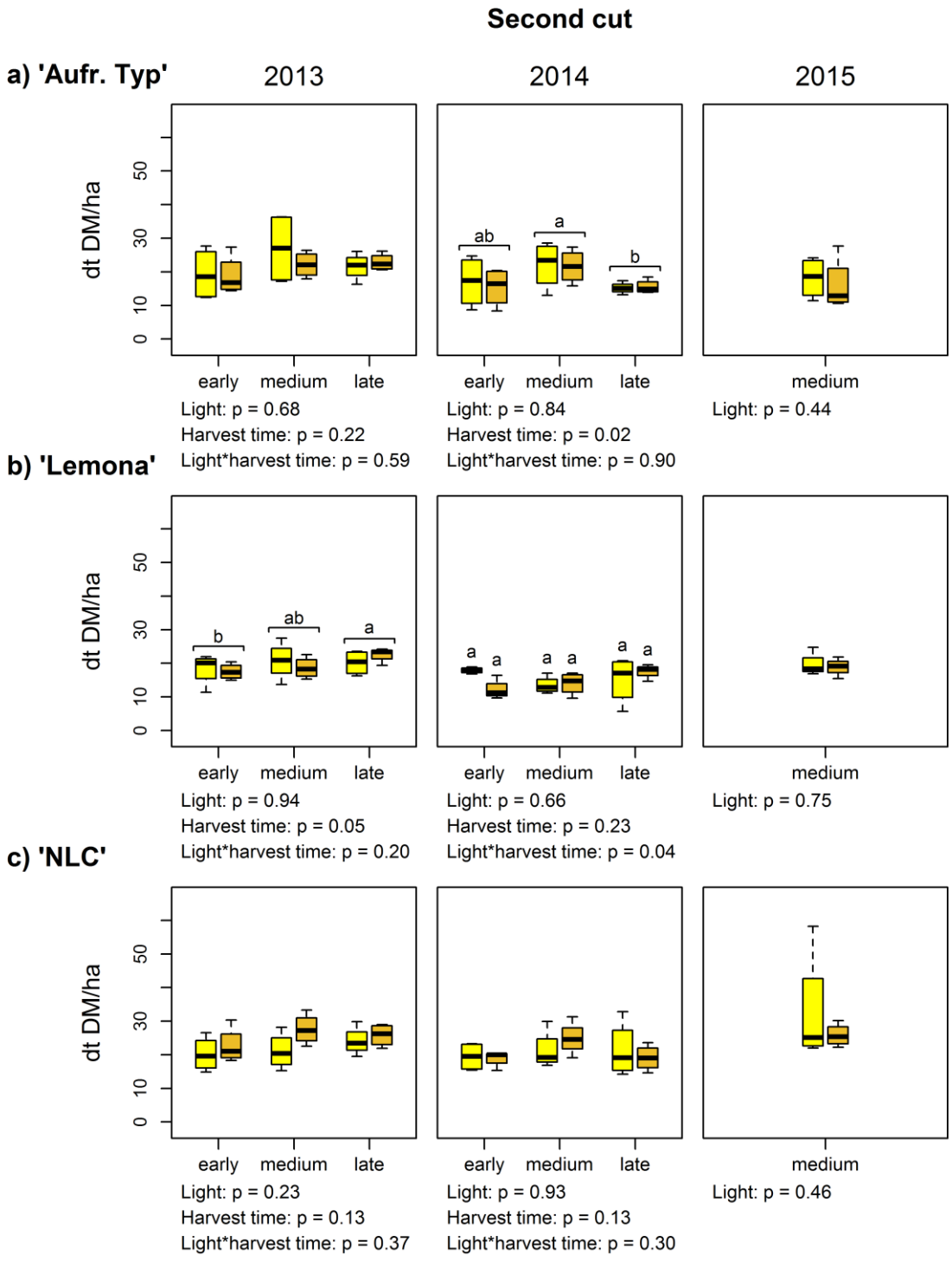

Fig. 35: Dry matter biomass yield [dt DM/ha] of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

4.1.2.3 Leaf yield (FM)

Leaf yield (FM) was found in a range from 42.1 to 115.5 dt FM/ha, depending on genotype, light intensity, harvest stage, year, and cut within the year (**Tab. A 7**). For the reference variant (natural light, medium harvest stage), annual sums of 137.5, 143.0, as well as 151.4 dt FM/ha were harvested for 'Aufrechter Typ', 128.5, 107.3, as well as 168.7 dt FM/ha for 'Lemona', and 122.8, 117.9, as well as 155.3 dt FM/ha for 'NLC' in the years 2013, 2014, and 2015, respectively. The genotypes differed significantly for cut 1 in 2013, as well as for cut 2 in 2014, with 'Lemona' having significantly lower leaf yield (FM) than 'NLC'. For the first cut in 2014, a genotype effect was observed only in combination with an interaction genotype*harvest time, whereas in 2015, no significant genotype effect occurred (**Tab. A 7**).

The results of the two-factorial analysis are presented in **Fig. 36** and **Fig. 37**. Generally, no significant effect of the moderate shading on leaf fresh matter yield could be observed in all three experimental years.

Regarding the harvest stages, no significant differences were seen in 2013 for the first cut for all investigated genotypes (**Fig. 36**). The same was true for 'Aufrechter Typ' for the second cut in 2013 (**Fig. 37 a**). However, leaf yield (FM) increased significantly from early to late harvest stage in genotype 'NLC' (**Fig. 37 c**), whereas in 'Lemona', an increase occurred from early to late harvest stage for the non-shaded plants, as well as from medium to late harvest stage in the shaded plants (**Fig. 37 b**).

An unexpected decrease of leaf yield (FM) over time could be observed in the first cut of 2014 for 'Aufrechter Typ' from early to late harvest stage (**Fig. 36 a**), as well as for 'NLC for the shaded plants (**Fig. 36 c**), and for 'Lemona' for the non-shaded plants from medium to late harvest stage (**Fig. 36 b**). A decreased yield over time was also seen for the second cut in 2014, where 'Aufrechter Typ' had a significantly decreased leaf fresh matter yield at late harvest stage compared to medium harvest stage (**Fig. 37 a**), and 'Lemona' for the non-shaded plants from early to medium harvest stage (**Fig. 37 b**).

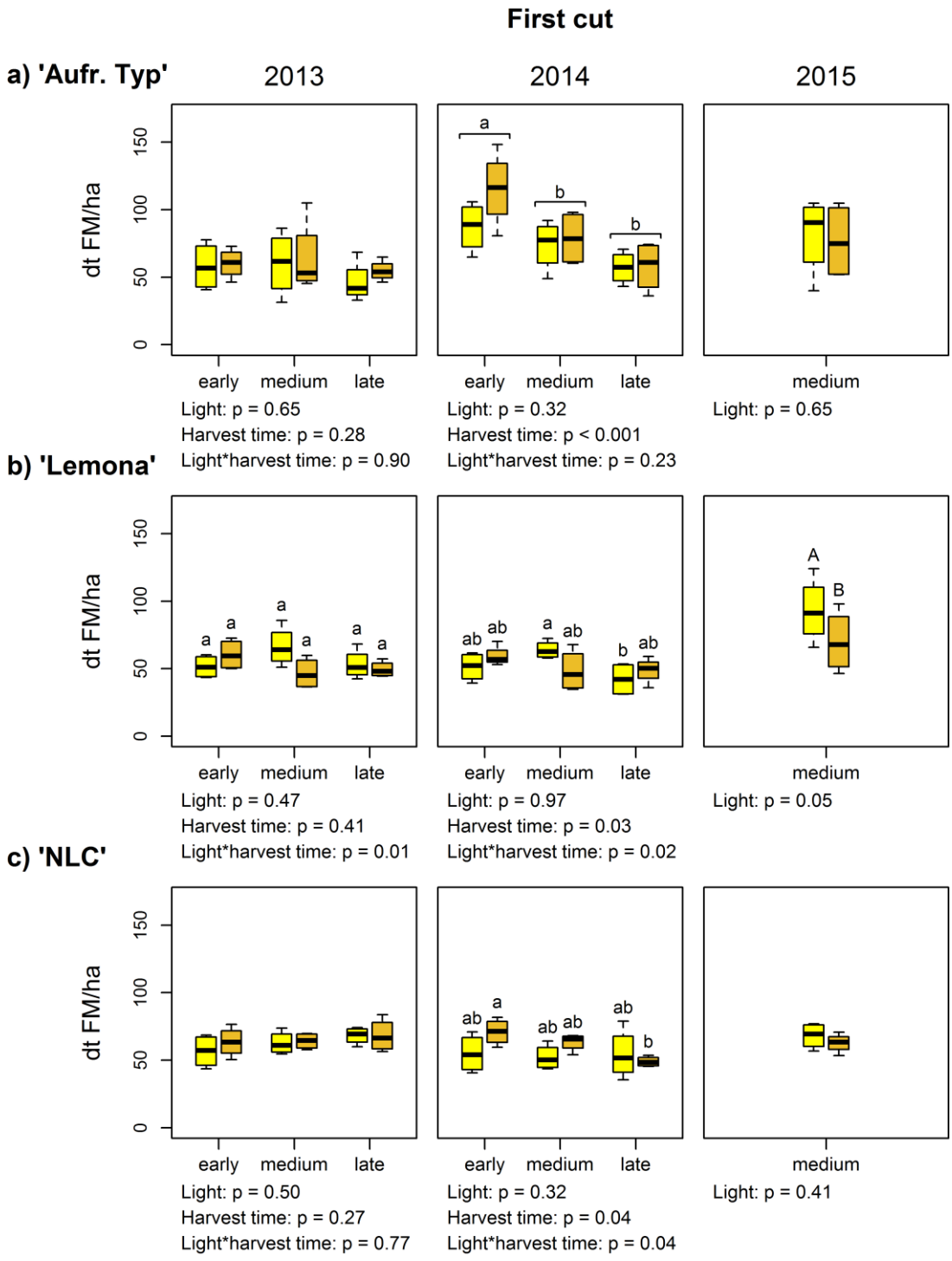

Fig. 36: Fresh matter leaf yield [dt FM/ha] of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

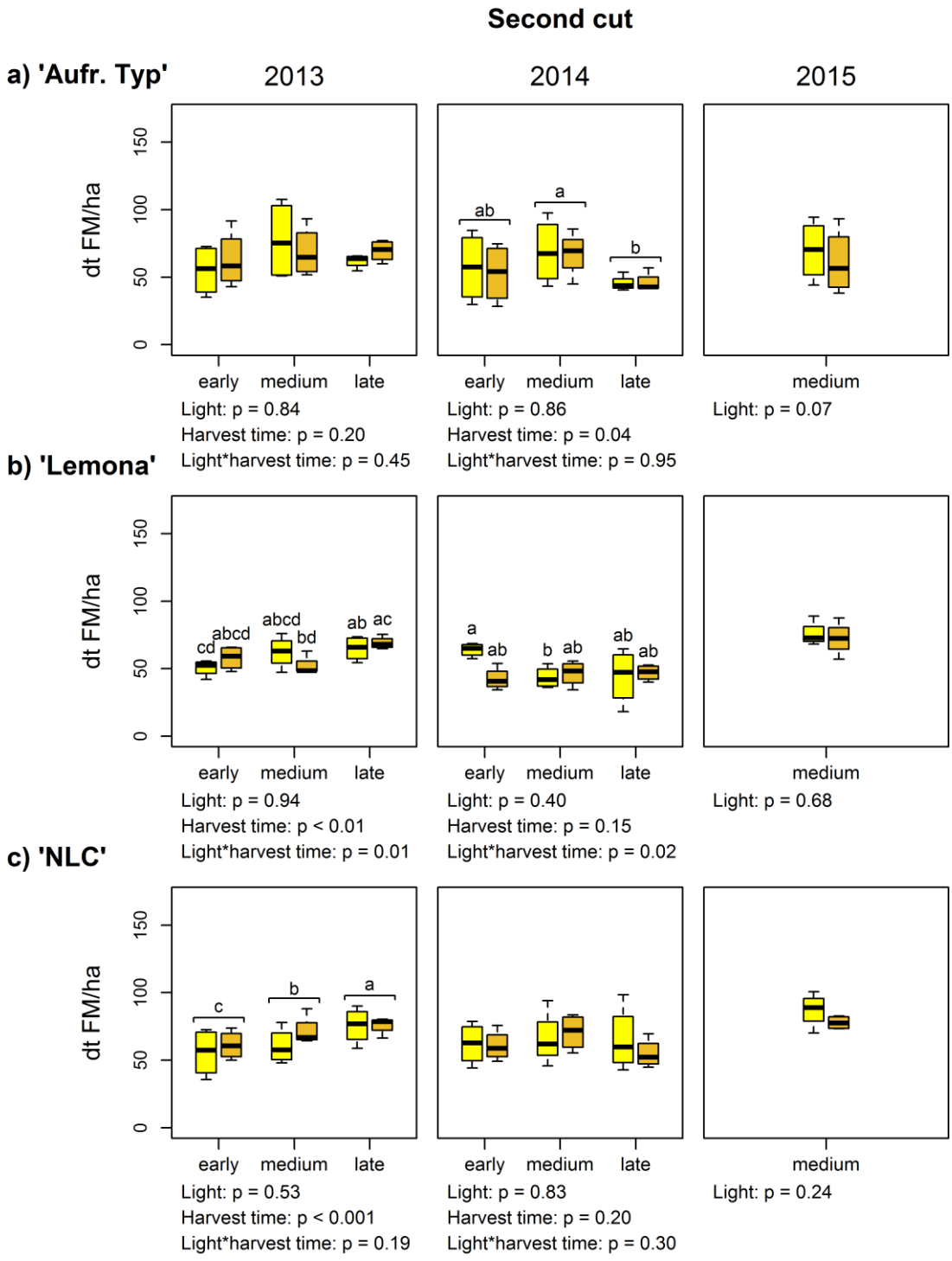

Fig. 37: Fresh matter leaf yield [dt FM/ha] of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

4.1.2.4 Leaf yield (DM)

Leaf yield (DM) was found in a range from 8.4 to 20.9 dt DM/ha, depending on genotype, light intensity, harvest stage, year, and cut within the year (**Tab. A 8**). For the reference variant (natural light, medium harvest stage), annual sums of 35.0, 29.0, as well as 29.4 dt DM/ha were harvested for 'Aufrechter Typ', 32.1, 21.8, as well as 32.4 dt DM/ha for 'Lemona', and 30.7, 26.1, as well as 32.4 dt DM/ha for 'NLC' in the years 2013, 2014, and 2015, respectively.

Regarding a genotype effect, significant differences between the genotypes were observed for the first cuts in 2013 and 2014, as well as for the second cuts in 2014 and 2015, albeit in combination with an interaction effect genotype*harvest time for cut 1 in 2013, as well as for cut 2 in 2014. 'Lemona' gave significantly lower DM leaf yields than 'NLC' for cut 1 in 2014 (14.5 vs. 17.4 dt DM/ha), and (accounting for the interaction effect), for the late harvest stage of cut 1 in 2013 (14.8 vs. 20.5 dt DM/ha), as well as for the medium harvest stage of cut 2 in 2014 (8.7 vs. 15.0 dt DM/ha). 'Aufrechter Typ' had, with 15.9 dt DM/ha, an even significantly higher DM leaf yield than 'NLC' for cut 1 in 2014, whereas for cut 2 in 2015 it reached only 11.9 dt DM/ha, which was significantly lower than 'NLC' (**Tab. A 8**).

The two-factorial analysis showed an expected increase of DM leaf yield for 'NLC' in the first cut in 2013 from early to late harvest stage, and for the non-shaded, but not for the shaded plants, in 2014 (**Fig. 38 c**). Also for 'Lemona', a different reaction of the shaded and non-shaded plants was observed for the first cut, with an increase of DM leaf yield in 2013 and 2014 from early to medium harvest stage only in the plants receiving full sunlight (**Fig. 38 b**). For 'Aufrechter Typ', no significant differences of the harvest stages were observed in the first cut in both years (**Fig. 38 a**). For the second cut, a significant increase from early to late harvest stage was found for 'Lemona' (**Fig. 39 b**) in 2013, and for 'NLC' from early to medium harvest stage in 2014 (**Fig. 39 c**). There was no significant effect of the shading on DM leaf yield in any of the investigated genotypes.

Leaf dry matter and fresh matter yield were highly correlated ($r = 0.80$, $p < 0.001$; **Fig. A 5**).

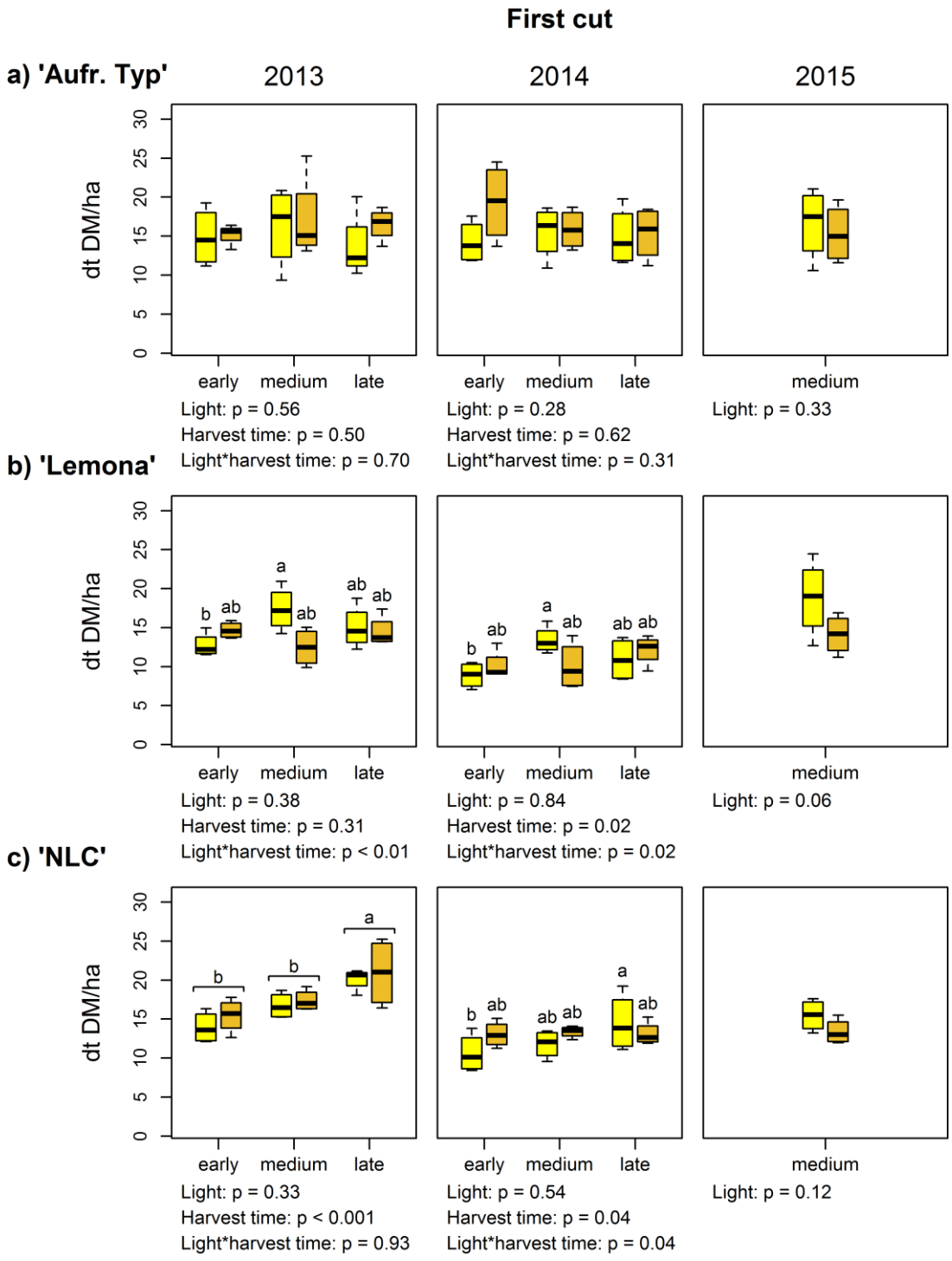

Fig. 38: Dry matter leaf yield [dt DM/ha] of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (■ = natural light; ■ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

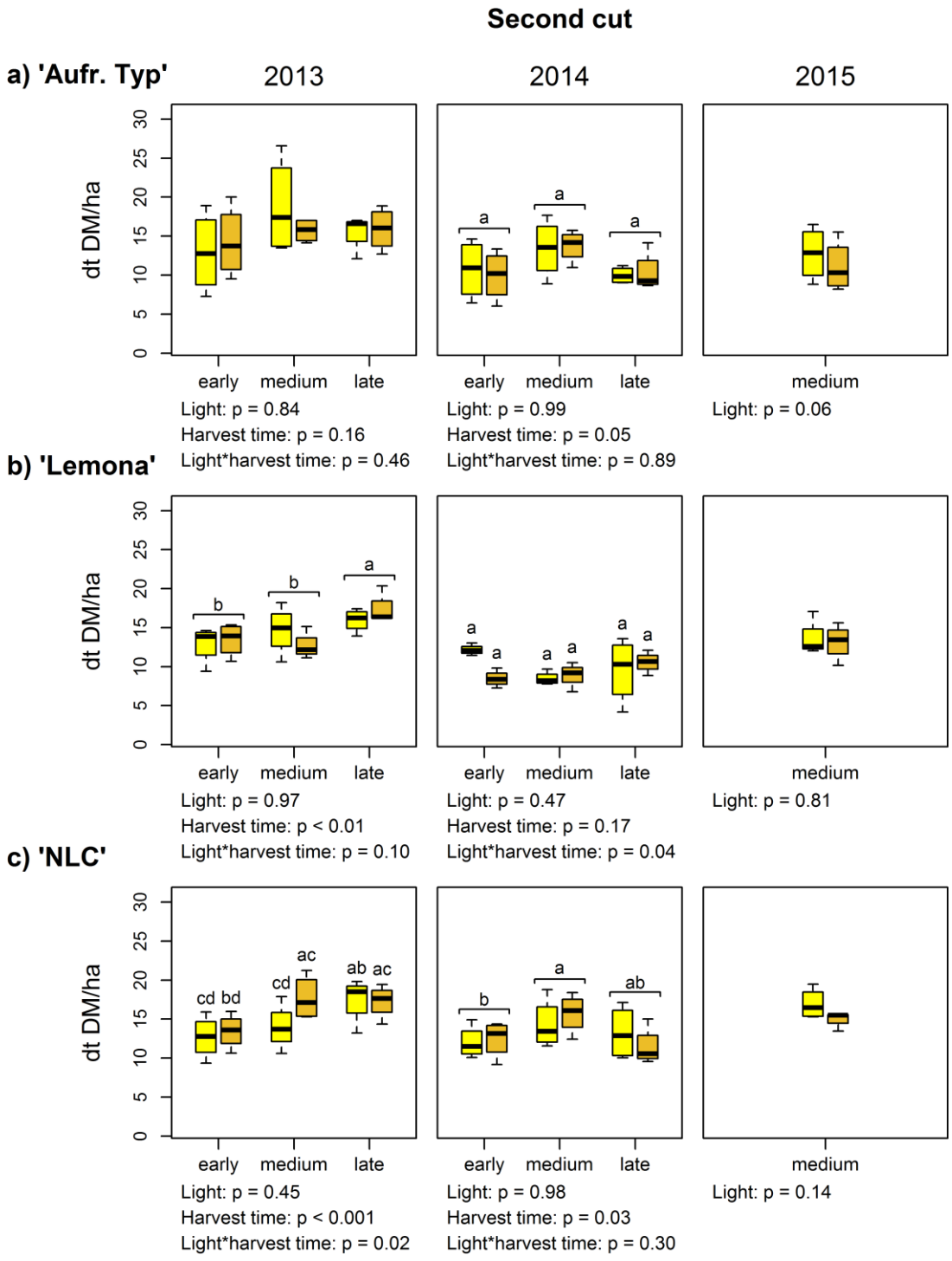

Fig. 39: Dry matter leaf yield [dt DM/ha] of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (■ = natural light; ■ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

4.1.2.5 DM content of the leaves

The DM content of the leaves was almost always above 20% in plants harvested at medium harvest stage, except for the second cut in 2015, where it reached only 18% (**Tab. A 9**).

There was no effect of the moderate shading on the DM content of the leaves. The three-factorial analysis (**Tab. A 9**) revealed no significant differences between the investigated genotypes, except for the first cut in 2014, where 'NLC' reached a significantly higher DM content than the two other genotypes.

The DM content of the leaves increased significantly for the first cut from early to late harvest stage in 2013 from 25.1% to 29.6%, as well as in 2014 from 17.6% to 26.4% (numbers averaged over all genotypes) (**Tab. A 9**). This clear pattern can also be seen in the box plots, separately presented for the three genotypes, in **Fig. 40**.

For the second cut, however, no significant effect of the harvest stage could be observed in 2013, whereas there was an interaction effect genotype*harvest stage in 2014 (**Tab. A 9** and **Fig. 41**). The DM content of the leaves increased significantly from early to late harvest stage in 'Aufrechter Typ' (from 19.5% to 22.1%; **Fig. 41 a**) as well as in 'Lemona' (from 19.6% to 22.2%; **Fig. 41 b**). In 'NLC', however, it increased from early (20.2%) to medium harvest stage (22.3%), with the late harvest stage not being significantly different from early and medium harvest stage (**Fig. 41 c**).

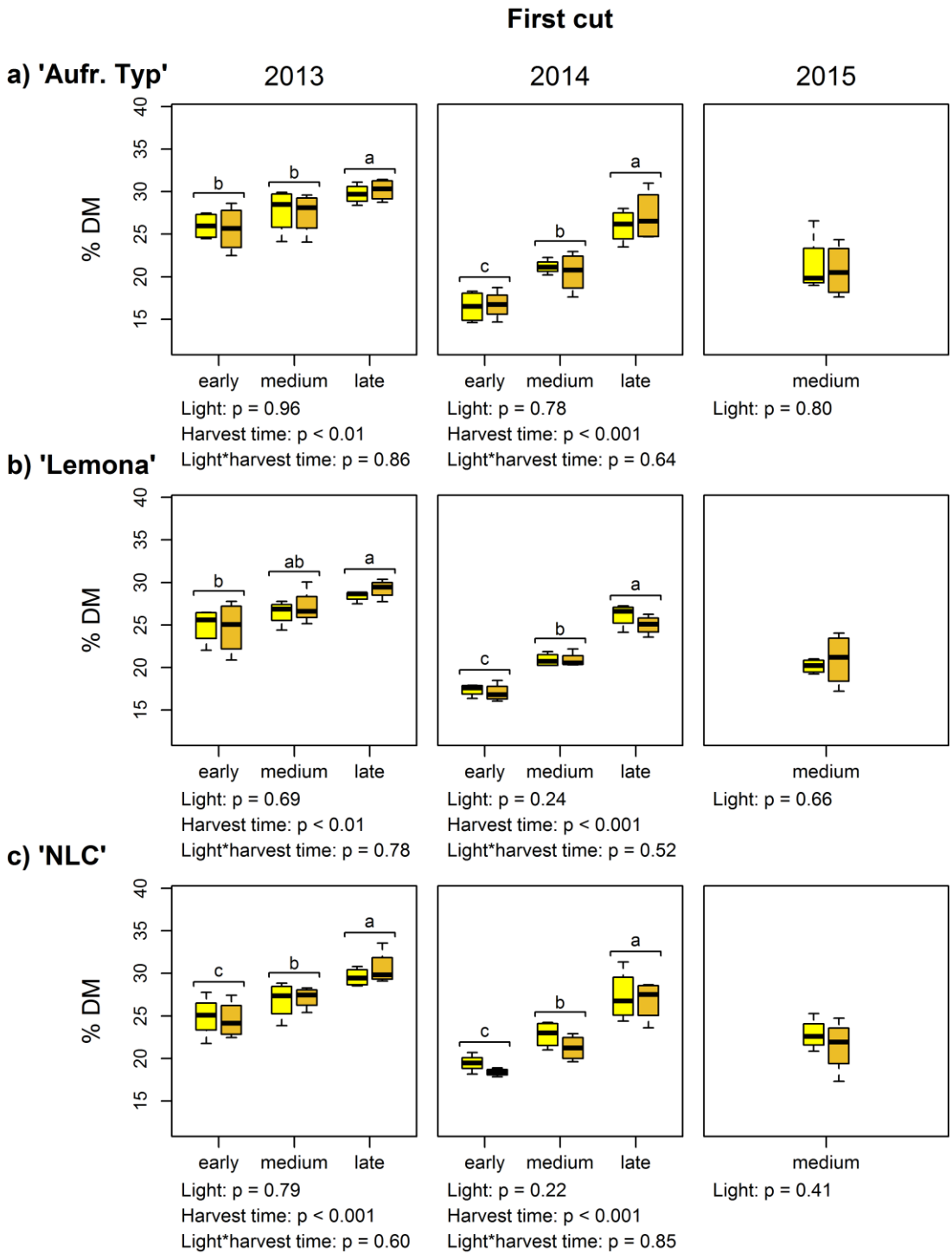

Fig. 40: DM content of the leaves [%] of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

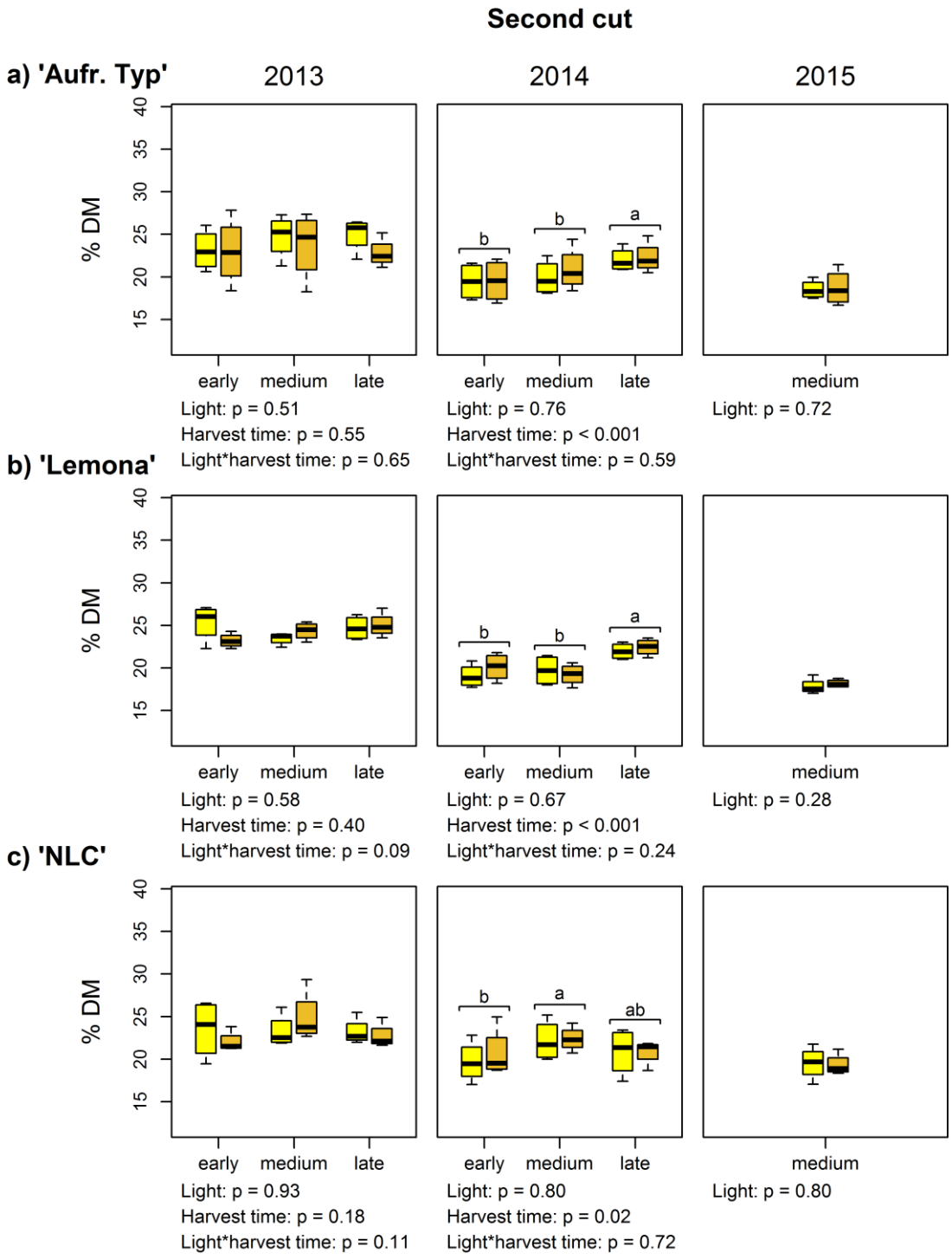

Fig. 41: DM content of the leaves [%] of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

4.1.2.6 Leaf:stem ratio

The values for the leaf:stem ratio were covering a range from below 1.0 until about 3.5 (**Tab. A 10**). No significant effect of the moderate shading on the leaf:stem ratio could be observed in all tested genotypes, years, and cuts.

The three-factorial analysis (**Tab. A 10**) revealed significant differences between the genotypes in all investigated years and cuts. For the second cut, the leaf:stem ratio of 'NLC' (1.93, 1.68, and 1.66 in the years 2013, 2014, and 2015) was always significantly lower than that of the other two genotypes 'Aufrechter Typ' and 'Lemona', which reached values of above 2.0. The same was true for the first cut in 2013. However, it was the other way round for the first cut in 2014, where 'NLC' had a significantly higher leaf:stem ratio than the other two genotypes, but on a very low level of less than 1.0. For the first cut in 2015, the leaf:stem ratio of 'NLC' (1.29) was significantly higher than 'Lemona' (0.92), whereas 'Aufrechter Typ' (1.11) was not significantly different from the other two genotypes (**Tab. A 10**).

The results of the two-factorial analysis, split up by the different genotypes, are presented in **Fig. 42** and **Fig. 43**. There was no significant effect of the harvest stage on the leaf:stem ratio of the first cut in 2013 for all genotypes (**Fig. 42**). The situation was similar for the second cut in 2013 (**Fig. 43**), where no differences between the harvest stages were observed in the genotypes 'Aufrechter Typ' and 'NLC' (**Fig. 43 a + c**). Although the two-factorial ANOVA calculated a significant interaction effect light*harvest time for 'Lemona', the following pairwise comparisons (adjusted according to Tukey) revealed no different groups for this genotype either (**Fig. 43 b**).

In 2014, however, a decrease of the leaf:stem ratio over time could be observed in all tested genotypes for the first cut, with significantly lower values for medium and late harvest time compared to the early harvest time (**Fig. 42**). For the second cut in 2014, the leaf:stem ratio decreased significantly only in the genotype 'Aufrechter Typ' from early to medium harvest time (**Fig. 43 a**), whereas no significant differences between the harvest times could be observed in 'Lemona' and 'NLC' (**Fig. 43 b + c**).

There was a significant negative correlation between leaf:stem ratio and plant height ($r = -0.79$, $p < 0.001$; **Fig. A 6**).

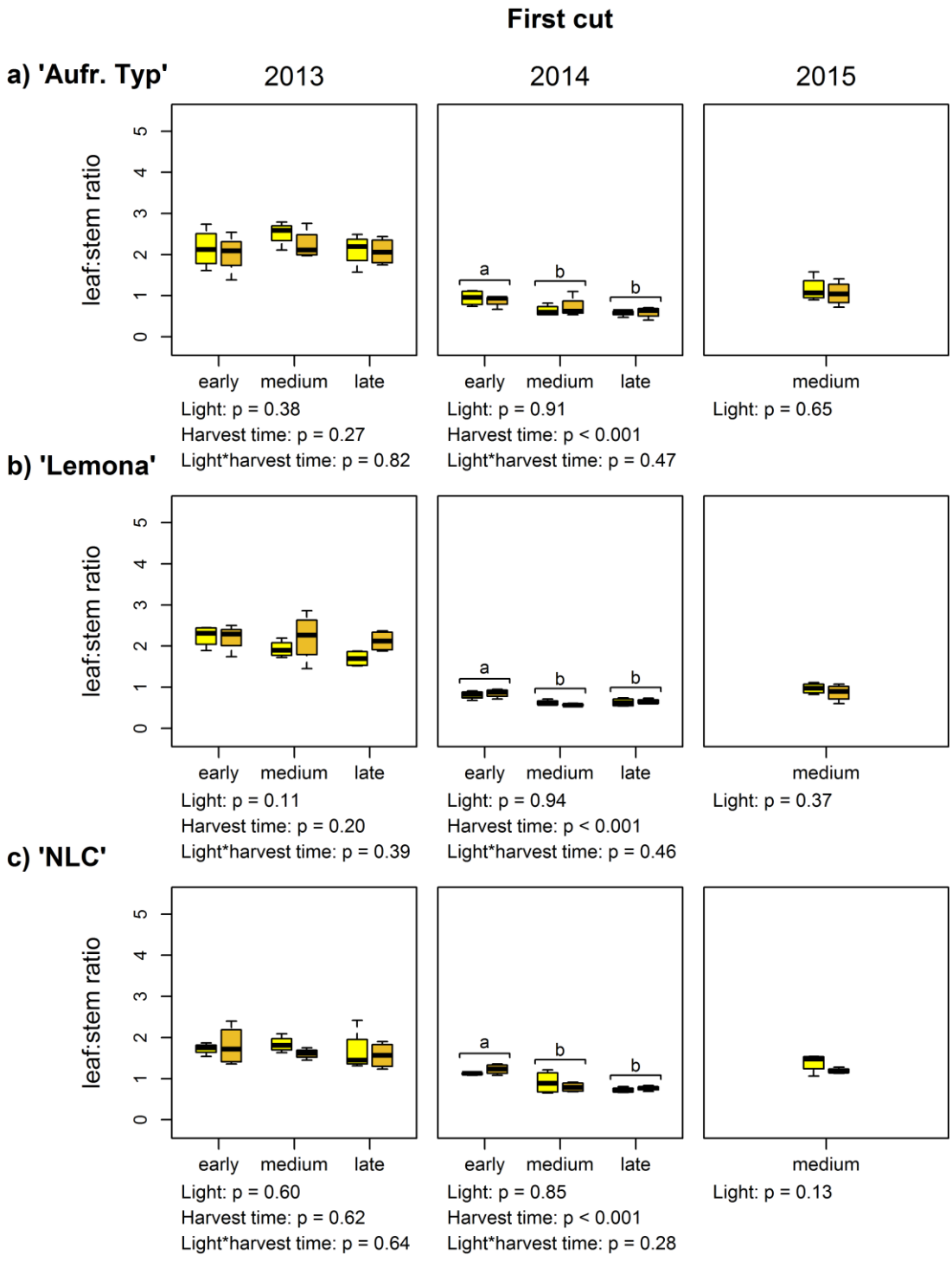

Fig. 42: Leaf:stem ratio of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

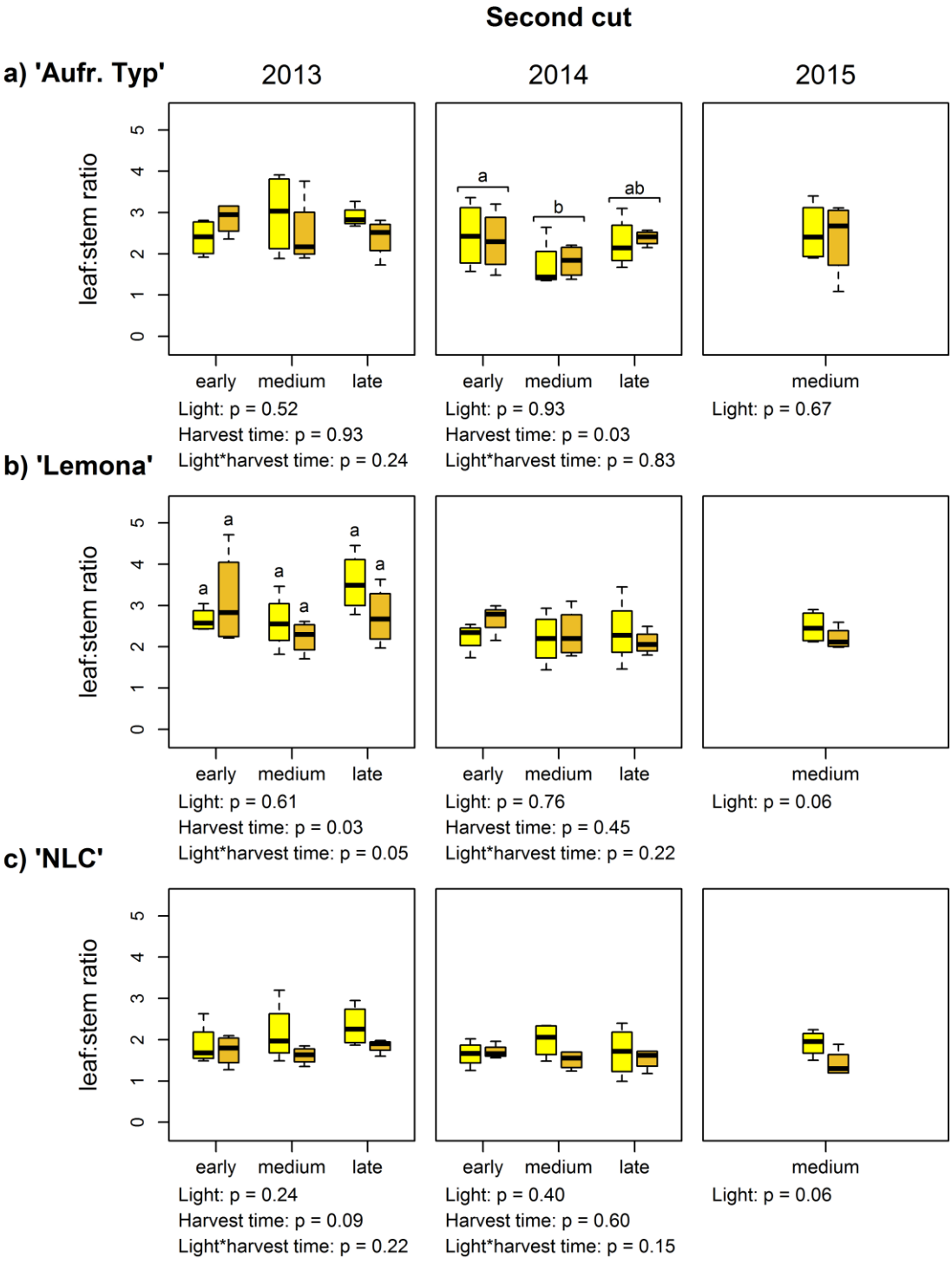

Fig. 43: Leaf:stem ratio of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; ■ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

4.1.3 Essential oil content

The essential oil (EO) content of the leaves was covering a range from less than 0.10% to more than 0.50%, depending on genotype, year, harvest stage, and cut (**Tab. A 11**). For the reference variants (natural light, medium harvest stage), the EO content of the first cut of the harvested leaves was determined as 0.17, 0.08 as well as 0.13% for 'Aufrechter Typ', 0.31, 0.15 as well as 0.28% for 'Lemona', and 0.15, 0.10 as well as 0.12% for 'NLC' (in the years 2013, 2014, and 2014). The EO content of the second cut of the reference variants was generally higher, yielding 0.60, 0.29 as well as 0.48% for 'Aufrechter Typ', 0.72, 0.53 as well as 0.65% for 'Lemona', and 0.52, 0.19 as well as 0.45% for 'NLC' in the years 2013, 2014, and 2015, respectively.

Not only for the reference variant, but also for the other treatments, the leaves of the second cuts had markedly higher EO contents compared to those of the first cuts. In 2013 and 2015, EO concentrations were reaching on average more than 0.50% in the second cut, compared to around 0.20% in the first cut. In 2014, the values were slightly lower with around 0.30% in the second cut, but still much higher than in the first cut with (on average over all treatments) 0.10% EO (**Tab. A 11**).

The three-factorial analysis (**Tab. A 11**) revealed statistically significant differences between the tested genotypes, however partially in combination with interaction effects genotype* harvest stage for the second cuts in 2013 and 2014 (see below). The genotype 'Lemona' proved to accumulate the highest EO contents, reaching on average around 0.1 or more percentage points than the two other genotypes.

Taking account of the interaction effect genotype*harvest stage and therefore regarding only the medium harvest stage, 'Lemona' reached 0.31% and 0.29% in the first cuts in 2013 and 2015. In the second cut, contents were around two times as high, with 0.74% and 0.66% EO for the medium harvest stage, respectively. In 2014, with generally lower EO contents, 'Lemona' still showed an EO content of 0.49% for the medium harvest stage in the second cut, being significantly higher compared to 'Aufrechter Typ' (0.30%) and 'NLC (0.18%). The genotype 'NLC' had the lowest content of EO. In the first cuts of 2013, 2014, and 2015, EO contents for medium harvest stage reached 0.15%, 0.09%, and 0.11%, respectively. The contents were higher in the second cuts, but with 0.49%, 0.18%, and 0.45% for the medium harvest stage in the years 2013, 2014, and 2015, respectively, still below the average of all genotypes. 'Aufrechter Typ' was in between the two other genotypes, or as low as 'NLC' (**Tab. A 11**).

The results of the two-factorial analysis (**Fig. 44** and **Fig. 45**) revealed some different reactions of the tested genotypes. For the first cut, an effect of the harvest stage was

observed only for 'Lemona' in 2013 (**Fig. 44 b**), with a decrease from early to medium harvest stage, as well as for 'NLC' in 2014 (**Fig. 44 c**), with an increase from early to medium harvest stage.

For the second cut (**Fig. 45**), an increase of the EO content from early to medium harvest stage was observed in the year 2013 for 'Aufrechter Typ' (**Fig. 45 a**) and 'Lemona' (**Fig. 45 b**), but not for 'NLC' (**Fig. 45 c**). However, in 2014, 'NLC' showed a decrease in EO content from early to medium, and then again an increase from medium to late harvest stage. While 'Lemona' did not show significant differences in EO content for the different harvest stages, 'Aufrechter Typ' showed a significant decrease from early and medium to late harvest stage only in the plants receiving natural sunlight, but not in the shaded plants (**Fig. 45 a**). At the same time, the essential oil content of the shaded plants was significantly higher at late harvest stage for the second cut in 2014, compared to the non-shaded plants. 'Aufrechter Typ' was also the only genotype that showed a reaction on the different light intensities for the second cut in 2013 and 2015, with a significantly higher EO content in the shaded plants. In 2015, it reached 0.58% EO in the shaded compared to 0.48% in the non-shaded plants. No effect of the shading could be observed for the first cut in all tested genotypes, and for 'Lemona' and 'NLC' neither for the second cut.

There was a positive correlation between EO and leaf:stem ratio ($r = 0.64$, $p < 0.001$; **Fig. A 7**), and a negative correlation between EO and plant height ($r = -0.67$, $p < 0.001$; **Fig. A 8**), while there was no correlation between EO and DM leaf yield ($r = -0.08$, $p = 0.15$; **Fig. A 9**).

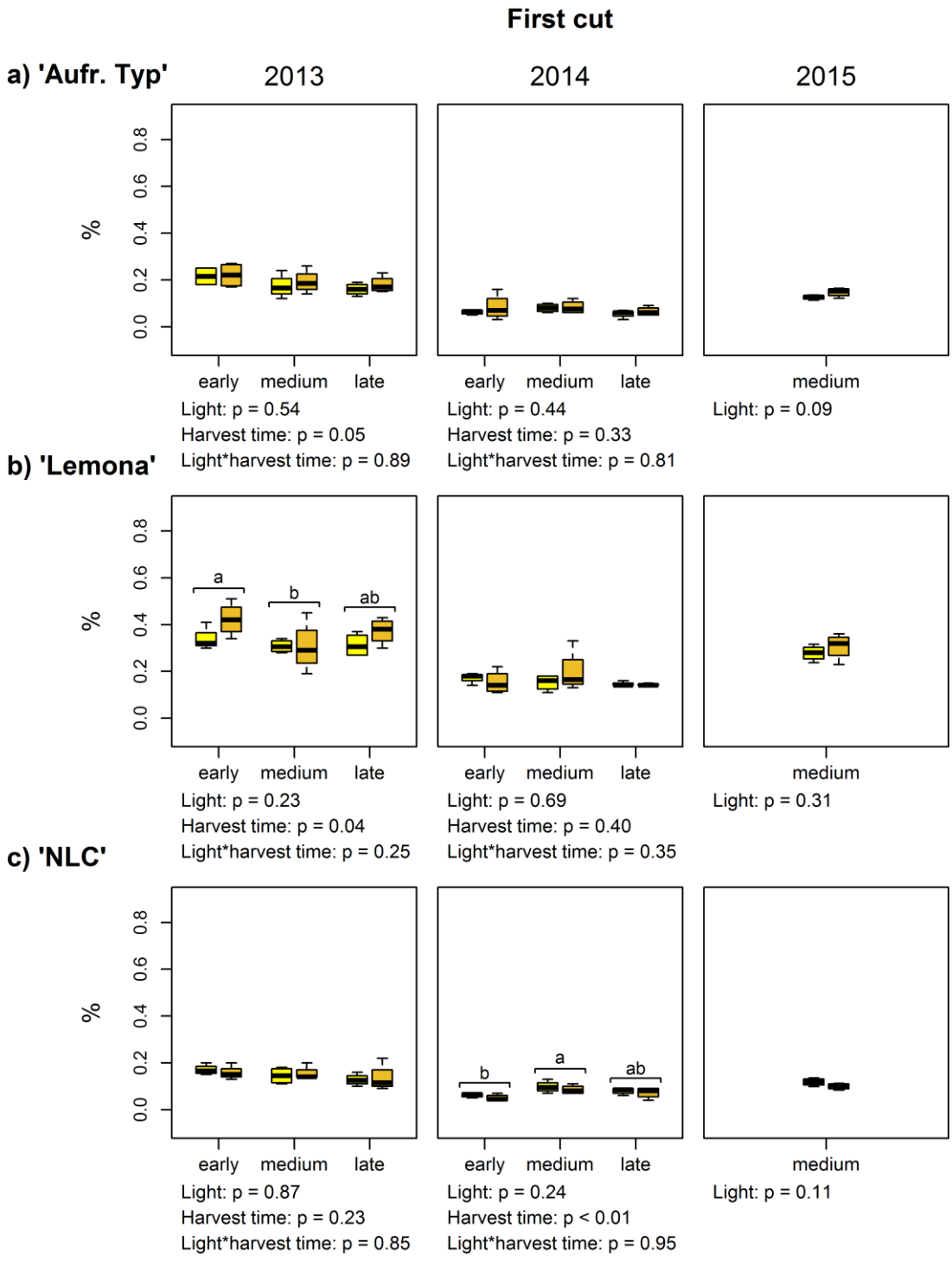

Fig. 44: Essential oil content [%] of lemon balm leaves. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (■ = natural light; ■ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

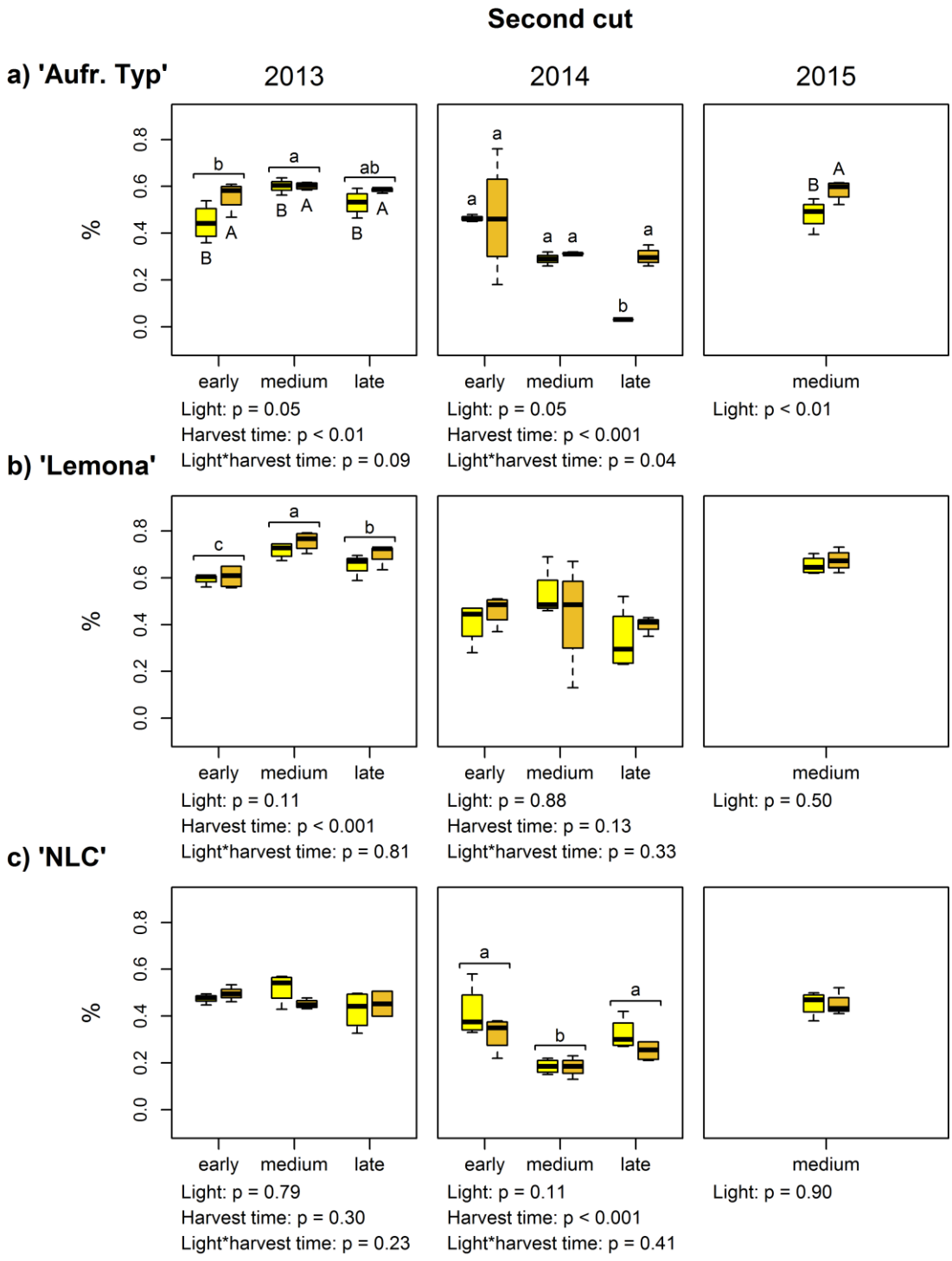

Fig. 45: Essential oil content [%] of lemon balm leaves. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (■ = natural light; ■ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.1.4 Total phenolic content

The total phenolic content (TPC) of the investigated lemon balm plants was found in a range from about 110 to more than 180 mg GAE/g DM, depending on genotype, year, harvest time, and cut (**Tab. A 12**).

For the reference variants (natural light, medium harvest stage), TPC values of the first cut of the harvested leaves were determined as 143.0, 149.1 as well as 153.1 mg GAE/g DM for 'Aufrechter Typ', 145.7, 147.0 as well as 159.2 mg GAE/g DM for 'Lemona', and 152.1, 185.6 as well as 161.1 mg GAE/g DM for 'NLC' (in the years 2013, 2014, and 2014). TPC values of the second cut of the reference variants were yielding 127.6, 134.5, as well as 125.8 mg GAE/g DM for 'Aufrechter Typ', 116.1, 147.7 as well as 138.1 mg GAE/g DM for 'Lemona', and 127.4, 134.0 as well as 147.6 mg GAE/g DM for 'NLC' in the years 2013, 2014, and 2015, respectively. Generally, TPC values of the second cut were on a lower level than those of the first cut.

The three-factorial analysis (**Tab. A 12**) revealed significant differences between the genotypes, except for the second cut in 2014. In the other five harvests, 'NLC' was always found in the group with the highest TPC values. However, for the first cut in 2013 as well as for the second cut in 2014, an interaction effect genotype*harvest time occurred, which will be further elucidated below. Except for the second cut in 2013, where TPC values of 'Aufrechter Typ' were not significantly different from 'NLC', this genotype had significantly lower TPC values than 'NLC'. In 2015, 'Aufrechter Typ' reached 146.9 and 129.0 mg GAE/g DM for the first and second cut, whereas 'NLC' had values of 165.4 and 143.3 mg GAE/g DM, respectively. While the genotype 'Lemona' had significantly lower TPC values than the genotype 'NLC' in the second cut in 2013 (119.4 vs. 128.3 mg GAE/g DM) as well as in the first cut in 2014 (155.9 vs. 174.3 mg GAE/g DM), with values of 161.2 as well as 140.4 mg GAE/g DM it was not significantly different from 'NLC' for cut 1 and cut 2 in 2015.

The two-factorial analysis revealed a decrease of TPC values over time for the first cut in 2013 for all three genotypes (**Fig. 46**). The interaction effect mentioned above can be seen in the pattern that TPC values of 'Lemona' and 'NLC' decreased from early to medium harvest stage (**Fig. 46 b + c**), with the late harvest stage not being significantly different from medium harvest stage, whereas TPC values of 'Aufrechter Typ' decreased not only from early to medium, but also from medium to late harvest stage (**Fig. 46 a**). For the first cut in 2014, 'NLC' showed decreasing TPC values from early and medium to late harvest stage (**Fig. 46 c**), whereas 'Aufrechter Typ' did not show significant differences between the harvest stages (**Fig. 46 a**). Although the ANOVA calculated a significant interaction effect light*harvest time for 'Lemona' for the first cut in 2014, the following

pairwise comparisons did not result in significantly different groups (**Fig. 46 b**). No significant main effect of the shading was observed at all for the first cut in all three investigated years.

For the second cut, the situation was more complex (**Fig. 47**). In the year 2013, no significant effect of the harvest stage on TPC could be observed for the second cut in all three genotypes. In the year 2014, it occurred only in combination with an interaction effect light*harvest time in the genotypes 'Aufrechter Typ' (**Fig. 47 a**) and 'Lemona' (**Fig. 47 b**). The interaction effect for 'Aufrechter Typ' could be seen in the pattern that for the non-shaded plants TPC values decreased from early to medium harvest stage, while the late harvest stage was not significantly different from the other two stages. For the shaded plants, however, early and medium harvest stage were not significantly different from each other, but TPC values of the late harvest stage were significantly decreased (**Fig. 47 a**).

For the genotype 'Lemona', a significant effect of the shading could be observed for the second cut in 2013 and 2014, but only in combination with an interaction effect light*harvest time (**Fig. 47 b**). In 2013, this resulted in a significant difference in the TPC values of the shaded plants at medium harvest stage from those of the non-shaded plants at late harvest stage. A more complex pattern occurred in 2014, where the non-shaded plants reached significantly higher TPC values than the shaded plants at the early harvest stage, but not at medium or late harvest stage. Additionally, the values decreased significantly from early and medium to late harvest stage in the non-shaded plants, whereas TPC values of the shaded plants were higher at medium harvest stage, compared to early and late harvest stage.

In 2015, no significant effect of the shading could be observed in all three genotypes for the second cut. 'NLC' did not show significant differences of TPC values at all in the second cut for the different light treatments, harvest stages, or their interaction (**Fig. 47 c**).

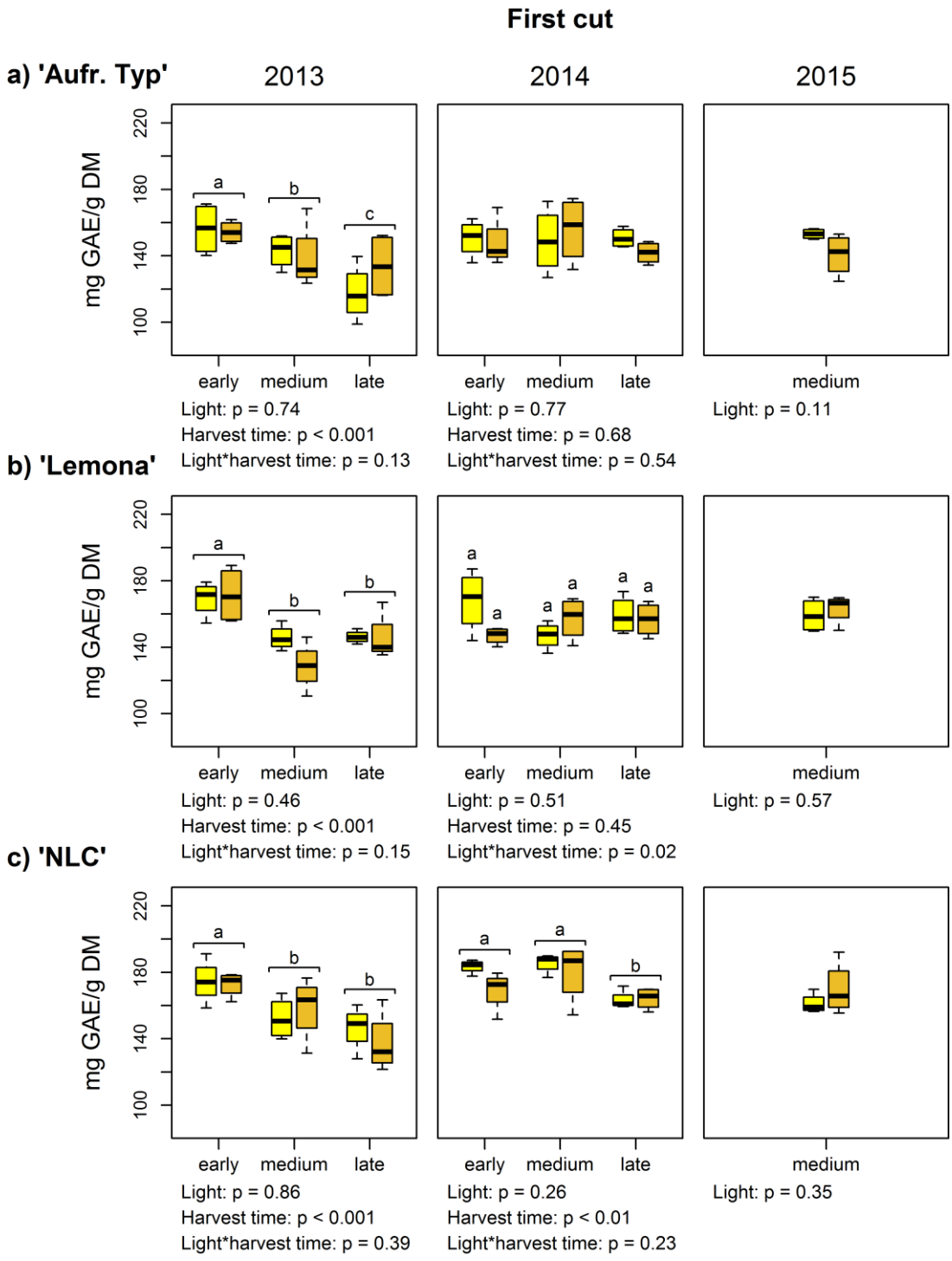

Fig. 46: Total phenolic content [mg GAE/g DM] of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

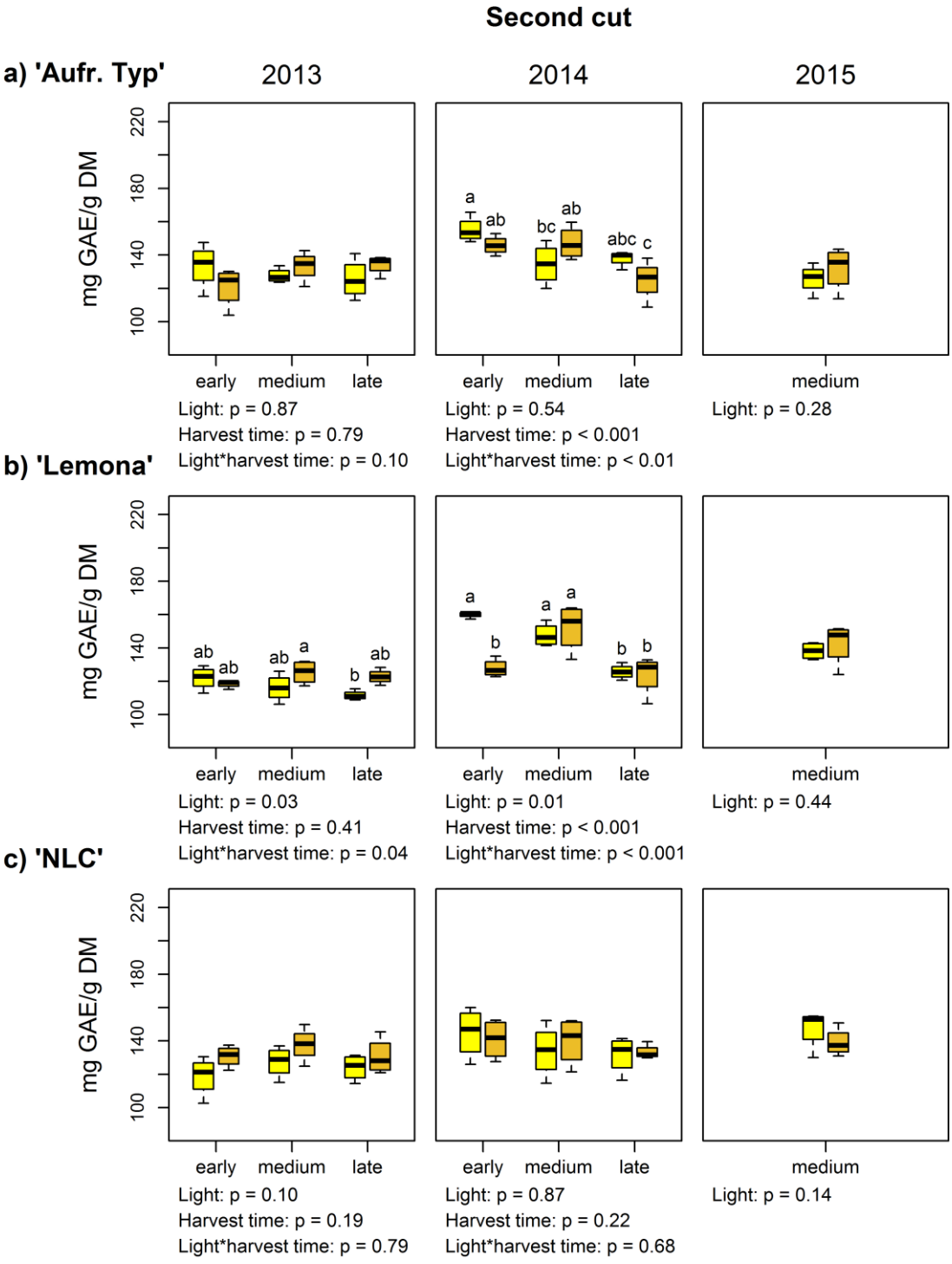

Fig. 47: Total phenolic content [mg GAE/g DM] of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

4.1.5 Antioxidant capacity

ORAC values for the antioxidant capacity of the methanolic lemon balm extracts covered a range from around 1700 to more than 2600 µmol TE/g DM (**Tab. A 13**). For the reference variants (natural light, medium harvest stage), ORAC values of the first cut of the harvested leaves were determined as 1985, 2156 as well as 2308 µmol TE/g DM for 'Aufrechter Typ', 2235, 2199 as well as 2264 µmol TE/g DM for 'Lemona', and 1937, 2663 as well as 2388 µmol TE/g DM for 'NLC' (in the years 2013, 2014, and 2014). ORAC values of the second cut of the reference variants were 2031, 2122 as well as 1955 µmol TE/g DM for 'Aufrechter Typ', 1925, 2413 as well as 2125 µmol TE/g DM for 'Lemona', and 1998, 2133 as well as 2020 µmol TE/g DM for 'NLC' in the years 2013, 2014, and 2015, respectively.

The three-factorial analysis showed a significant genotype effect for the second cut in 2013, as well as for both cuts in 2014, albeit in combination with an interaction effect genotype*harvest time for cut 2 in 2014. For the second cut in 2013, the antioxidant capacity of 'Aufrechter Typ' reached 2081 µmol TE/g DM, which was significantly higher than 'NLC' with 1976 µmol TE/g DM. For the first cut in 2014, however, the pattern was inverted, with significantly higher ORAC values for 'NLC' (2593 µmol TE/g DM) compared to 'Aufrechter Typ' (2357 µmol TE/g DM). 'Lemona' was not significantly different from the two other genotypes for the second cut in 2013 (2039 µmol TE/g DM), and not significantly different from 'Aufrechter Typ', but significantly lower than 'NLC' for the first cut in 2014 (2377 µmol TE/g DM). In 2015, no significant genotype effect could be observed for both cuts (**Tab. A 13**).

The results of the two-factorial analysis are presented in **Fig. 48** (first cut) and **Fig. 49** (second cut). For the first cut in 2013, the antioxidant capacity of 'Aufrechter Typ' (**Fig. 48 a**) and 'NLC' (**Fig. 48 c**) decreased from early to medium harvest stage, while the late harvest stage was not different from the medium harvest stage. For 'Lemona', however, an interaction effect light*harvest time was observed: ORAC values decreased from early to medium harvest stage only in the shaded plants, with the late harvest stage not being significantly different from the other two stages (**Fig. 48 b**). Additionally, the shaded 'Lemona' plants had significantly lower ORAC values than the non-shaded plants at medium harvest stage, but not at the other two stages (**Fig. 48 b**).

For the first cut in 2014, no effect of the harvest stage on the antioxidant capacity was observed. However, a significant effect of the shading could be seen in the genotype 'NLC', with significantly reduced ORAC values for the shaded plants (**Fig. 48 c**), but not in 'Aufrechter Typ' and 'Lemona' (**Fig. 48 a + b**). For the first cut in 2015, no significant effect of the shading could be seen in all three genotypes (**Fig. 48**).

For the second cut in 2013 (**Fig. 49**), no effect of the harvest stage, light intensity, or their interaction could be observed. The same was true for the second cut in 2014 for 'NLC' (**Fig. 49 c**). For 'Aufrechter Typ', the results of the ANOVA indicated a significant interaction effect. However, the following pairwise comparisons did not result in significantly different groups (**Fig. 49 a**). For 'Lemona', both a harvest time effect as well as a light effect were observed for the second cut in 2014 (**Fig. 49 b**), with the late harvest stage having a significantly lower antioxidant capacity than early and medium harvest stage, as well as the shaded plants having significantly lower values than the non-shaded plants.

For the second cut in 2015, no effect of the shading on the antioxidant capacity could be observed in the genotypes 'Aufrechter Typ' (**Fig. 49 a**) and 'Lemona' (**Fig. 49 b**). However, the ORAC values of the shaded plants of the genotype 'NLC' were significantly lower than those of the non-shaded plants (**Fig. 49 c**).

There was a positive correlation between ORAC and TPC values calculated over all years and growth cycles ($r = 0.71$, $p < 0.001$; **Fig. A 10**).

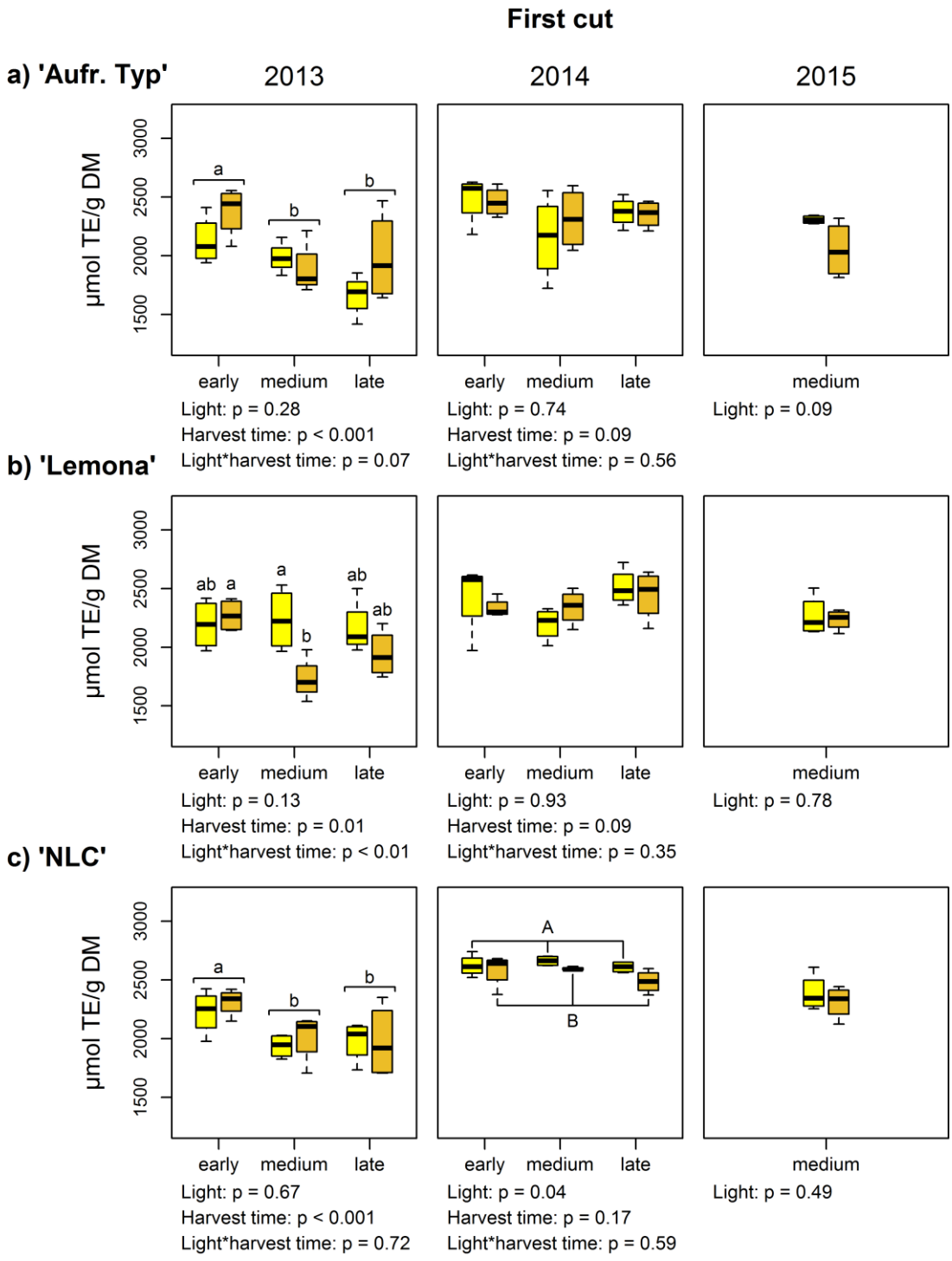

Fig. 48: Antioxidant capacity [µmol TE/g DM] of lemon balm. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

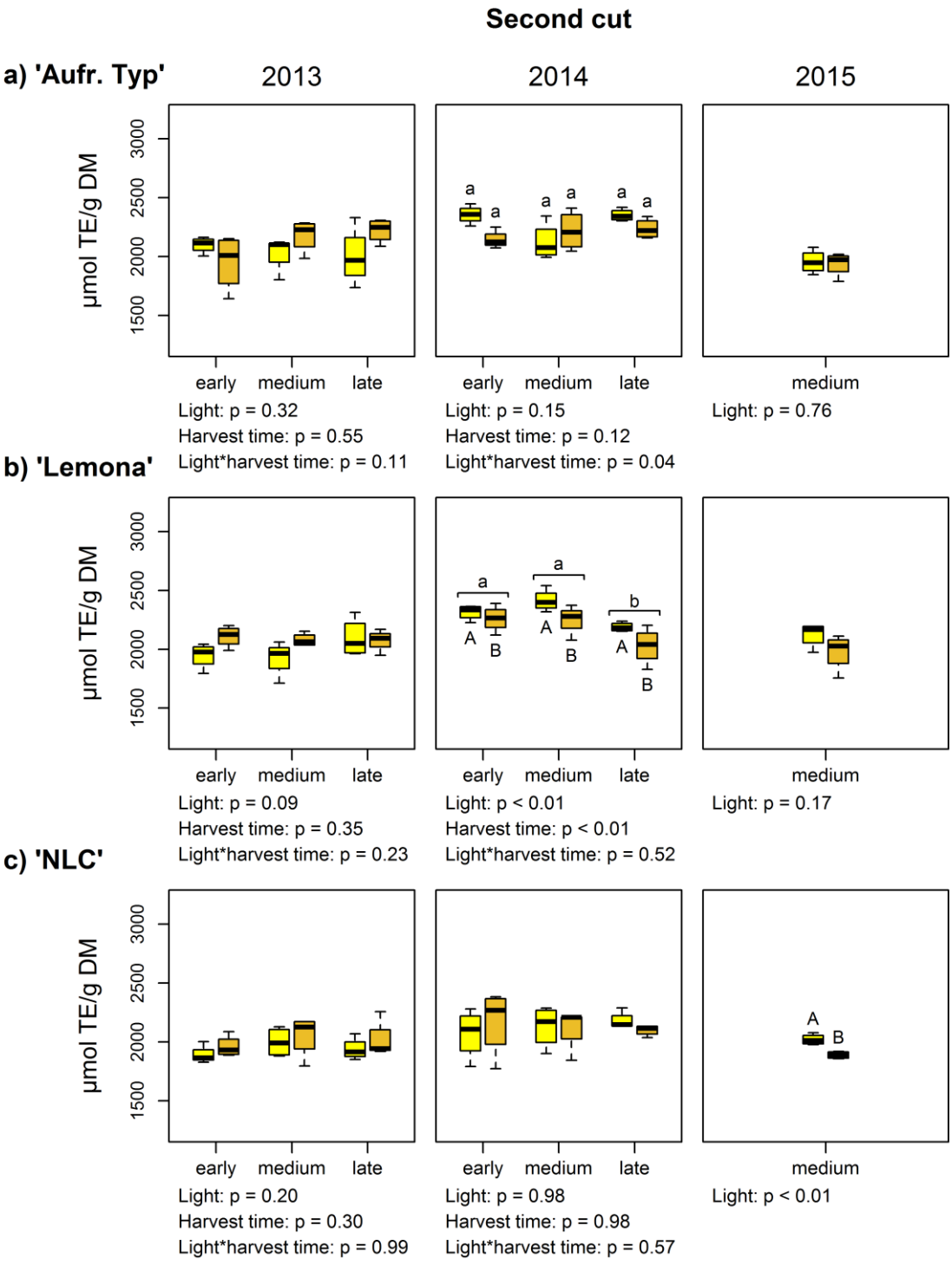

Fig. 49: Antioxidant capacity [µmol TE/g DM] of lemon balm. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.1.6 Rosmarinic acid content

The rosmarinic acid (RA) content covered a range from about 2.7% to more than 6.0% RA in the dried leaves, depending on genotype, harvest stage, year, and cut (**Tab. A 14**). For the reference variants (natural light, medium harvest stage), RA content of the first cut of the harvested leaves was determined as 4.2%, 4.2% as well as 5.2% for 'Aufrechter Typ', 4.3%, 4.4% as well as 5.4% for 'Lemona', and 3.9%, 6.1% as well as 5.9% for 'NLC' (in the years 2013, 2014, and 2014). RA content of the second cut of the reference variants was determined as 4.7%, 4.3% as well as 4.7% for 'Aufrechter Typ', 4.3%, 5.0% as well as 5.2% for 'Lemona', and 4.5%, 4.1% as well as 4.4% for 'NLC' in the years 2013, 2014, and 2015, respectively.

The results of the three-factorial analysis revealed a significant genotype effect for the first cuts in 2014 and 2015, with 'NLC' (5.8% and 5.7%) having significantly higher RA contents than 'Aufrechter Typ' (4.7% and 4.9%). In these two cases, 'Lemona' was either not significantly different from 'Aufrechter Typ' (2013: 5.2%), or from 'NLC' (2015: 5.6%) (**Tab. A 14**).

Also for the first cut in 2013 as well as for the second cut in 2014, the genotypes differed significantly, but this was found in combination with a two-way interaction effect genotype*harvest stage. For the medium harvest stage of cut 2 in 2014, 'NLC' reached a RA content of 4.0%, which was significantly lower than 'Lemona' (4.8%). For cut 1 in 2013, a three-way interaction effect light*genotype*harvest stage was found as well. The interaction effects will be further elucidated by the two-factorial results below, which are presented in form of box plots in **Fig. 50** (first cut) and **Fig. 51** (second cut).

An effect of the harvest stage for the first cut in 2013 could be seen in all three genotypes, however, in combination with an interaction effect light*harvest stage (**Fig. 50**). For 'Aufrechter Typ', a significant decrease of the RA content from early and medium to late harvest stage could be observed in the non-shaded, but not in the shaded plants (**Fig. 50 a**). For 'Lemona', RA content was significantly higher at early harvest stage, compared to medium and late harvest stage, in both shaded and non-shaded plants (**Fig. 50 b**). For 'NLC' (**Fig. 50 c**), a significant decrease of the RA content from early to medium harvest stage occurred in the non-shaded plants, where the late harvest stage was not significantly different from the other two harvest stages. In the shaded plants, however, only the late harvest stage had a significantly lower RA content than the early harvest stage. Additionally, the shaded plants had a significantly lower RA content than the non-shaded plants at the late harvest stage (**Fig. 50 c**).

For the first cut in 2014, no significant effect of the moderate light reduction could be observed. A significant effect of the harvest stage was only observed in 'NLC' (**Fig. 50 c**), with the late harvest stage showing a significantly decreased RA content at late harvest stage, compared to early and medium harvest stage. For the genotypes 'Aufrechter Typ' (**Fig. 50 a**) and 'Lemona' (**Fig. 50 b**), there was only a tendency of a decreasing RA content over time, with the p-values of the harvest stage effect only almost reaching significance ('Aufrechter Typ': $p = 0.051$; 'Lemona': $p = 0.057$). For the first cut in 2015, no effect of the light reduction was seen in all three genotypes (**Fig. 50**).

For the second cut, no effect of the harvest stage was observed in 2013 (**Fig. 51**). However, a significant effect of the moderate shading on the RA content was observed in 'Lemona', with slightly higher values for the shaded plants (**Fig. 51 b**).

In 2014, a significant harvest time effect could be seen for the second cut in all three genotypes, with the early harvest stage exhibiting significantly higher RA contents than the medium harvest stage (**Fig. 51**). However, the tested genotypes differed in their reaction at the late harvest stage, where the RA content was either not significantly different from the early harvest stage ('Aufrechter Typ', **Fig. 51 a**), or from the medium harvest stage ('Lemona', **Fig. 51 b**), or from both ('NLC', **Fig. 51 c**).

No effect of the moderate light reduction on the RA content of the second cut could be observed in the years 2014 and 2015 in all three genotypes (**Fig. 51**).

There was a positive correlation of RA content with ORAC values ($r = 0.72$, $p < 0.001$; **Fig. A 11**) as well as with TPC ($r = 0.63$, $p < 0.001$; **Fig. A 12**).

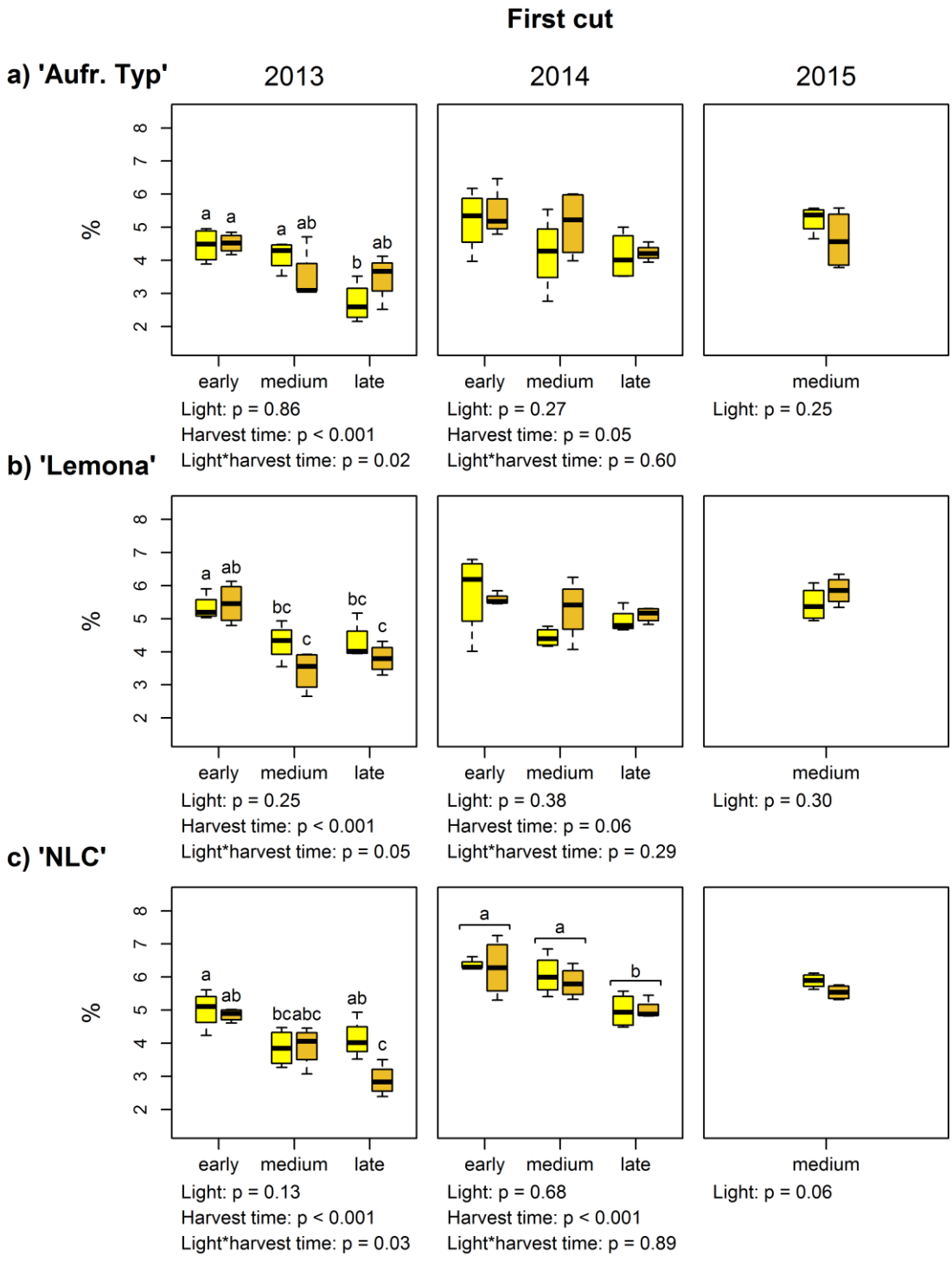

Fig. 50: Rosmarinic acid content [%] of dried lemon balm leaves. First cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

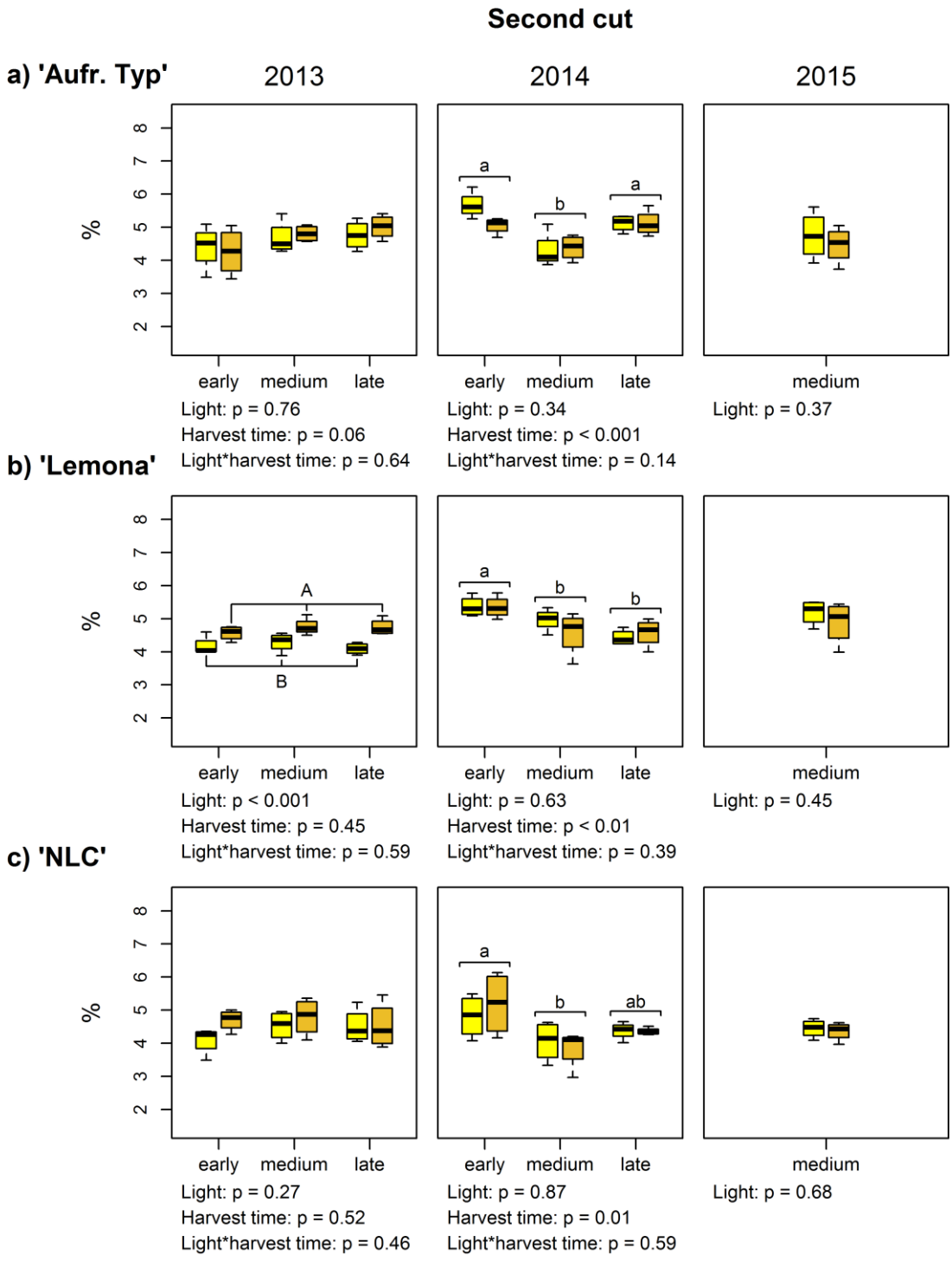

Fig. 51: Rosmarinic acid content [%] of dried lemon balm leaves. Second cut in Gross-Gerau, 2013–2015. Three lemon balm genotypes (a–c) cultivated under different light conditions (□ = natural light; □ = moderate shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2 Field experiment Rauischholzhausen

4.2.1 Plant parameters

4.2.1.1 Plant height

The lemon balm plants in Rauischholzhausen were reaching heights of about 20 cm to more than 100 cm, depending on genotype, harvest stage, cut, and year. Generally, the second cuts had lower plant heights than the first cuts.

The three-factorial analysis (**Tab. A 15**) revealed a significant genotype effect for the first cut in 2013, with higher plants for the genotype 'Lemona' (81.0 cm) compared to' Aufrechter Typ' (73.7 cm). However, for the first cuts in 2014 and 2015, no significant differences between the genotypes were observed. Also for the second cut in 2015, the genotypes did not differ significantly, whereas for the second cuts in 2013 and 2014, significant genotype effects were observed in combination with interaction effects light*genotype*harvest stage.

The results of the two-factorial analysis, split by the genotypes, are presented in **Fig. 52** (first cut) and **Fig. 53** (second cut). For the first cut in 2013 and 2014, 'Aufrechter Typ' showed an expected significant increase of plant height over the three harvest stages (**Fig. 52 a**). The same was true for 'Lemona' in 2013, and for the non-shaded plants in 2014, whereas plant height of the shaded plants increased from early to medium harvest stage, in parallel to the non-shaded plants, whereas a drop in plant height was seen for the late harvest stage (**Fig. 52 b**). No significant differences for the two light treatments were seen for the first cut in 2015 for both genotypes (**Fig. 52**).

For the second cut, however, significant light effects were observed in all three years and both genotypes, albeit partially in combination with interaction effects (**Fig. 53**). For the genotype 'Aufrechter Typ', a significantly increased plant height of the shaded plants compared to the non-shaded plants was observed in 2013 only for early and late, but not for medium harvest stage (**Fig. 53 a**). In 2014, however, shaded plants were higher than non-shaded plants at medium and late harvest stage. For the second cut in 2015, shaded plants were also significantly higher than non-shaded plants (**Fig. 53 a**).

For the genotype 'Lemona', a light effect in the second cut with a higher plant height of the shaded plants was significant in 2013 and 2015, as well as for the medium harvest stage in 2014. For the early and late harvest stage in 2014, however, only a tendency of higher plants in the shade was observed (**Fig. 53 b**).

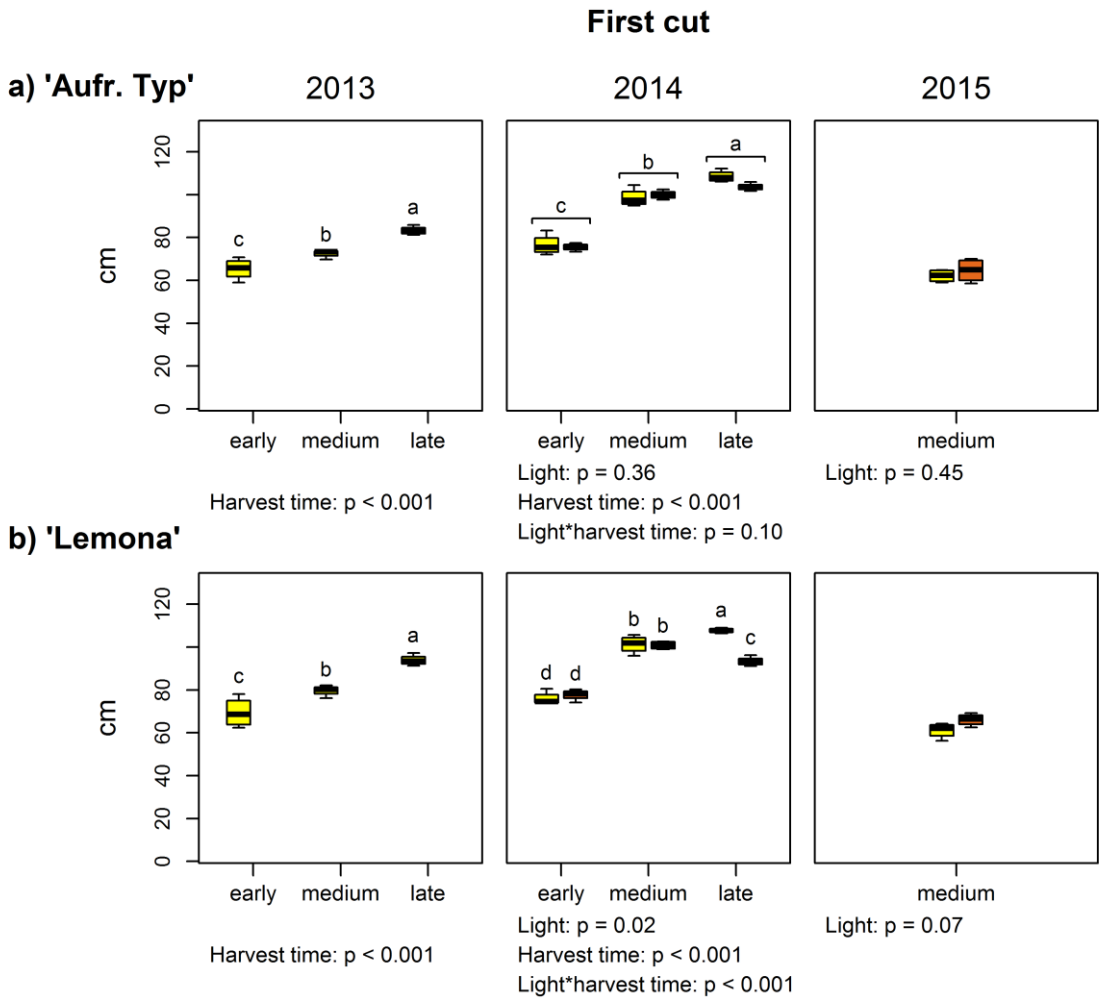

Fig. 52: Plant height [cm] of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

The situation regarding a harvest stage effect of the second cut was a little bit more complex. While the differences between the harvest stages did not reach significance for 'Aufrechter Typ' in 2013, plant height of this genotype decreased from early to medium, and again from medium to late harvest stage in the non-shaded plants in 2014 (**Fig. 53 a**). In the shaded plants of 'Aufrechter Typ', however, plant height increased from early to medium harvest stage, and then decreased from medium to late harvest stage (**Fig. 53 a**).

For 'Lemona', on the other hand, plant height decreased from early and medium to late harvest stage in both light treatments in 2013 (**Fig. 53 b**). The same was true for the shaded plants in 2014, whereas the non-shaded plants showed a decreased plant height from early to medium, and again from medium to late harvest stage (**Fig. 53 b**).

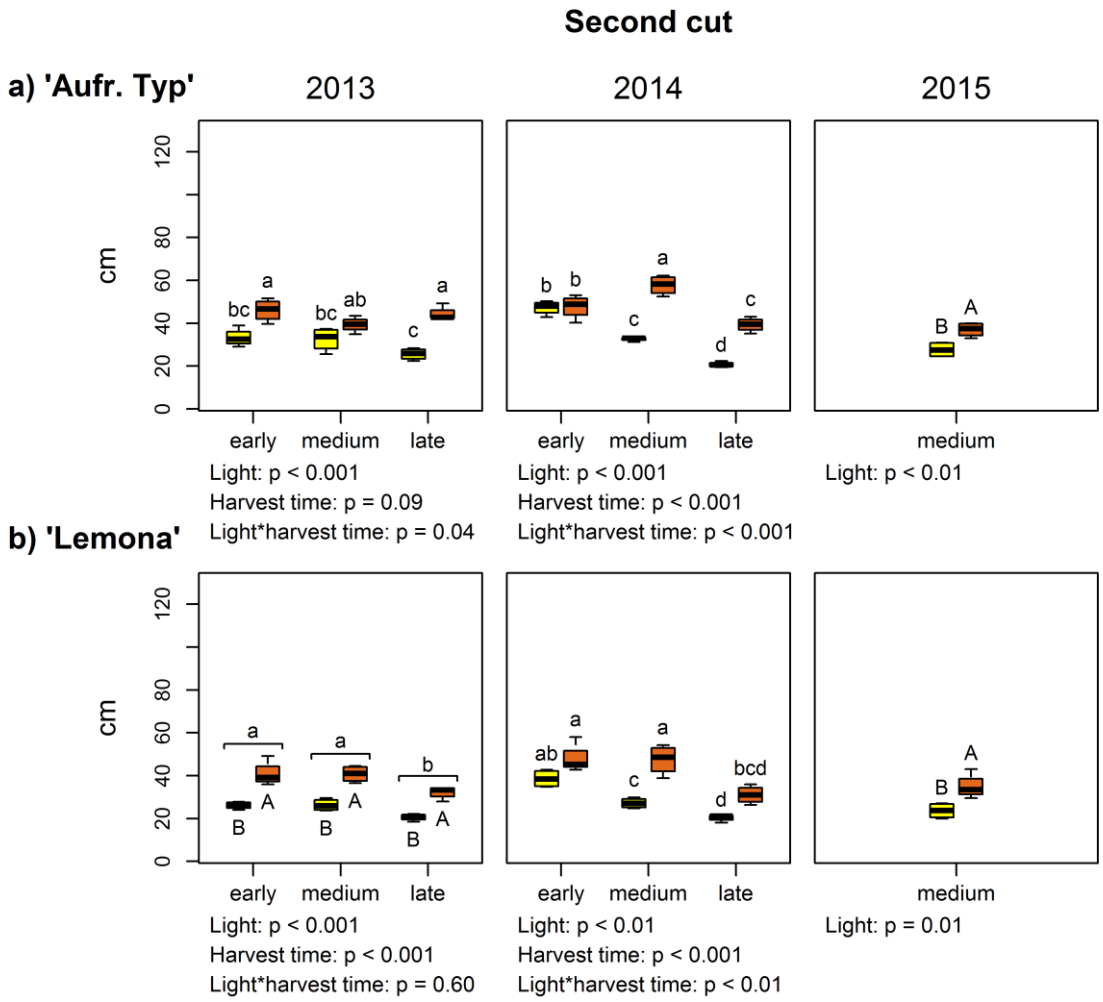

Fig. 53: Plant height [cm] of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (■ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.1.2 Leaf area index (LAI)

Leaf Area Index (LAI) values, registered for the years 2014 and 2015, were found in a range from 3.0 to 14.6, depending on the investigated conditions (**Tab. A 16**).

The three-factorial analysis showed no significant genotype effect regarding LAI for the first cut. However, it revealed significant differences between the two genotypes for the second cut, with 'Lemona' having significantly lower LAI values (6.1 and 6.8) than 'Aufrechter Typ' (7.8 and 8.5) for cut 2 in 2014 and 2015 (**Tab. A 16**).

The results of the following two-factorial analysis are presented in **Fig. 54**. For the first cut, a significant harvest time effect was observed for both genotypes, with a decrease from early to medium harvest stage in both genotypes, and another decrease to late harvest stage only for 'Aufrechter Typ' (**Fig. 54 a**, left-hand side), but not for 'Lemona' (**Fig. 54 b**, left-hand side). No significant effect of the shading on LAI was observed for the first cut in both genotypes.

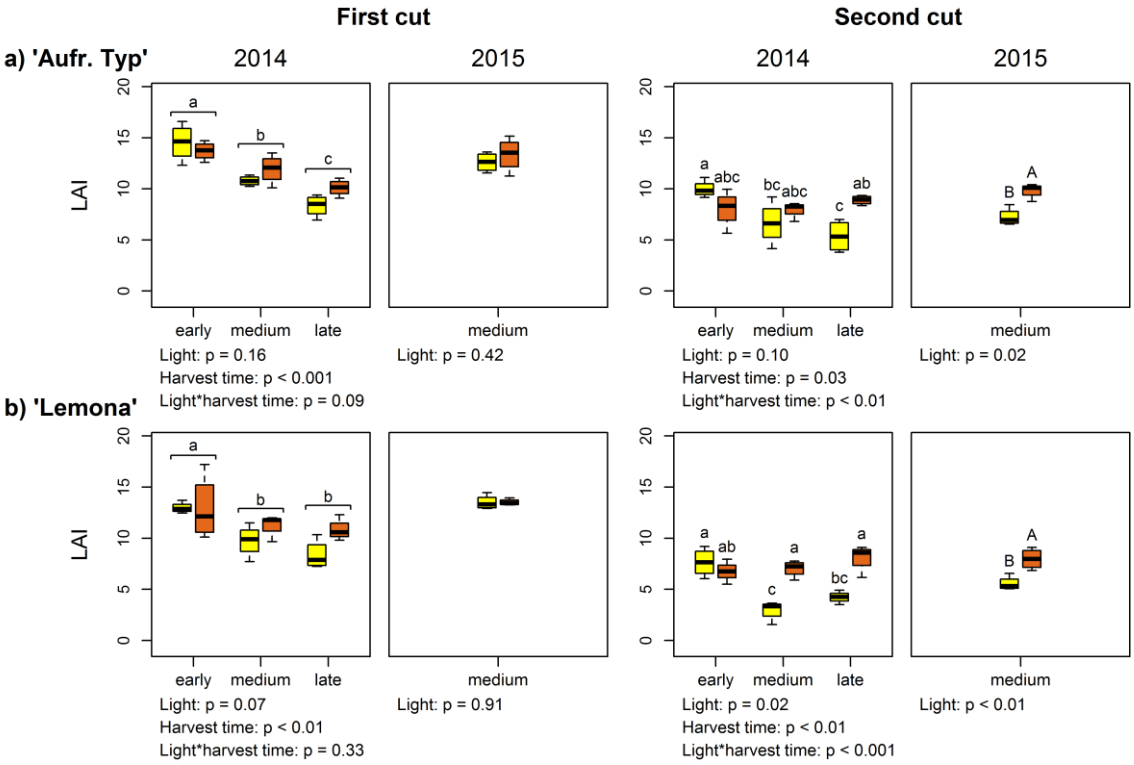

Fig. 54: Leaf Area Index (LAI) of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (■ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late) in two cuts (left-hand side: first cut; right-hand side: second cut). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

For the second cut, however, a significant effect of the shading on LAI was observed in both genotypes, albeit in combination with an interaction effect light*harvest stage for the year 2014 (**Fig. 54**, right-hand side). A significant decrease of LAI values over time was only observed for the non-shaded plants in 2014. A significant effect of the shading, with increased LAI values of the shaded plants, was observed in both genotypes for the second cut in 2015 (**Fig. 54**, right-hand side). The same was true for the late harvest stage in 2014, and for 'Lemona' also for the medium harvest stage (**Fig. 54 b**, right-hand side).

4.2.1.3 SPAD values

SPAD values were ranging from 22.7 to 40.7 SPAD units (**Tab. A 17**). According to the three-factorial analysis, no significant genotype effect was found for the first cut in the investigated years. For the second cut, however, 'Lemona' showed significantly higher SPAD values than 'Aufrechter Typ' in 2013 and 2015, as well as (due to an interaction effect) for the late harvest stage in 2014 (**Tab. A 17**).

The results of the two-factorial analysis are presented in **Fig. 55** and **Fig. 56**. For the first cut, 'Aufrechter Typ' showed no significant harvest time effect in 2013 (**Fig. 55 a**). In 2014, an increase from early to medium harvest stage, followed by a decrease from medium to late harvest stage, could be seen for both shaded and non-shaded plants, albeit to different extents. This appeared in combination with a significant effect of the light intensity, with the shaded plants showing higher SPAD values than the non-shaded plants for the medium and late, but not for the early harvest stage. For the first cut in 2015, no significant effect of the shading on SPAD values could be observed in 'Aufrechter Typ' (**Fig. 55 a**).

The reaction of 'Lemona' on the investigated factors during the first cut was different (**Fig. 55 b**). In 2013, a significant harvest time effect was found, with significantly higher SPAD values at late harvest stage, compared to medium harvest stage. In 2014, SPAD values at medium and late harvest stage were significantly higher than those at early harvest stage, and the shaded plants showed significantly higher values than the non-shaded plants. Also in 2015, a significant effect of the reduced light intensity, leading to higher SPAD values of the shaded plants, could be observed for 'Lemona' (**Fig. 55 b**).

For the second cut, 'Aufrechter Typ' did not show a significant reaction of the SPAD values on light intensity or harvest stage in 2013 (**Fig. 56 a**). In 2014, however, both factors were significant. SPAD values increased from early to medium harvest stage, and the late harvest stage was not significantly different from the medium harvest stage. Additionally, shaded plants had significantly higher SPAD values than the non-shaded plants. In 2015, on the other hand, no significant influence of the light intensity on SPAD values could be observed (**Fig. 56 a**).

While the reaction of 'Lemona' was similar to 'Aufrechter Typ' for the second cut in 2014 and 2015, it differed distinctly in 2013 (**Fig. 56 b**). In this case, the harvest time effect was seen similar to the first cut of this genotype in 2013, with significantly higher SPAD values at late harvest stage, compared to medium harvest stage, and the early harvest stage not being significantly different from the other two stages. However, shaded plants showed decreased SPAD values, compared to the non-shaded plants (**Fig. 56 b**).

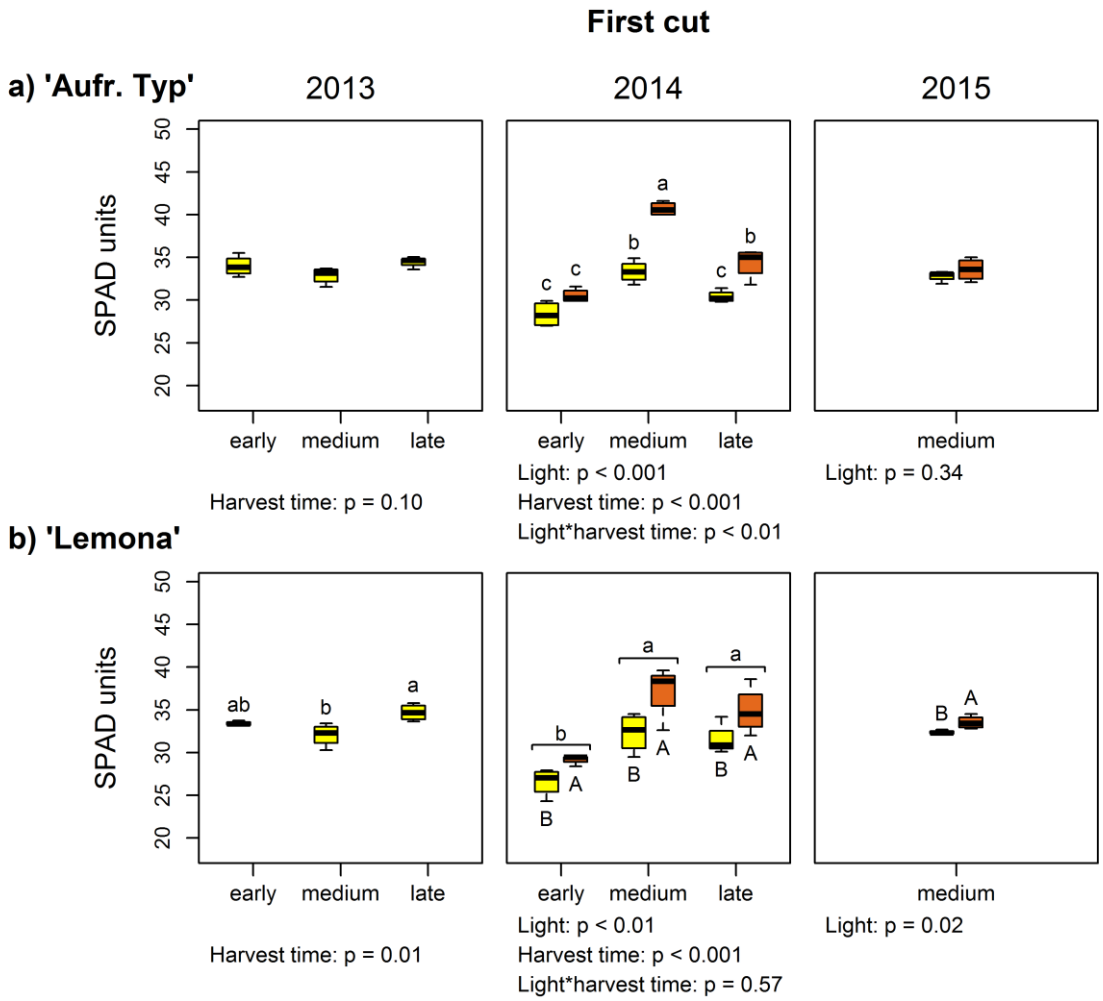

Fig. 55: SPAD values of lemon balm (first fully developed leaf). First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

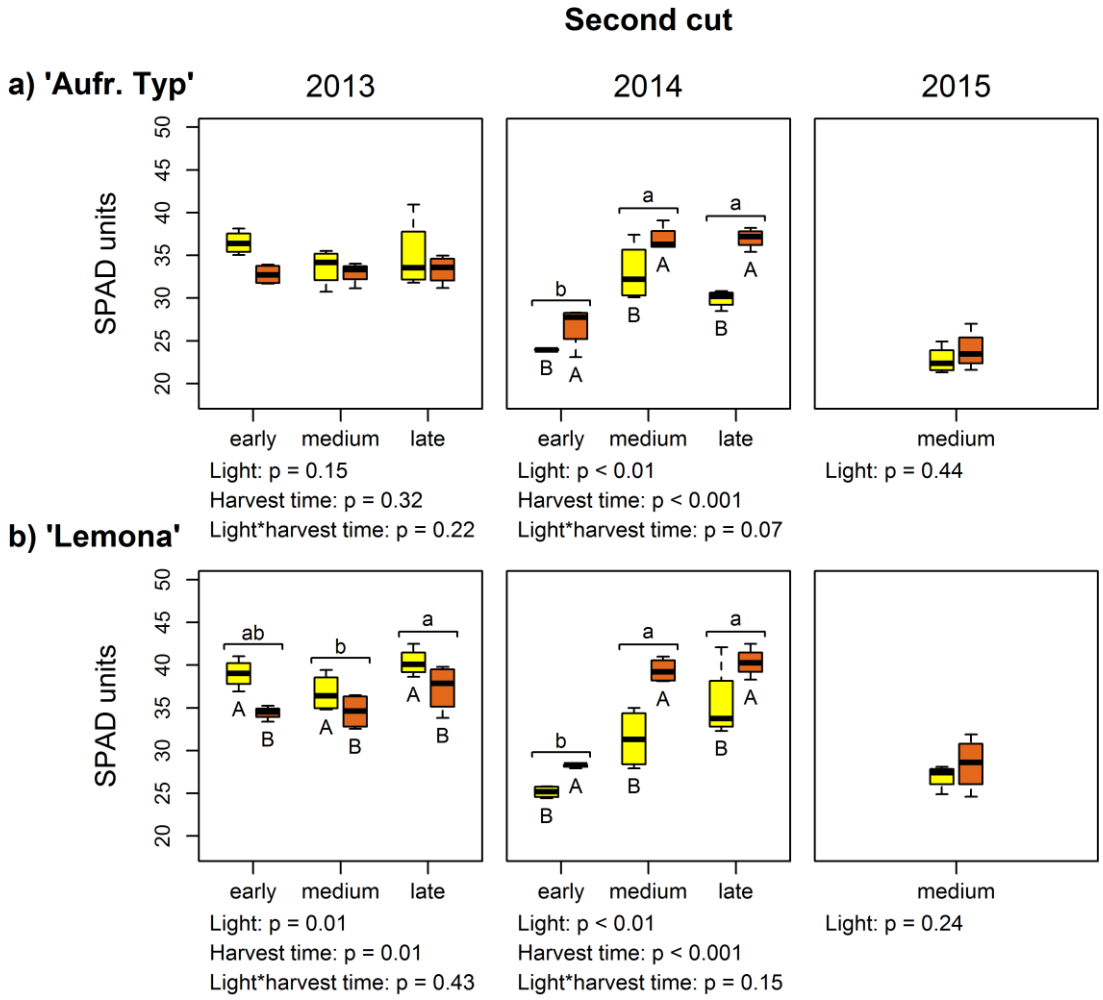

Fig. 56: SPAD values of lemon balm (first fully developed leaf). Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.1.4 Number of shoots per plant

The numbers of shoots per plant were found in a range from 44 to 110 in the investigated years, genotypes, light conditions and harvest times (**Tab. A 18**). The three-factorial analysis did not show significant differences between the two genotypes, except for the first cut in 2015, with 'Lemona' having a higher number of shoots per plant than 'Aufrechter Typ' (**Tab. A 18**). The results of the following two-factorial analysis are presented in **Fig. 57** and **Fig. 58**.

For the first cut, no significant differences in the number of shoots per plant between the harvest stages could be seen in 2013, neither was an effect of the reduced light intensity significant in 2015 (**Fig. 57**). For the first cut in 2014, however, both genotypes showed a significantly higher number of shoots per plant at early harvest stage, compared to late harvest stage. Additionally, the shaded plants exhibited significantly lower numbers of shoots per plant than the non-shaded plants (**Fig. 57**).

Also for the second cut in 2013, no significant effect of the harvest stage could be seen in both genotypes (**Fig. 58**). The light effect was not significant in this case either. The genotype 'Aufrechter Typ' showed an increased number of shoots at late harvest stage, compared to early harvest stage, for the second cut in 2014 (**Fig. 58 a**).

Additionally, the shaded plants exhibited significantly less shoots per plant than the non-shaded plants. Also for the second cut in 2015, this genotype showed a reduced number of shoots per plant for the shaded plants (**Fig. 58 a**).

'Lemona' did not show a significant reaction on light intensity or harvest time for the second cut in 2013 (**Fig. 58 b**). In 2014, however, the number of shoots was increased at late harvest stage for the shaded plants, but not for the non-shaded plants. At early and medium harvest stage, the shaded plants showed significantly less shoots per plant than the non-shaded plants, while this light effect was not reaching significance for the second cut in 2015 (**Fig. 58 b**).

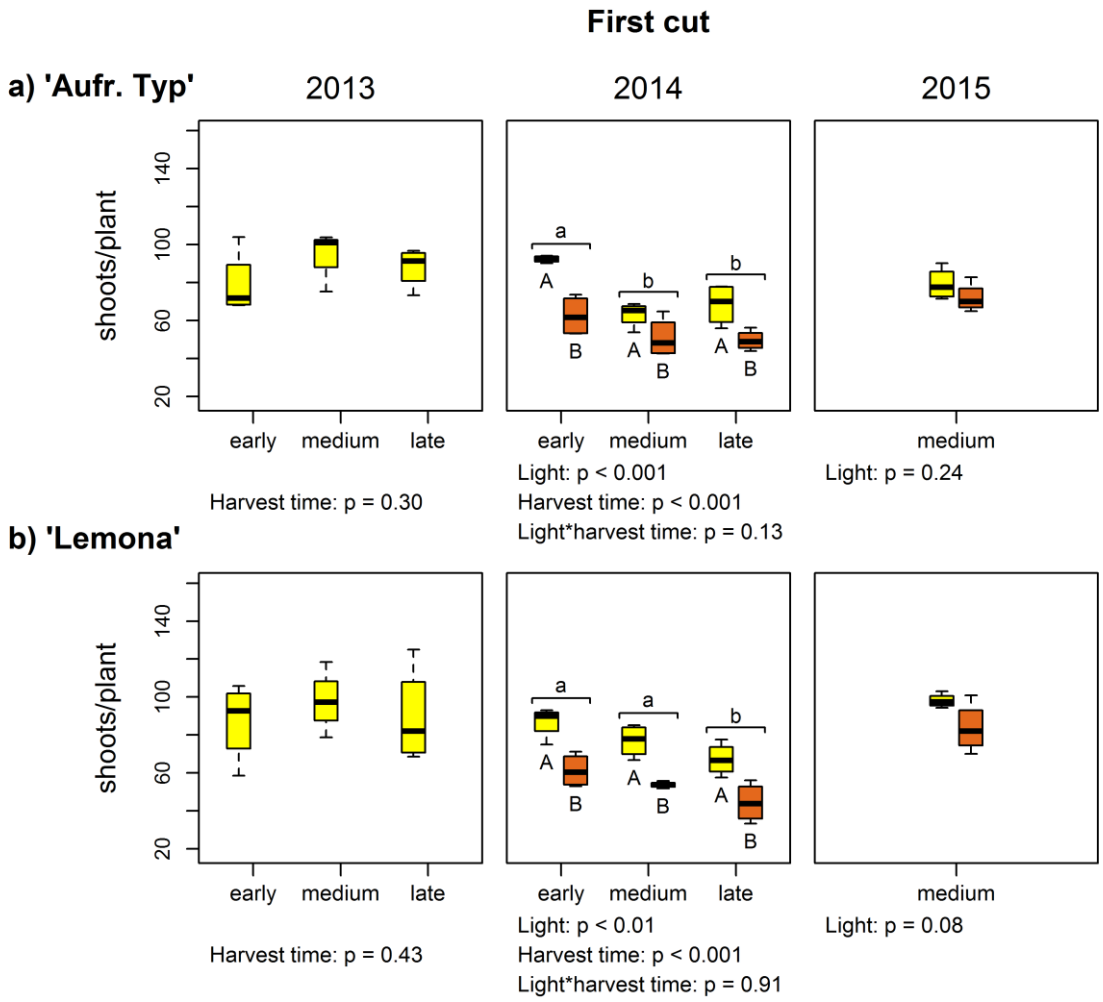

Fig. 57: Shoots per plant of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

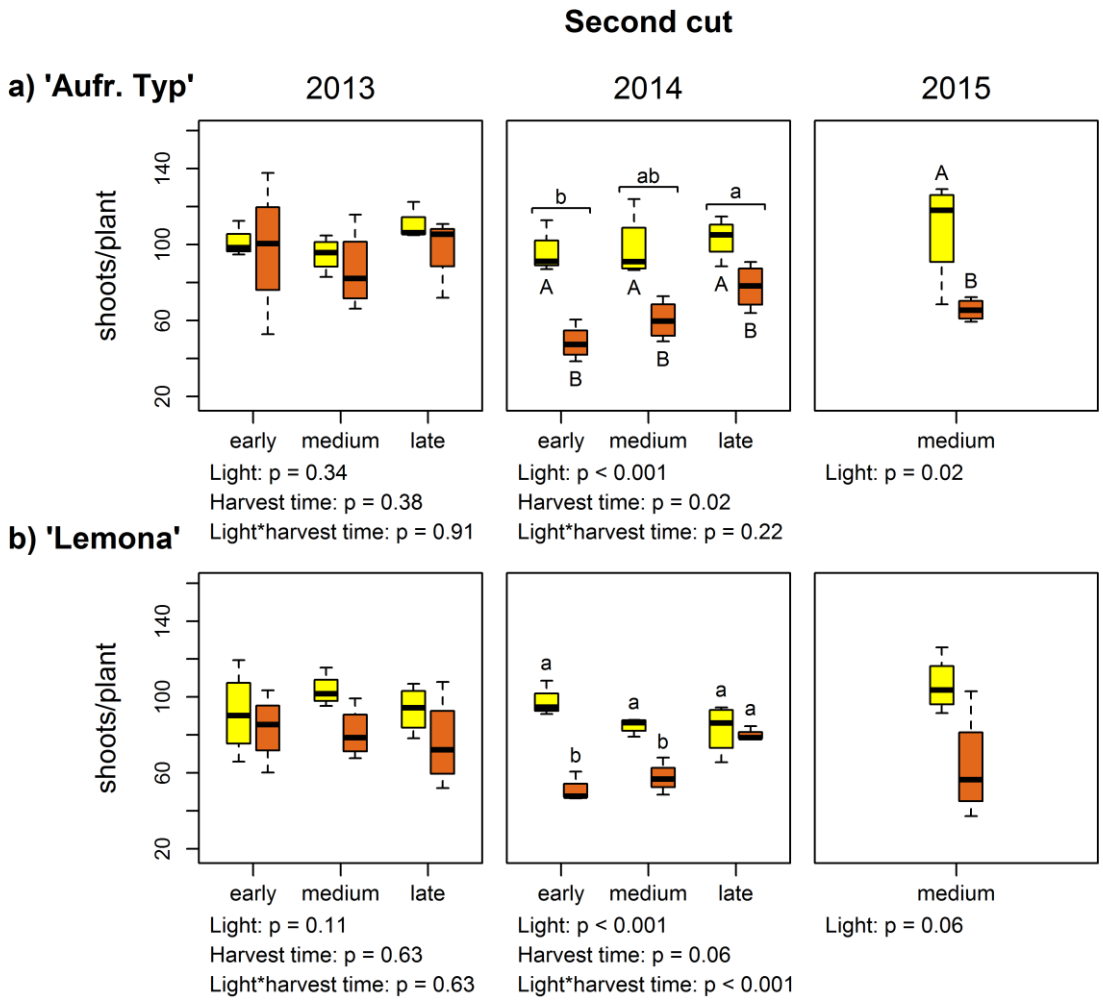

Fig. 58: Shoots per plant of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (■ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.2 Yield parameters

4.2.2.1 Biomass yield (FM)

Biomass yield (FM) was found in a range from 63.9 to 438.9 dt FM/ha, depending on genotype, light intensity, harvest stage, year, and cut within the year (**Tab. A 19**). For the reference variant (natural light, medium harvest stage), annual sums of 428.0, 459.0, as well as 315.2 dt FM/ha were harvested for 'Aufrechter Typ', and 446.3, 423.8, as well as 341.0 dt FM/ha for 'Lemona' in the years 2013, 2014, and 2015, respectively. Generally, biomass yield (FM) of the first cut was higher than the second cut.

The three-factorial analysis showed a significant genotype effect for both cuts in 2013, as well as for the second cut in 2014, and the first cut in 2015 (**Tab. A 19**). For the first cuts in 2013 and 2015, 'Lemona' gave significantly higher biomass yield (FM) (310.0 and 268.7 dt FM/ha) than 'Aufrechter Typ' (279.5 and 232.9 dt FM/ha). However, for the second cuts in 2013 and 2014, an inverse effect was observed, with 'Lemona' (114.6 and 93.0 dt FM/ha) showing a significantly lower biomass yield (FM) than 'Aufrechter Typ' (16.9 and 115.2 dt FM/ha) (**Tab. A 19**).

The results of the following two-factorial analysis are presented in **Fig. 59** (first cut) and **Fig. 60** (second cut). For the first cut in 2013, no significant differences between the harvest stages were observed in any of the genotypes (**Fig. 59**). For the first cut in 2014, however, 'Aufrechter Typ' showed a decrease of biomass yield (FM) in the non-shaded plants, with significantly higher yield at early harvest stage, compared to late harvest stage (**Fig. 59 a**). The non-shaded plants, on the other hand, gave a significantly lower yield at early harvest stage, compared to late harvest stage. While the differences between shaded and non-shaded plants did not reach significance for medium and late harvest stage, the yield of the shaded plants was significantly lower than that of the non-shaded plants at early harvest stage (**Fig. 59 a**).

For the genotype 'Lemona', biomass yield (FM) at early harvest stage was also higher than at late harvest stage in the non-shaded plants of the first cut in 2014 (**Fig. 59 b**). The shaded plants, however, did not show a significant harvest time effect. However, biomass yield (FM) of shaded 'Lemona' plants was significantly lower than that of the non-shaded plants at all three harvest stages, albeit to different extents (**Fig. 59 b**). For the first cut in 2015, no significant effect of the light reduction on biomass yield (FM) was observed (**Fig. 59**).

For the second cut, no significant differences in biomass yield (FM) were observed between the harvest stages in 2013 in any of the two genotypes (**Fig. 60**). 'Aufrechter Typ', however, showed a significant reduction of biomass yield (FM) by the light reduction

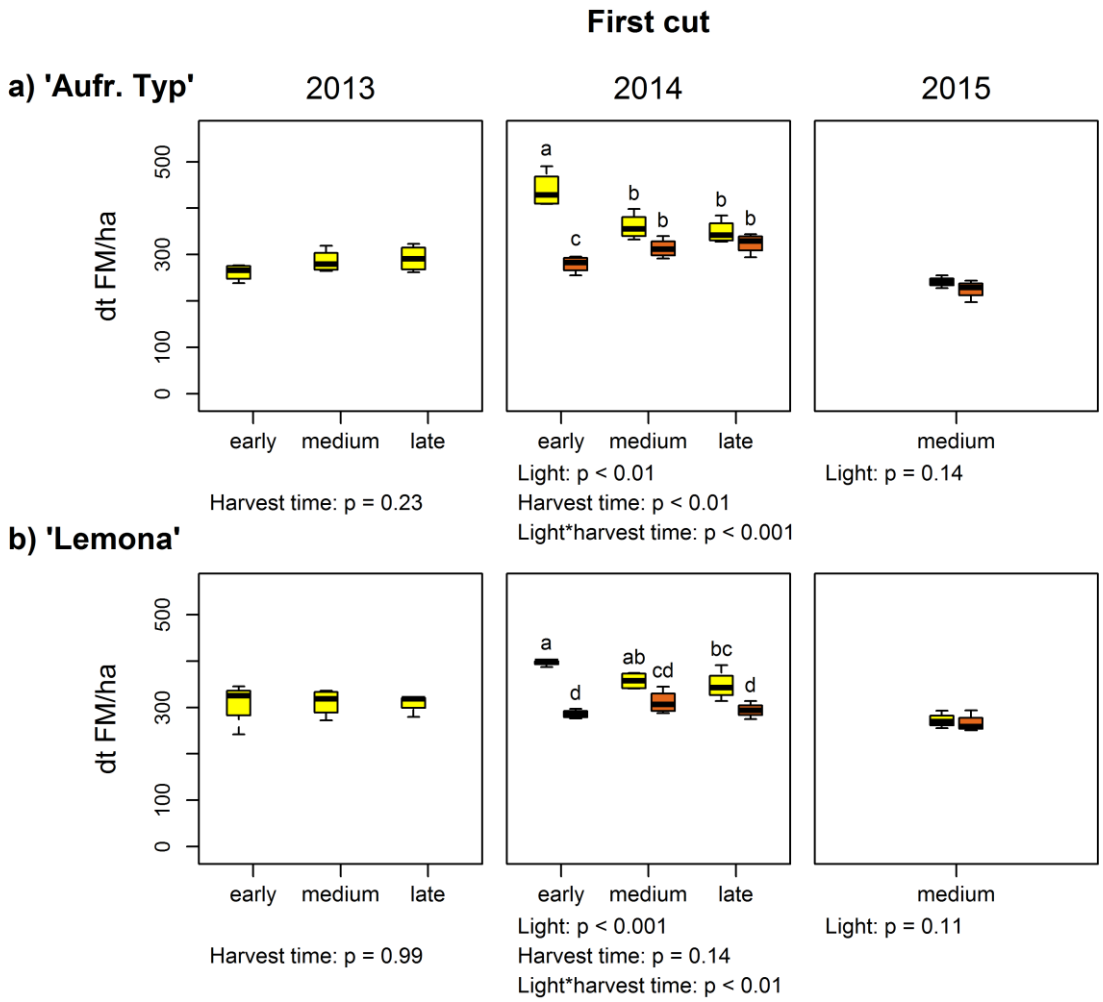

Fig. 59: Fresh matter biomass yield [dt FM/ha] of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

in this case (**Fig. 60 a**). In 2014, this genotype exhibited an interaction effect light*harvest stage. Biomass yield (FM) at medium and late harvest stage was significantly lower than at early harvest stage for the non-shaded plants, while the shaded plants did not differ significantly among the harvest stages. Additionally, shaded plants of 'Aufrechter Typ' gave a significantly decreased biomass yield (FM) at early harvest stage, whereas it was significantly higher at medium and late harvest stage, compared to the non-shaded plants (**Fig. 60 a**). 'Lemona' only showed a significant decrease of biomass yield (FM) from early to medium harvest stage in the non-shaded plants, remaining at the same level at late harvest stage, and no significant differences between the two light conditions (**Fig. 60 b**). In 2015, none of the two genotypes exhibited a significant effect of the reduced light intensity on biomass yield (FM) for the second cut (**Fig. 60**).

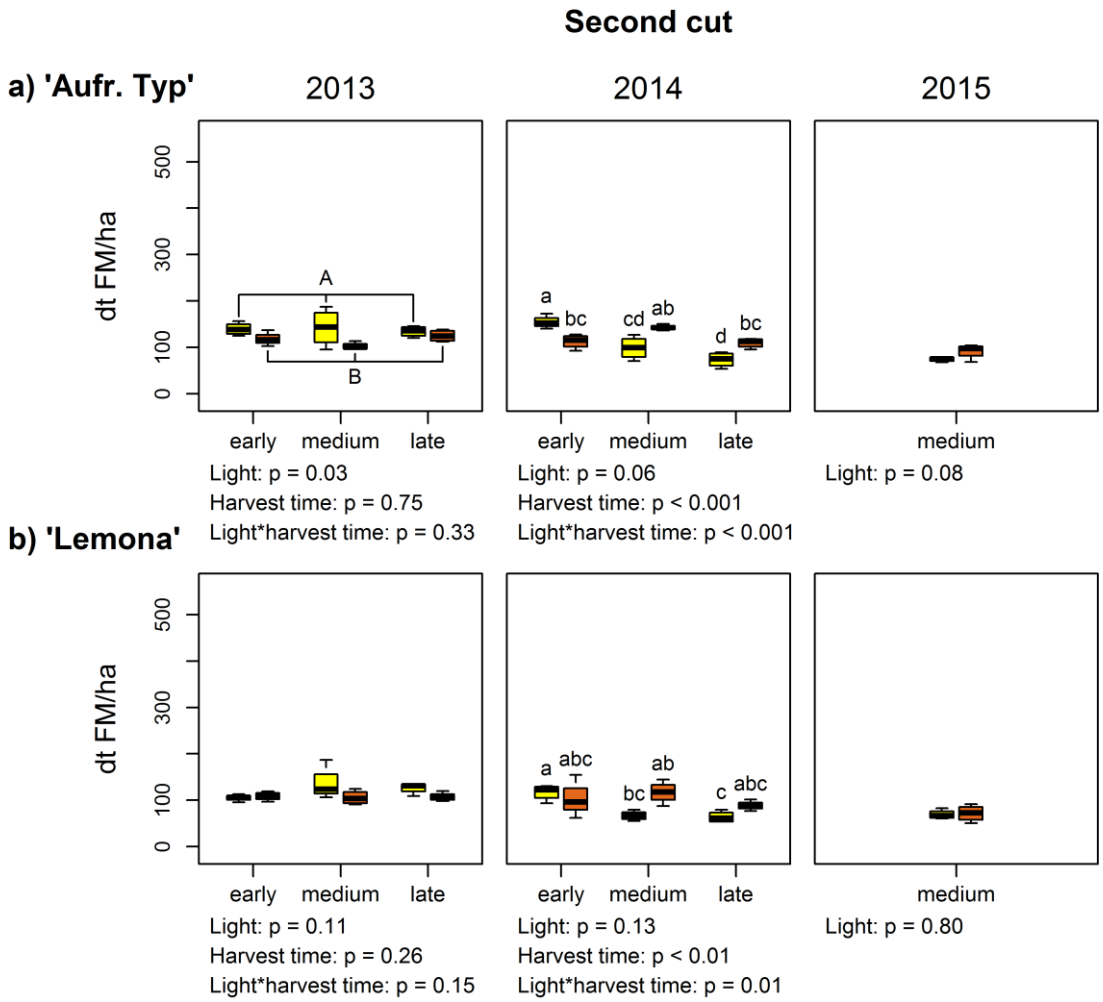

Fig. 60: Fresh matter biomass yield [dt FM/ha] of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; □ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.2.2 Biomass yield (DM)

Biomass yield (DM) was ranging from 16.2 to 93.1 dt DM/ha in the investigated years, genotypes, harvest stages, light conditions, and cuts within a year (**Tab. A 20**). For the reference variant (natural light, medium harvest stage), annual sums of 91.2, 106.7, as well as 71.7 dt DM/ha were harvested for 'Aufrechter Typ', and 86.2, 94.4, as well as 75.0 dt DM/ha for 'Lemona' in the years 2013, 2014, and 2015, respectively. As for biomass yield (FM), values obtained in the second cuts were lower compared to the first cuts.

The three-factorial analysis resulted in significant differences between the two genotypes for the second cut in 2013, both cuts in 2014, as well as for the first cut in 2015 (**Tab. A 20**). 'Aufrechter Typ' gave significantly higher yields than 'Lemona' in the second cut of 2013 (28.8 vs. 25.3 dt DM/ha), in the first cut of 2014 (67.7 vs. 62.5 dt DM/ha), as well as in the second cut of 2014 (25.8 vs. 20.7 dt DM/ha). In the first cut of 2015, however, 'Aufrechter Typ' (46.0 dt DM/ha) had a significantly lower biomass yield (DM) than 'Lemona' (51.9 dt DM/ha) (**Tab. A 20**).

The results of the two-factorial analysis are presented in **Fig. 61** and **Fig. 62**. For the first cut in 2013, both genotypes showed an increase from early to late harvest stage (**Fig. 61**). While for 'Lemona' (**Fig. 61 a**), early and medium harvest stage did not differ, all three harvest stages were significantly different, with an increasing pattern, in 'Aufrechter Typ' (**Fig. 61 b**). This increase from early to medium, and again from medium to late harvest stage was also observed for the first cut in 2014 in both genotypes, however (because of an interaction effect) separately for the two light treatments in 'Aufrechter Typ' (**Fig. 61 a**), and as a main effect in 'Lemona' (**Fig. 61 b**). In both cases, a light effect, with significantly lower values for the shaded plants, was observed as well (for 'Lemona' as a main effect; for 'Aufrechter Typ', due to the interaction light*harvest stage, separately for each harvest stage). For the first cut in 2015, both genotypes gave significantly lower DM biomass leaf yields for the shaded plants, compared to the non-shaded plants (**Fig. 61**).

The results for the second harvest stage, presented in **Fig. 62**, showed this significant effect of the reduced light intensity on the biomass yield (DM) as well for 2013. In 2014, however, this was only the case for the early harvest stage of 'Aufrechter Typ' (**Fig. 62 a**). For the second cut in 2015, no significant differences between the two light treatments could be observed in any of the two genotypes (**Fig. 62**).

FM and biomass yield (DM) were highly correlated ($r = 0.90$, $p < 0.001$) (**Fig. A 13**).

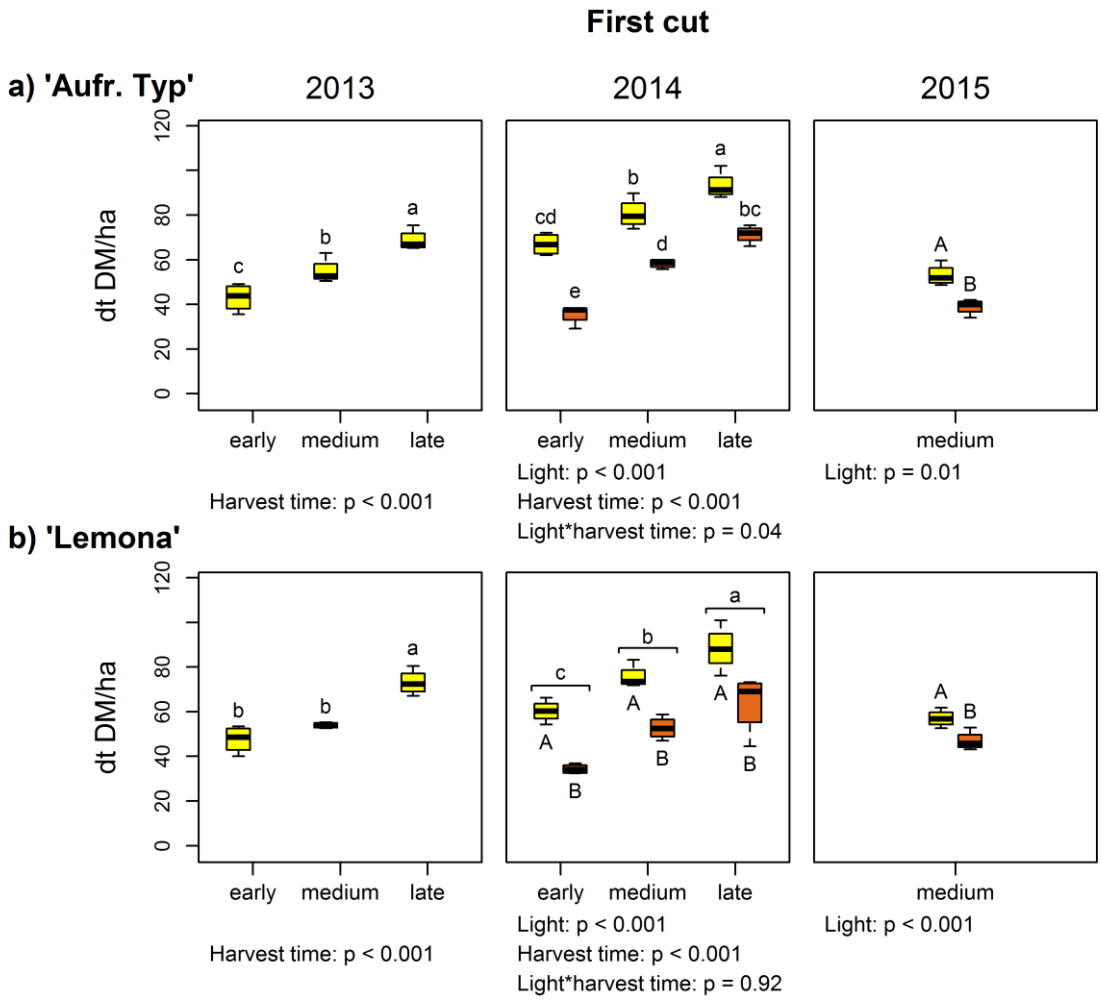

Fig. 61: Dry matter biomass yield [dt DM/ha] of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

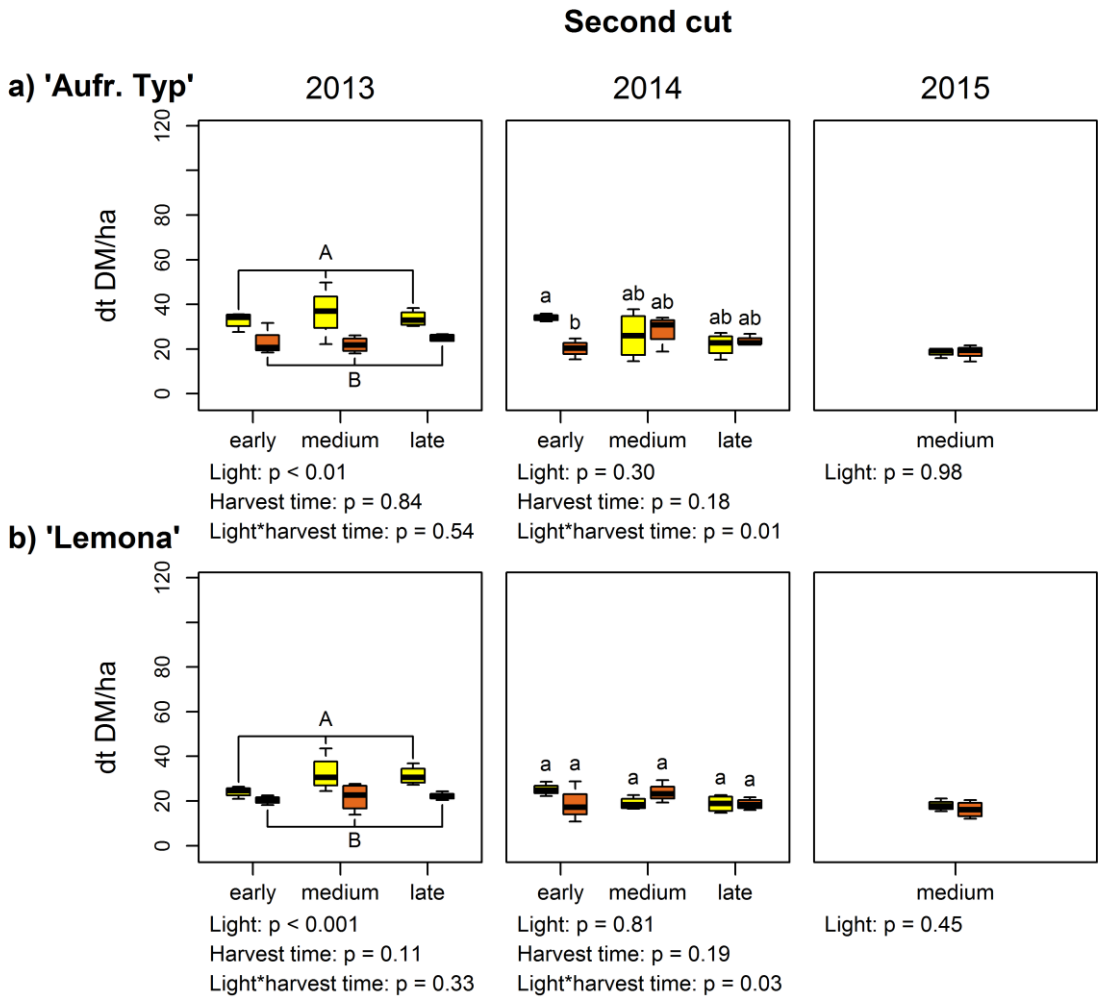

Fig. 62: Dry matter biomass yield [dt DM/ha] of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.2.3 Leaf yield (FM)

Leaf yield (FM) in Rauischholzhausen was found in a range from 27.0 to 181.4 dt/ha in the investigated years, depending on the experimental conditions (**Tab. A 21**). For the reference variant (natural light, medium harvest stage), annual sums of 237.4, 196.7, as well as 177.4 dt FM/ha were harvested for 'Aufrechter Typ', and 237.0, 160.2, as well as 182.2 dt FM/ha for 'Lemona' in the years 2013, 2014, and 2015, respectively. Values for the second cuts were lower than for the first cuts in all three years.

The three-factorial analysis revealed significant differences between the tested genotypes for the first cut in 2013, where 'Lemona' exhibited a significantly higher leaf fresh matter yield (143.7 dt FM/ha) than 'Aufrechter Typ' (134.6 dt FM/ha), as well as for both cuts in 2014, where on the contrary 'Lemona' gave significantly lower yields (cut 1: 118.4 dt FM/ha; cut 2: 48.3 dt FM/ha) than 'Aufrechter Typ' (cut 1: 135.7 dt FM/ha; cut 2: 59.7 dt FM/ha) (**Tab. A 21**). No significant differences between the genotypes were observed for the second cut in 2013, as well as for both cuts in 2015.

The results of the two-factorial analysis are presented in **Fig. 63** and **Fig. 64**. For the first cut in 2013, leaf yield (FM) at late harvest stage was significantly higher than at early harvest stage, and medium harvest stage was either not significantly different from late harvest stage ('Aufrechter Typ'; **Fig. 63 a**), or from early and late harvest stage ('Lemona'; **Fig. 63 b**). In 2014, however, late harvest stage showed significantly lower leaf fresh matter yields for both light treatments in 'Lemona' (**Fig. 63 b**), but only for the non-shaded plants in 'Aufrechter Typ' (**Fig. 63 a**). A significant light effect, with the shaded plants giving lower leaf fresh matter yields than the non-shaded plants, was significant for both genotypes in 2015, for 'Lemona' at all harvest stages in 2014, and in 'Aufrechter Typ' only for the early harvest stage in 2014 (**Fig. 63**).

For the second cut, a significantly reduced leaf yield (FM) of the shaded plants was only observed for 'Aufrechter Typ' in 2013, as well as for the early harvest stage in 2014 (**Fig. 64 a**). The latter was found in combination with a higher yield at early harvest stage, compared to medium and late harvest stage, only in the non-shaded plants, while there were no significant differences between the harvest stages of the shaded plants (**Fig. 64 a**). For 'Lemona', this pattern of a significantly higher leaf fresh matter yield at early harvest stage only in the non-shaded plants was observed as well in the same year (**Fig. 64 b**). In 2015, none of the two genotypes exhibited a significant reaction on the reduced light intensity for the second cut (**Fig. 64**).

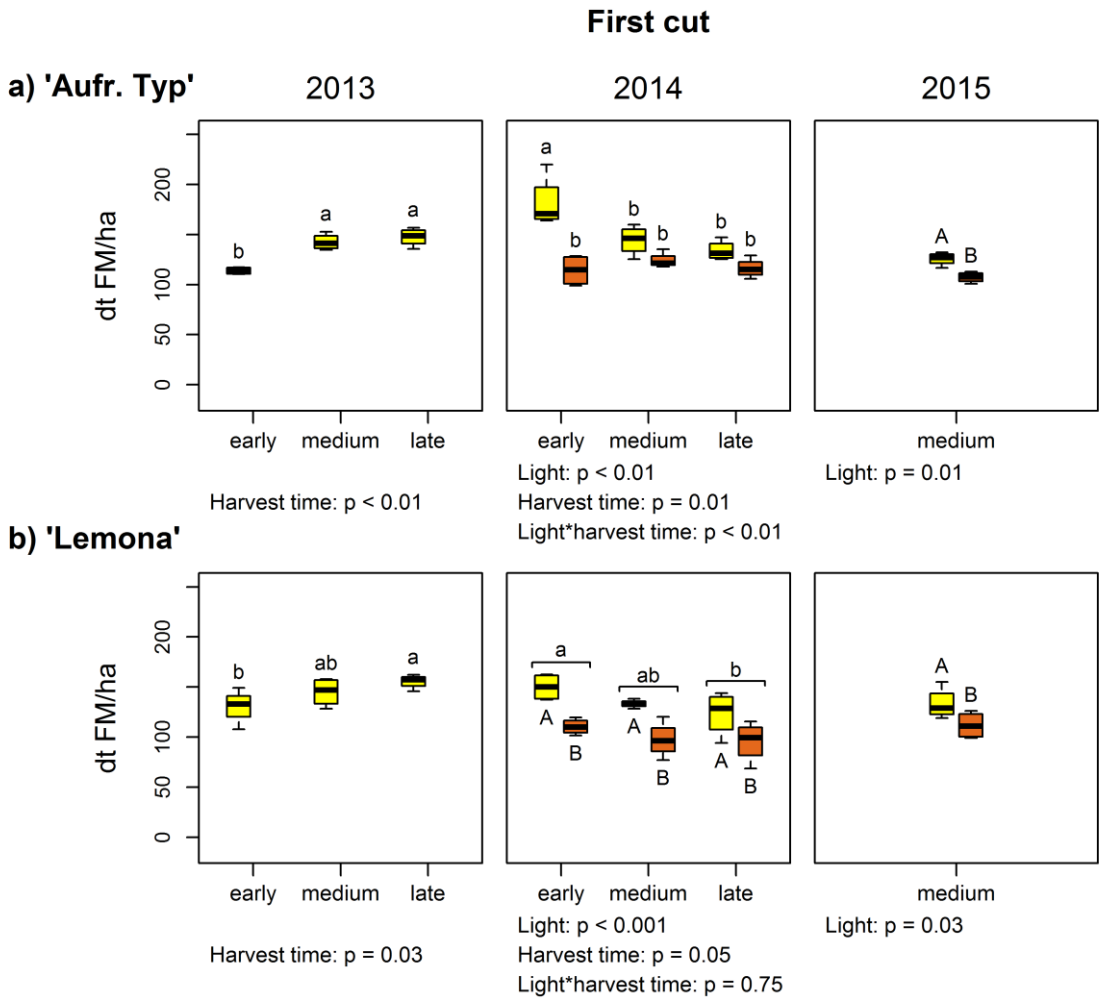

Fig. 63: Fresh matter leaf yield [dt FM/ha] of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

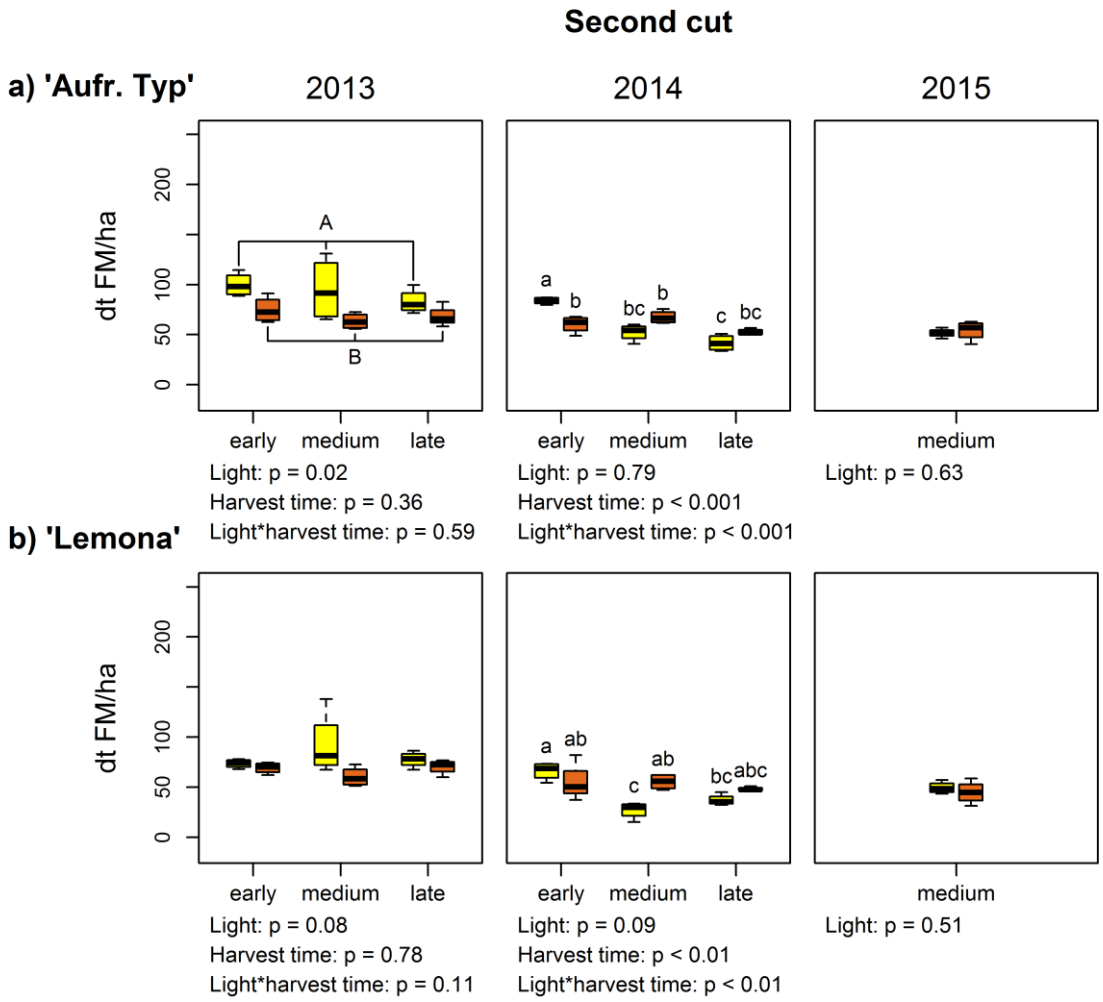

Fig. 64: Fresh matter leaf yield [dt FM/ha] of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.2.4 Leaf yield (DM)

DM leaf yield was covering a range from 9.5 to 40.7 dt DM/ha under the investigated conditions in the years 2013–2015 (**Tab. A 22**). For the reference variant (natural light, medium harvest stage), annual sums of 46.8, 49.2, as well as 44.4 dt DM/ha were harvested for 'Aufrechter Typ', and 47.0, 40.3, as well as 44.0 dt DM/ha for 'Lemona' in the years 2013, 2014, and 2015, respectively.

Similar to the leaf fresh matter yield, 'Lemona' gave a significantly higher leaf dry matter yield (31.0 dt DM/ha) than 'Aufrechter Typ' (27.0 dt DM/ha) for the first cut in 2013, whereas the opposite was true for the first cut in 2014 (24.2 vs. 27.1 dt DM/ha) (**Tab. A 22**). For the second cut in 2014, however, due to an interaction effect genotype*harvest stage, 'Aufrechter Typ' exhibited a significantly higher yield than 'Lemona' only for the medium harvest stage (**Tab. A 22**).

For the first cut in 2013, the results of the two-factorial analysis showed a significant effect of the harvest stage in both genotypes, with the late harvest stage giving a significantly higher yield than early or medium harvest stage (**Fig. 65**). For the first cut in 2014, however, a harvest stage effect was significant only for the shaded plants of 'Aufrechter Typ', where the late harvest stage exhibited a significantly higher yield than the early harvest stage (**Fig. 65 a**). Despite this interaction effect, shaded plants of 'Aufrechter Typ' still showed significantly decreased values compared to the non-shaded plants at all harvest stages (**Fig. 65 a**). For 'Lemona', the light effect, with the shaded plants giving lower DM leaf yields than the non-shaded plants, was significant in 2014 as a main effect (**Fig. 65 b**). Also for the first cut in the year 2015, both genotypes showed a significant reduction of DM leaf yield in the shaded plants (**Fig. 65**).

The same was true for the second cut in 2013 for both genotypes (**Fig. 66**). In 2014, due to an interaction effect light*harvest stage, shaded plants of 'Aufrechter Typ' gave a significantly lower yield than the non-shaded plants only at early harvest stage (**Fig. 66 a**). This was found in a combination of the shaded plants not differing in their yield among the three harvest stages, whereas the non-shaded plants gave a significantly higher DM leaf yield at early harvest stage, compared to medium and late harvest stage (**Fig. 66 a**). In 'Lemona', an interaction effect was observed as well in 2014, with a decrease from early to medium harvest stage only in the non-shaded plants. In 2015, the light effect with a reduced DM leaf yield of the shaded plants reached significance only for 'Aufrechter Typ' (**Fig. 66 a**).

FM and DM leaf yield were highly correlated ($r = 0.91$, $p < 0.001$) (**Fig. A 14**).

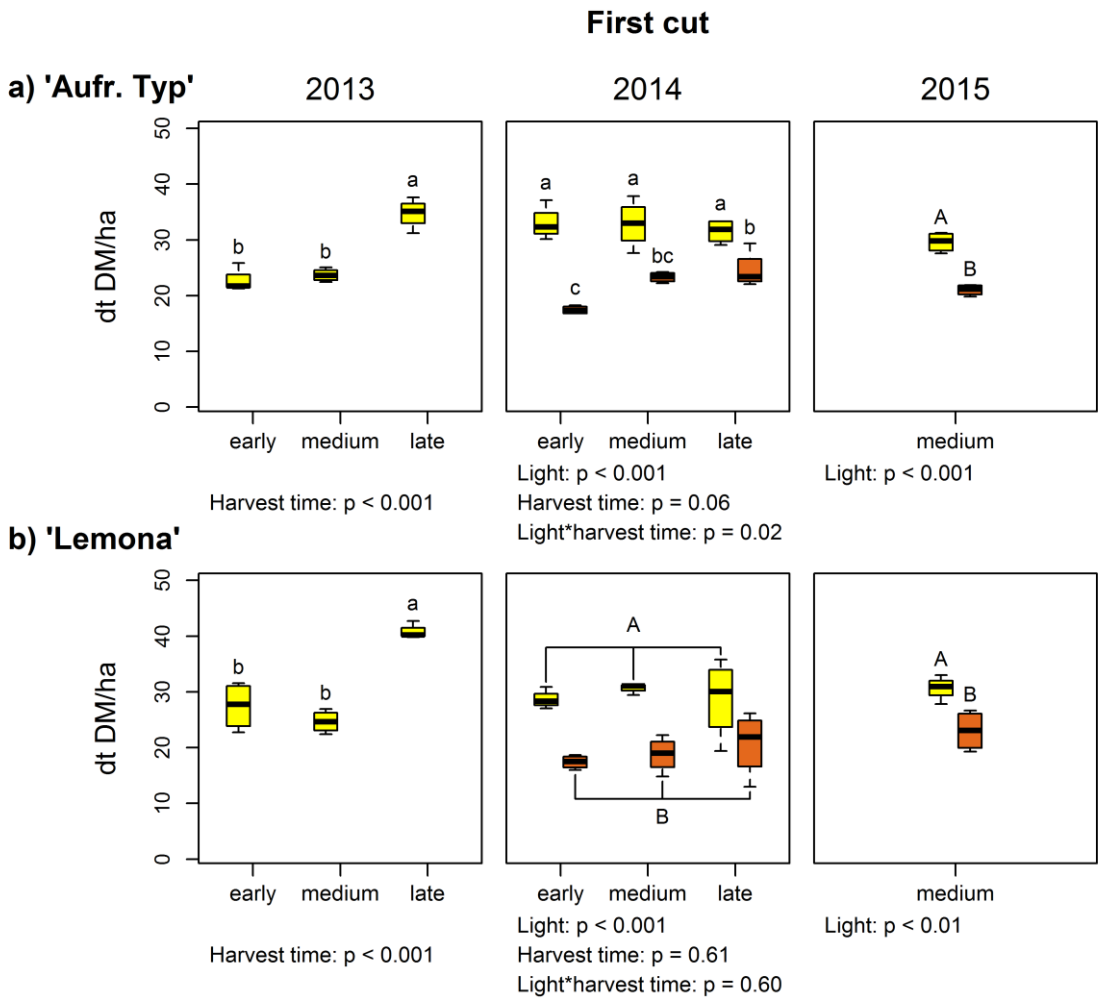

Fig. 65: Dry matter leaf yield [dt DM/ha] of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

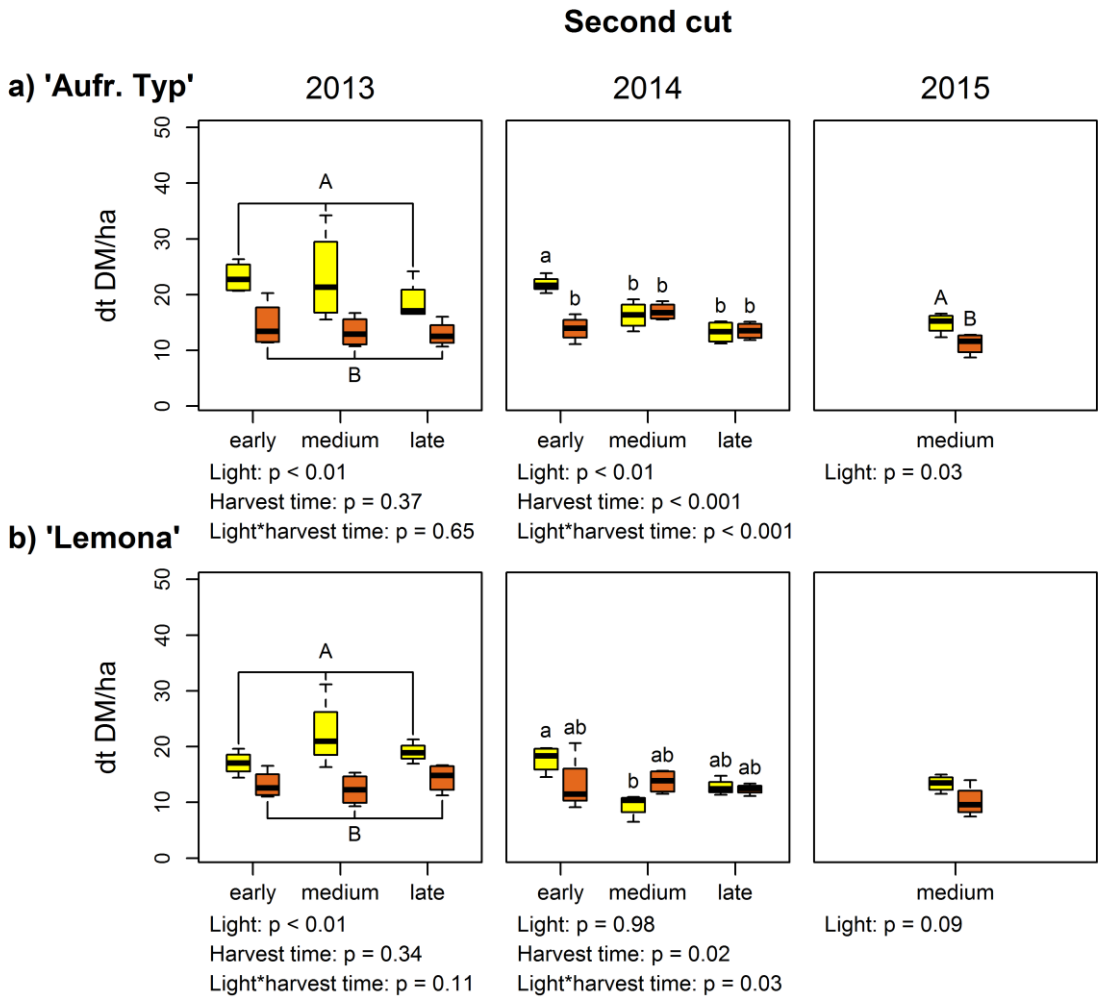

Fig. 66: Dry matter leaf yield [dt DM/ha] of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.2.5 DM content of the leaves

DM contents of the leaves were found in a range from 15.4 to 36.5%, depending on the experimental conditions (**Tab. A 23**). The three-factorial analysis showed significant differences between the tested genotypes in some cases, with 'Lemona' exhibiting higher values, however only for the first cut in 2013 ('Aufrechter Typ': 20.0%; 'Lemona': 21.4%), as well as for the second cut in 2014 ('Aufrechter Typ': 27.2%; 'Lemona': 28.7%) (**Tab. A 23**). In the remaining four cases, the genotypes did not differ significantly.

The results of the two-factorial analysis are presented in **Fig. 67** (first cut) and **Fig. 68** (second cut). For the first cut, a significant effect of the harvest stage was observed in both genotypes in 2013 as well as in 2014 (**Fig. 67**). In 2013, both genotypes showed a significant decrease from early to medium, and then a significant increase from medium to late harvest stage ('Aufrechter Typ': 19.9%, 16.7%, and 23.6%; 'Lemona': 21.0%, 17.1%, and 26.2%; **Fig. 67 a + b**). For the first cut in 2014, all three harvest stages were significantly different in 'Aufrechter Typ', with an increasing pattern (16.9%, 20.8%, and 22.3%; **Fig. 67 a**), whereas in 'Lemona', only the early harvest stage was significantly lower than medium and harvest stage, which did not differ significantly (17.5%, 21.2%, 22.2%; **Fig. 67 b**). Additionally, a significant reduction of leaf DM content in the shaded plants, compared to the non-shaded plants, could be observed in both genotypes (**Fig. 67 a + b**; 'Aufrechter Typ': 18.4% vs. 21.5%; 'Lemona': 18.8% vs. 21.8%; calculated as the main effect over all three harvest stages, cf. **Tab. A 23**). The significant effect of the light reduction was also visible for the first cut in 2015 (**Fig. 67**).

For the second cut in 2013, no significant harvest stage effect was observed in both genotypes (for 'Aufrechter Typ' only almost significant) (**Fig. 68**). However, once again the effect of the light reduction was significant in both genotypes, with reduced leaf DM content for the shaded plants, compared to the non-shaded plants (**Fig. 68 a + b**; 'Aufrechter Typ': 19.7% vs. 23.3%; 'Lemona': 19.9% vs. 24.1%; calculated as the main effect over all three harvest stages, cf. **Tab. A 23**). For the second cut in 2014, 'Aufrechter Typ' exhibited both a harvest stage effect (early harvest stage significantly lower than medium or late harvest stage), as well as a significant effect of the light reduction (**Fig. 68 a**). For 'Lemona', however, an interaction effect occurred, with the pattern of significantly lower values at early harvest stage only for the non-shaded plants, whereas the shaded plants did not differ significantly in their leaf DM content between the harvest stages. A significant reduction of the DM content of the leaves in the shaded 'Lemona' plants was seen only at medium and late harvest stage, but not at early harvest stage (**Fig. 68 b**). For the second cut in 2015, significantly reduced leaf DM contents of the shaded plants, compared to the non-shaded plants, were again observed in both genotypes ('Aufrechter Typ': 20.6% vs. 28.7%; 'Lemona': 22.7% vs. 27.2%; **Fig. 68 a + b**).

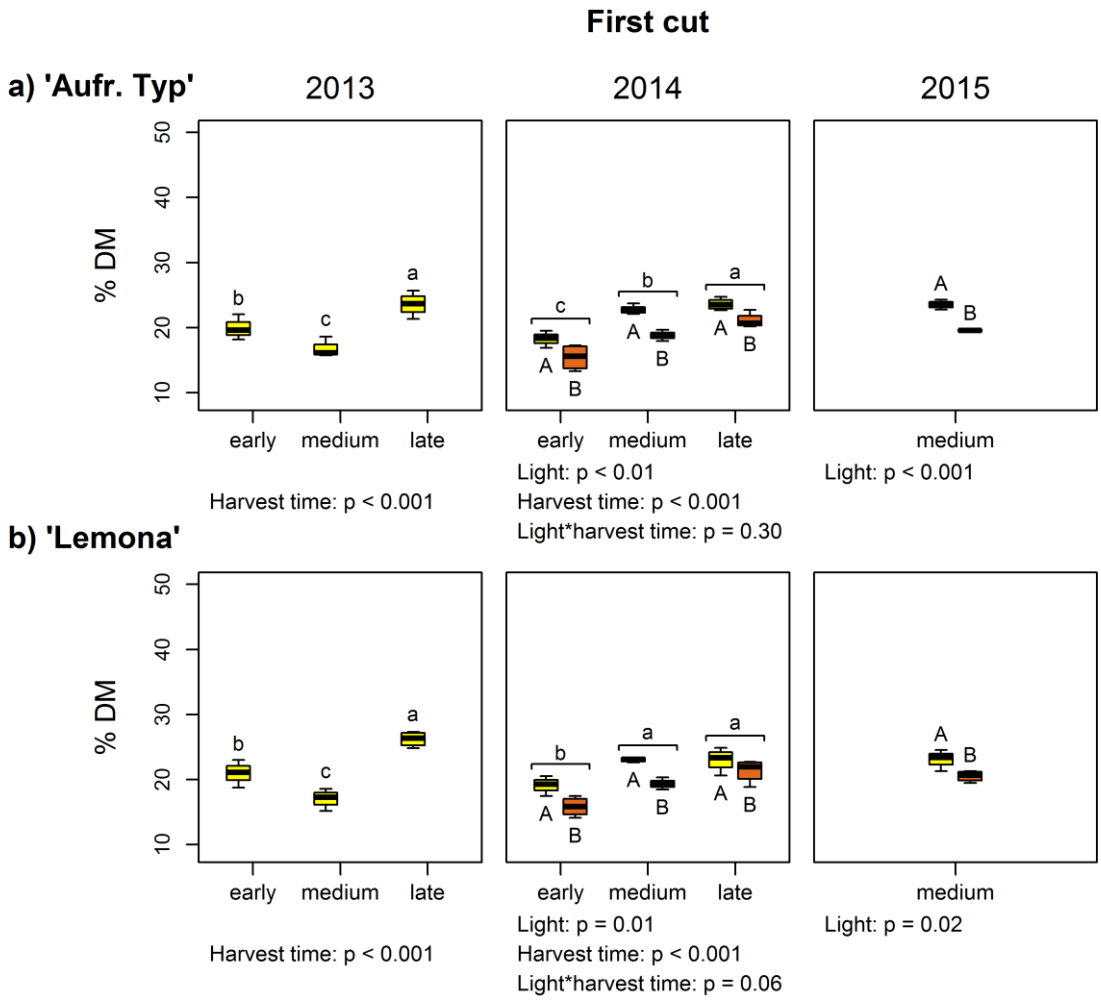

Fig. 67: DM content of the leaves [%] of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (■ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

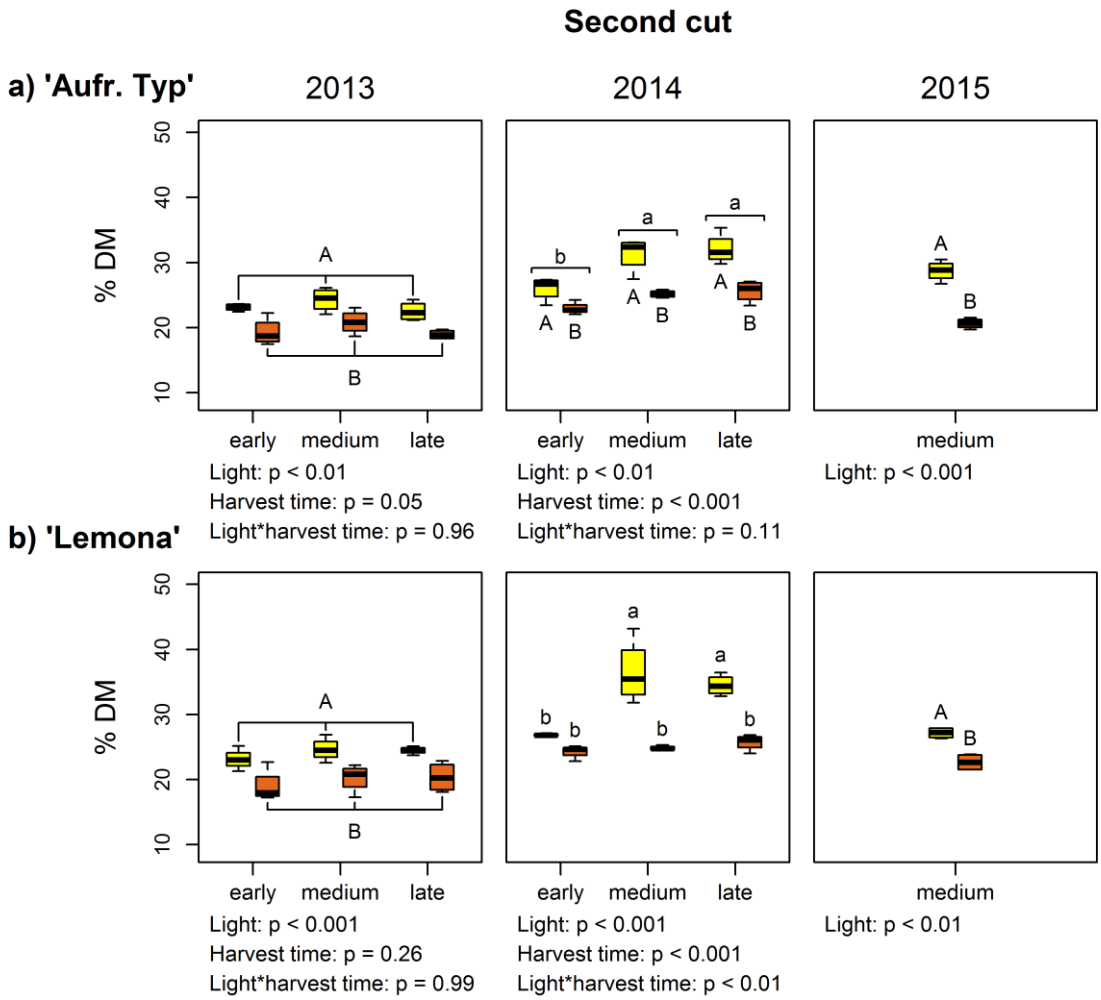

Fig. 68: DM content of the leaves [%] of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.2.6 Leaf:stem ratio

The leaf:stem ratio was covering a range from 0.5 to 2.6 in our experiment. Except for the first cut in 2014, values were normally around 1.0 or above. Values of the second cuts were generally higher than those of the first cuts (**Tab. A 24**).

The three-factorial analysis revealed no significant differences between the two genotypes in 2013 and 2014, whereas 'Aufrechter Typ' reached significantly higher values than 'Lemona' for the first cut in 2015 (1.2 vs. 1.0), whereas the opposite was true for the second cut in 2015 (2.0 vs. 2.4) (**Tab. A 24**).

The results of the two-factorial analysis did not show a harvest stage effect for the first cut in 2013, whereas all three harvest stages differed significantly from each other for the first cut in 2014, with a decreasing pattern ('Aufrechter Typ': 0.9, 0.7, and 0.5; 'Lemona': 1.0, 0.6, and 0.5) (**Fig. 69**). No significant influence of the light reduction was observed in this case, neither did 'Aufrechter Typ' show a significant reaction on the light reduction in 2015 (**Fig. 69 a**). However, 'Lemona' exhibited a reduced leaf:stem ratio of the shaded plants for the first cut in 2015 (**Fig. 69 b**).

Regarding the second cut, 'Aufrechter Typ' showed a significant harvest stage effect in 2013, with the late harvest stage exhibiting a significantly lower leaf:stem ratio than the early harvest, and the medium harvest stage not being significantly different from the other two stages. In 2014, a significant drop from early to medium harvest stages was observed, and the late harvest stage did not differ significantly from the other two stages (**Fig. 70 a**). The genotype 'Lemona' showed a similar behavior to 'Aufrechter Typ' in 2013 only for the non-shaded plants, with the late harvest stage having a significantly lower leaf:stem ratio than the early harvest stage, whereas the shaded plants did not differ significantly from each other (**Fig. 70 b**). In 2014, this genotype exhibited the significantly lowest leaf:stem ratio at medium harvest stage, whereas early and late harvest stage were not significantly different from each other (**Fig. 70 b**). For the second cut in 2015, where the leaf:stem ratio was on a quite high level, significant differences between the two light treatments were found in both genotypes, with values of 2.4 vs. 1.6 for 'Aufrechter Typ', as well as 2.6 vs. 2.1 for 'Lemona' for the non-shaded vs. the shaded plants, respectively.

There was a significant negative correlation between leaf:stem ratio and plant height ($r = -0.68$, $p < 0.001$; **Fig. A 15**).

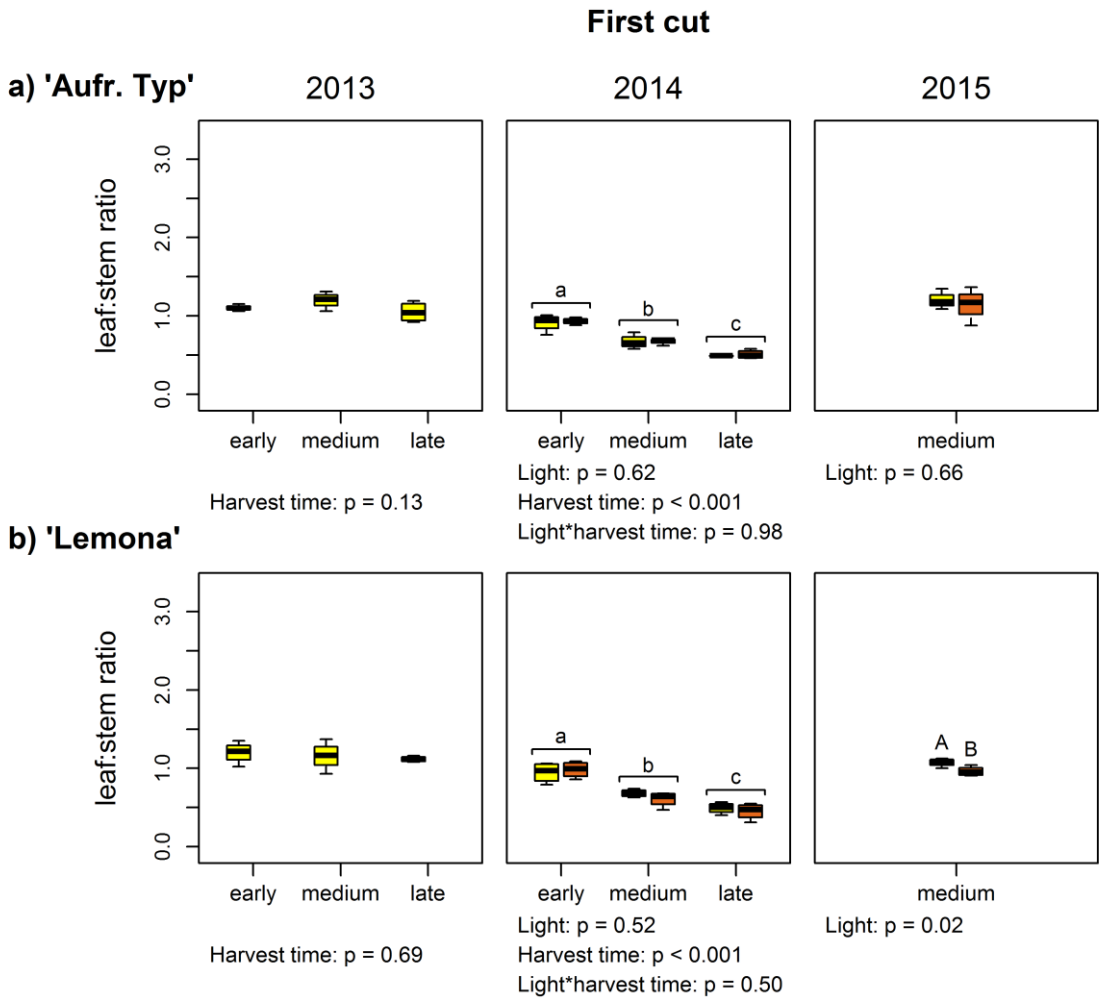

Fig. 69: Leaf:stem ratio of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

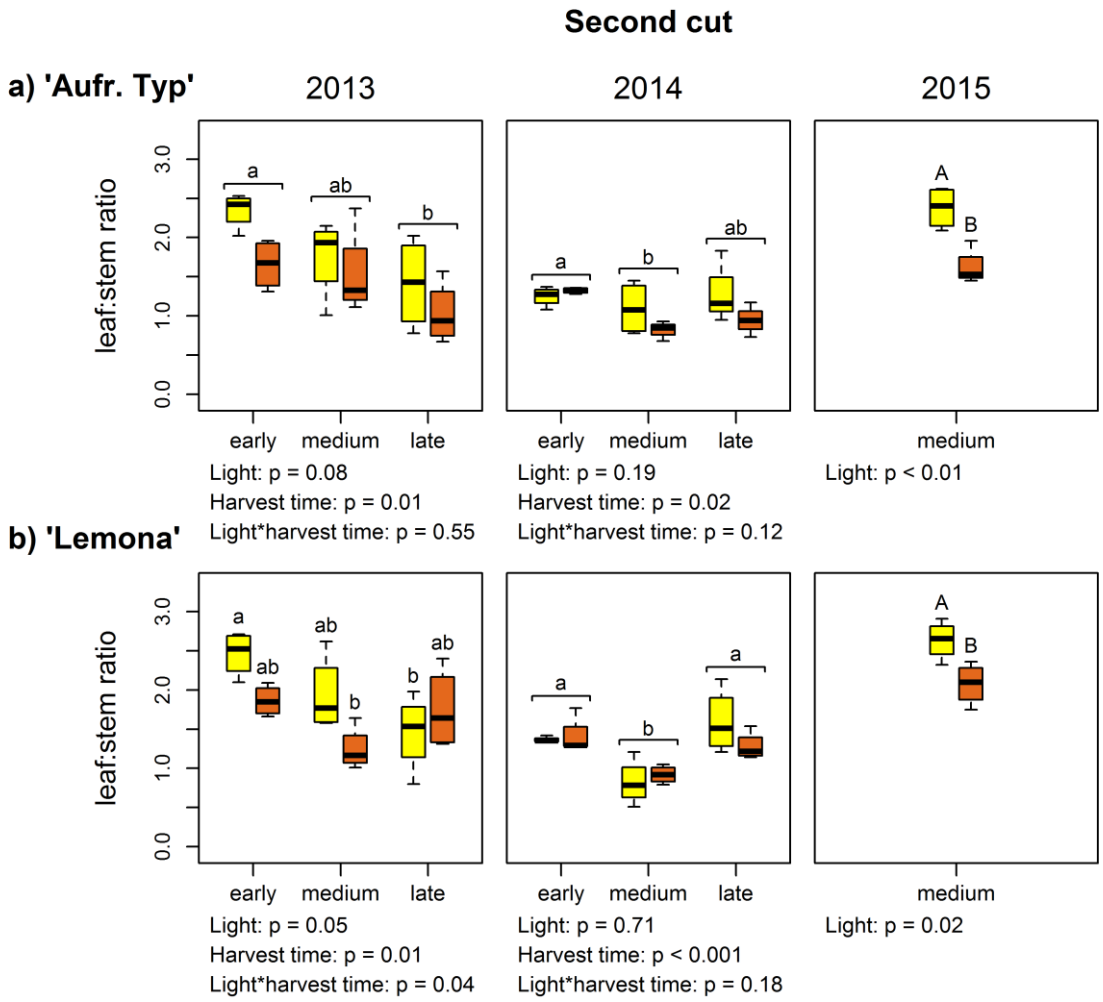

Fig. 70: Leaf:stem ratio of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.3 Essential oil content

The EO content of the leaves was covering a range from 0.05% to 0.76% under the different experimental conditions in the investigated years and cuts (**Tab. A 25**).

For the reference variants (natural light, medium harvest stage), the EO content of the harvested leaves of the first cut was determined as 0.17, 0.10 as well as 0.09% for 'Aufrechter Typ', and 0.29, 0.19 as well as 0.19% for 'Lemona' (in the years 2013, 2014, and 2014). For the second cut, the EO content of the reference variants reached 0.34, 0.30 as well as 0.67% for 'Aufrechter Typ', and 0.45, 0.30 as well as 0.76% for 'Lemona' in the years 2013, 2014, and 2015, respectively. Generally, values of the second cuts were higher than those of the first cuts. 'Lemona' exhibited a significantly higher EO content than 'Aufrechter Typ' in all three years, and in each of the two cuts per year, albeit in combination with interaction effects light*genotype as well as genotype*harvest stage for the first cut in 2014, as well as the interaction light*genotype for the first cut in 2015 (**Tab. A 25**).

The results of the following two-factorial analysis are presented in **Fig. 71** and **Fig. 72**. For the first cut in 2013, a significant effect of the harvest stage was observed only for 'Aufrechter Typ', where the late harvest stage (0.21%) showed a significantly higher EO content than the early harvest stage (0.13%), and the medium harvest stage (0.17%) not being significantly different from the other two stages (**Fig. 71 a**). For 'Lemona', a similar tendency of higher EO content at late harvest stage (0.35%) than at early harvest stage (0.26%) was not reaching significance, due to a higher variability for the medium harvest stage (0.29%) (**Fig. 71 b**). For the first cut in 2014, an interaction effect light*harvest stage was observed for both genotypes. In the non-shaded plants, the early harvest stage showed a significantly lower EO content than medium and late harvest stage in 'Aufrechter Typ' (0.05%, 0.10%, and 0.12%; **Fig. 71 a**), as well as in 'Lemona' (0.10%, 0.19%, and 0.23%; **Fig. 71 b**). For the shaded plants, EO content increased significantly from early to medium, and again from medium to late harvest stage in both genotypes ('Aufrechter Typ': 0.05%, 0.11%, and 0.23%; **Fig. 71 a**; 'Lemona': 0.10%, 0.26%, and 0.35%; **Fig. 71 b**). For the first cut in 2015, differences between shaded and non-shaded plants were not reaching significance, neither in 'Aufrechter Typ' (0.09% vs. 0.12%; **Fig. 71 a**) nor in 'Lemona' (0.19% vs. 0.14%; **Fig. 71 b**).

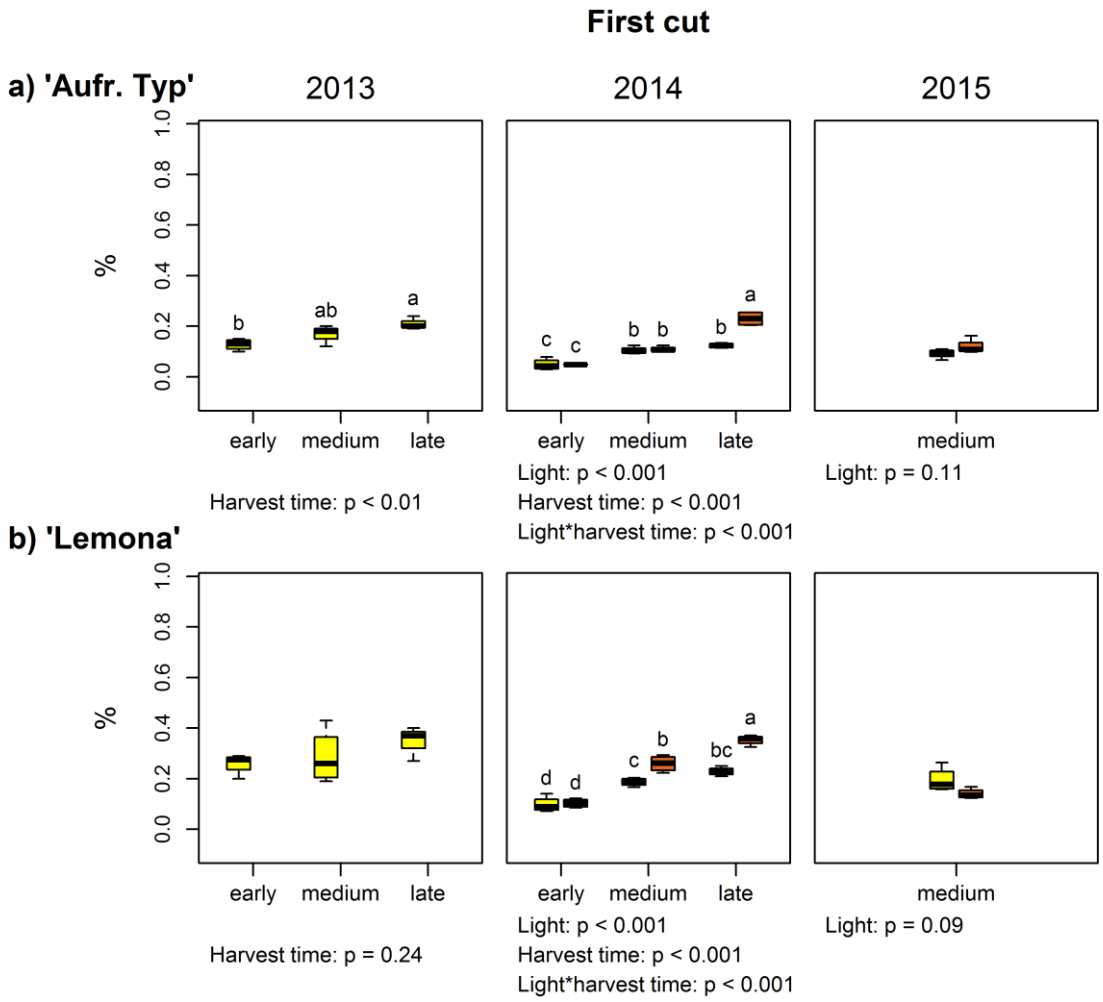

Fig. 71: Essential oil content [%] of lemon balm leaves. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$).

For the second cut in 2013, an effect of the harvest stage was observed for 'Aufrechter Typ', with the early harvest stage exhibiting significantly higher values than the late harvest stage (**Fig. 72 a**). A similar pattern did not reach significance for 'Lemona', but in this case a significant effect of the light reduction was observed (**Fig. 72 b**). For the second cut in 2014, both genotypes reached significantly higher EO contents at early harvest stage, compared to medium and late harvest stage ('Aufrechter Typ': 0.42%, 0.25%, and 0.24%; **Fig. 72 a**; 'Lemona': 0.53%, 0.30%, and 0.28%; **Fig. 72 b**). Additionally, 'Aufrechter Typ' showed significantly reduced values under reduced light intensity (**Fig. 72 a**), whereas such a tendency was not reaching significance for 'Lemona' for the second cut in 2014 (**Fig. 72 b**), as well as for both genotypes in 2015 (**Fig. 72 a + b**).

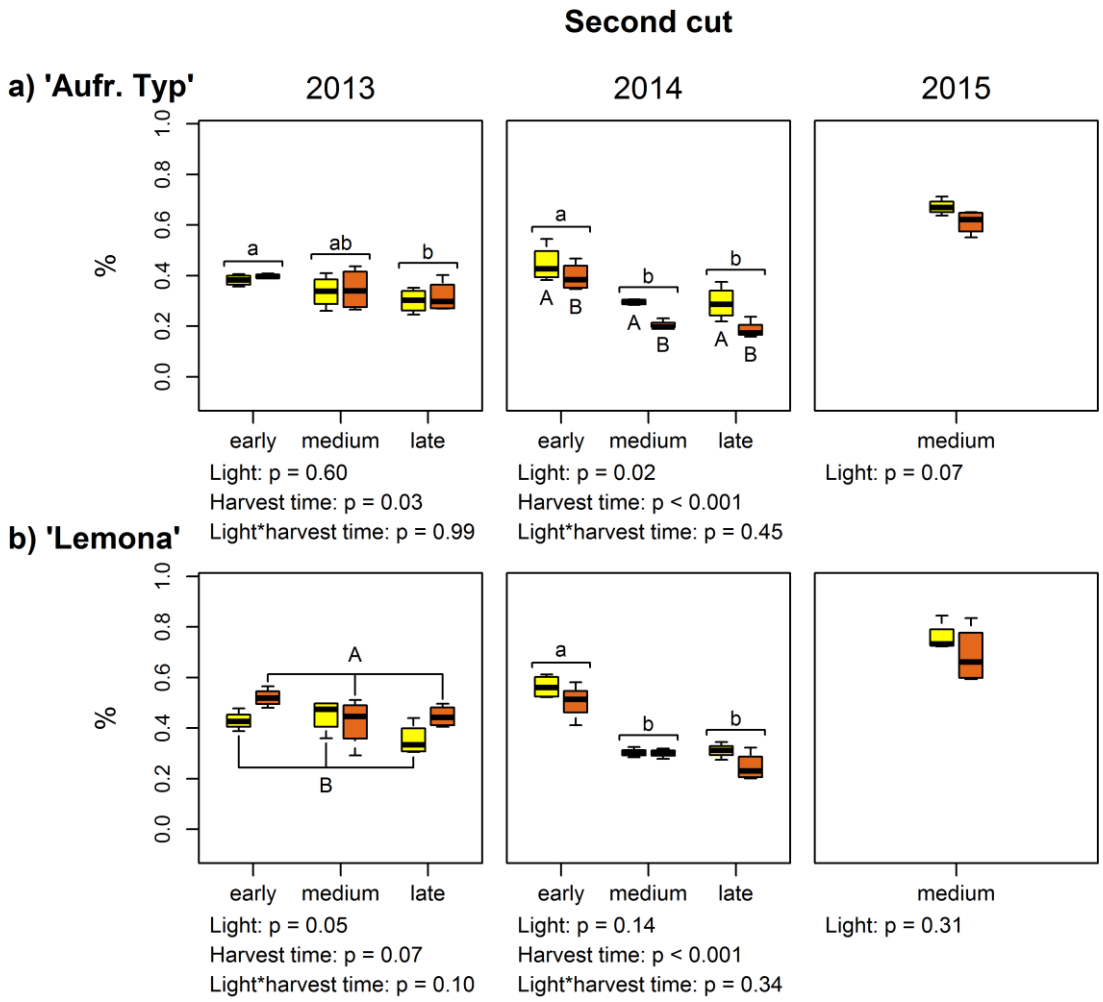

Fig. 72: Essential oil content [%] of lemon balm leaves. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.4 Total phenolic content

The values of the total phenolic content (TPC) were covering a range from 51.2 to 187.4 mg GAE/g DM under the experimental conditions in the investigated years (**Tab. A 26**). For the reference variants (natural light, medium harvest stage), TPC values of the first cut of the harvested leaves were determined as 143.8, 115.9 as well as 168.1 mg GAE/g DM for 'Aufrechter Typ', and 148.0, 119.1 as well as 187.4 mg GAE/g DM for 'Lemona' (in the years 2013, 2014, and 2014). TPC values of the second cut of the reference variants were in most cases lower, yielding 95.6, 127.6 as well as 170.3 mg GAE/g DM for 'Aufrechter Typ', and 98.0, 100.3 as well as 157.4 mg GAE/g DM for 'Lemona' in the years 2013, 2014, and 2015, respectively. No significant genotype effect was observed in the three-factorial analysis (**Tab. A 26**).

The following two-factorial analysis did not show significant differences between harvest stage for the first cut in 2013 in any of the genotypes (**Fig. 73**). For the first cut in 2014, however, both genotypes exhibited a significant harvest stage effect, with the early harvest stage being significantly lower than medium or late harvest stage. Additionally, shaded plants showed significantly reduced TPC values compared to non-shaded plants. For 'Aufrechter Typ', TPC values of the non-shaded plants at early, medium, and late harvest stage were 91.1, 115.9, and 124.4 mg GAE/g DM, and for the shaded plants 51.2, 92.5, and 99.6 mg GAE/g DM (**Fig. 73 a**). 'Lemona' showed a similar pattern, with 93.9, 119.1, and 127.1 mg GAE/g DM for the non-shaded, as well as 57.0, 103.2, and 99.4 mg GAE/g DM for the shaded plants (**Fig. 73 b**). For the first cut in 2015, the pattern of decreased TPC values of the shaded plants was reaching significance only for 'Lemona', but not for 'Aufrechter Typ' (**Fig. 73**).

For the second cut in 2013, significantly reduced TPC values under the light reduction were observed in both genotypes (**Fig. 74**). In 2014, this was also observed in 'Aufrechter Typ', and additionally a harvest stage effect was significant, with the late harvest stage exhibiting lower TPC values than early or medium harvest stage (**Fig. 74 a**). For 'Lemona', an interaction genotype*harvest stage occurred, with the early harvest stage reaching significantly higher values than medium and late harvest stage for the non-shaded plants, whereas the late harvest stage was significantly lower than early and medium harvest stage in the shaded plants (**Fig. 74 b**). For the second cut in 2015, significantly higher TPC values for the non-shaded plants, compared to the shaded plants, were observed in both 'Aufrechter Typ' (170.3 vs. 119.0 mg GAE/g DM; **Fig. 74 a**) and 'Lemona' (157.4 vs. 128.4 mg GAE/g DM; **Fig. 74 b**).

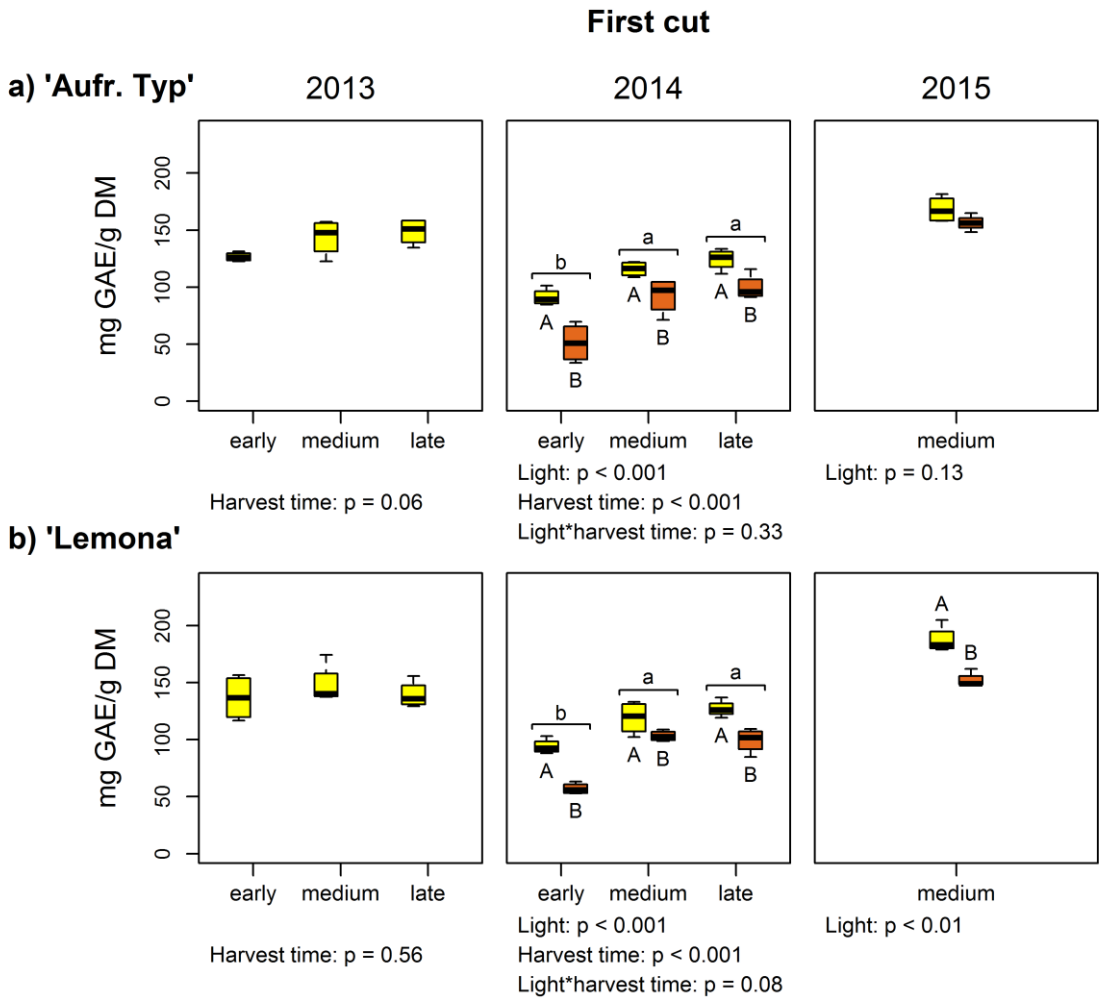

Fig. 73: Total phenolic content [mg GAE/g DM] of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

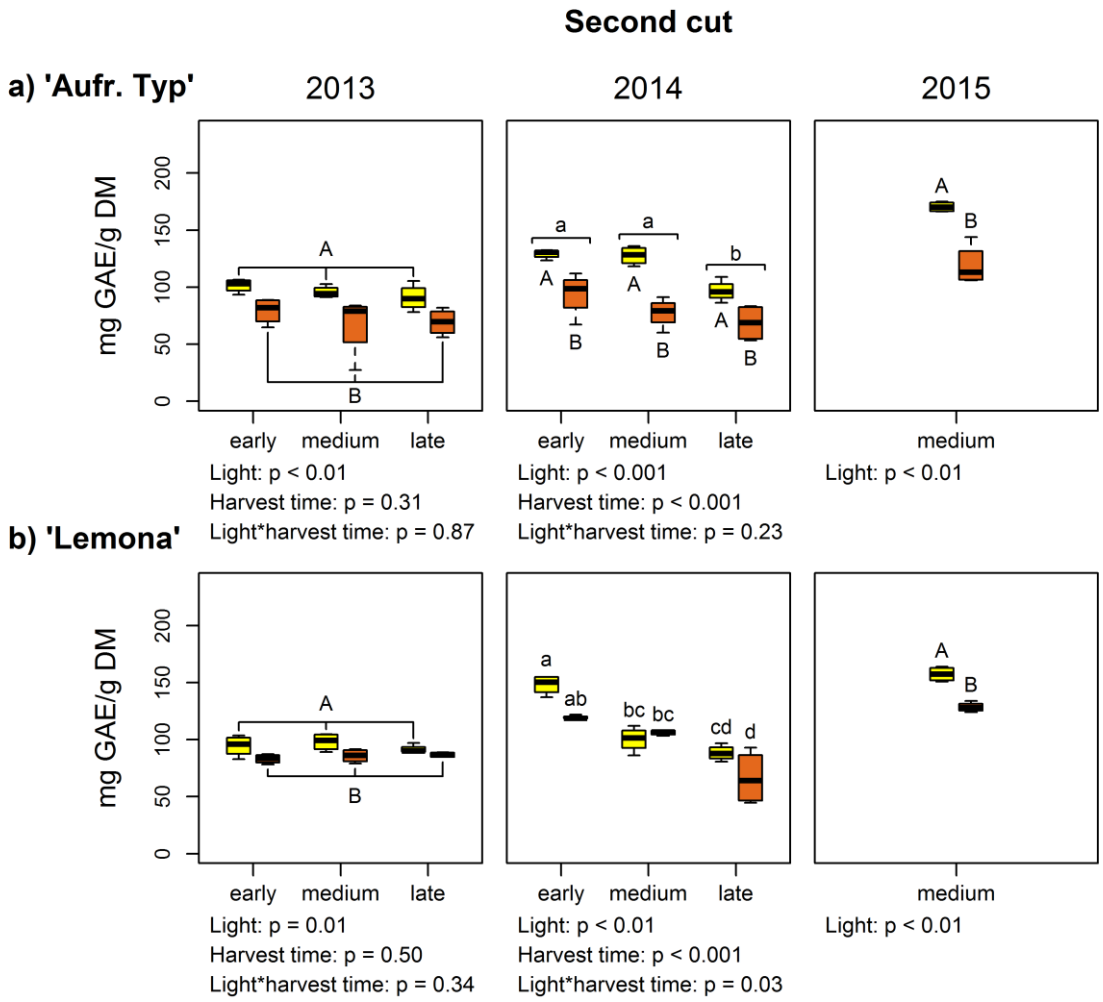

Fig. 74: Total phenolic content [mg GAE/g DM] of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.5 Antioxidant capacity

Values for the antioxidant capacity were ranging from 963 to 2894 µmol TE/g DM under the investigated conditions in the years 2013, 2014, and 2015 (**Tab. A 27**). For the reference variants (natural light, medium harvest stage), ORAC values of the harvested leaves of the first cut were determined as 2091, 1915 as well as 2781 µmol TE/g DM for 'Aufrechter Typ', and 2238, 2005 as well as 2893 µmol TE/g DM for 'Lemona' (in the years 2013, 2014, and 2014). Measurements of the ORAC values of the second cut of the reference variants gave 1760, 2075 as well as 2787 µmol TE/g DM for 'Aufrechter Typ', and 1863, 1658 as well as 2665 µmol TE/g DM for 'Lemona' in the years 2013, 2014, and 2015, respectively.

A significant difference between the two genotypes was observed only in two cases, with 'Aufrechter Typ' showing significantly lower values than 'Lemona' for the second cut in 2013 (1548 vs. 1677 µmol TE/g DM), as well as for the first cut in 2014 (1617 vs. 1741 µmol TE/g DM) (**Tab. A 27**).

The following two-factorial analysis revealed a significant harvest stage effect in 'Aufrechter Typ' for the first cuts in 2013 and 2014, with the late harvest stage exhibiting significantly higher values than early harvest stage, and the medium harvest stage not being significantly different from early and late (2013), or from late harvest stage (2014) (**Fig. 75 a**). For the first cut in 2014, shaded plants exhibited significantly lower values (1052, 1573, and 1631 µmol TE/g DM) than non-shaded plants (1476, 1915, and 2052 µmol TE/g DM) at early, medium, and late harvest stage (**Fig. 75 a**). The same pattern occurred for 'Lemona' for the first cut in 2014, where an interaction effect light*harvest stage was significant, with values of 1265, 1703, and 1613 µmol TE/g DM for the shaded plants, compared to 1654, 2005, and 2204 µmol TE/g DM in the non-shaded plants, at early, medium, and late harvest stage (**Fig. 75 a**). For the first cut in 2015, both genotypes showed a significantly reduced antioxidant capacity under reduced light intensity, compared to the non-shaded plants ('Aufrechter Typ': 2479 vs. 2781 µmol TE/g DM, **Fig. 75 a**; 'Lemona': 2496 vs. 2894 µmol TE/g DM, **Fig. 75 b**).

For the second cut, a significant reduction of the antioxidant capacity under reduced light intensity was observed in both genotypes in all investigated years (**Fig. 76**). For the year 2013, only the main effect of the light reduction was significant, whereas in 2014, an effect of the harvest stage was observed as well. In both genotypes, the late harvest stage exhibited significantly lower values than early and medium harvest stage, which were not significantly different from each other. This was observed in 'Aufrechter Typ', with 1299, 1223, and 963 µmol TE/g DM for the shaded as well as 2057, 2076, and 1512 µmol TE/g DM for the non-shaded plants (**Fig. 76 a**), and also in 'Lemona, with

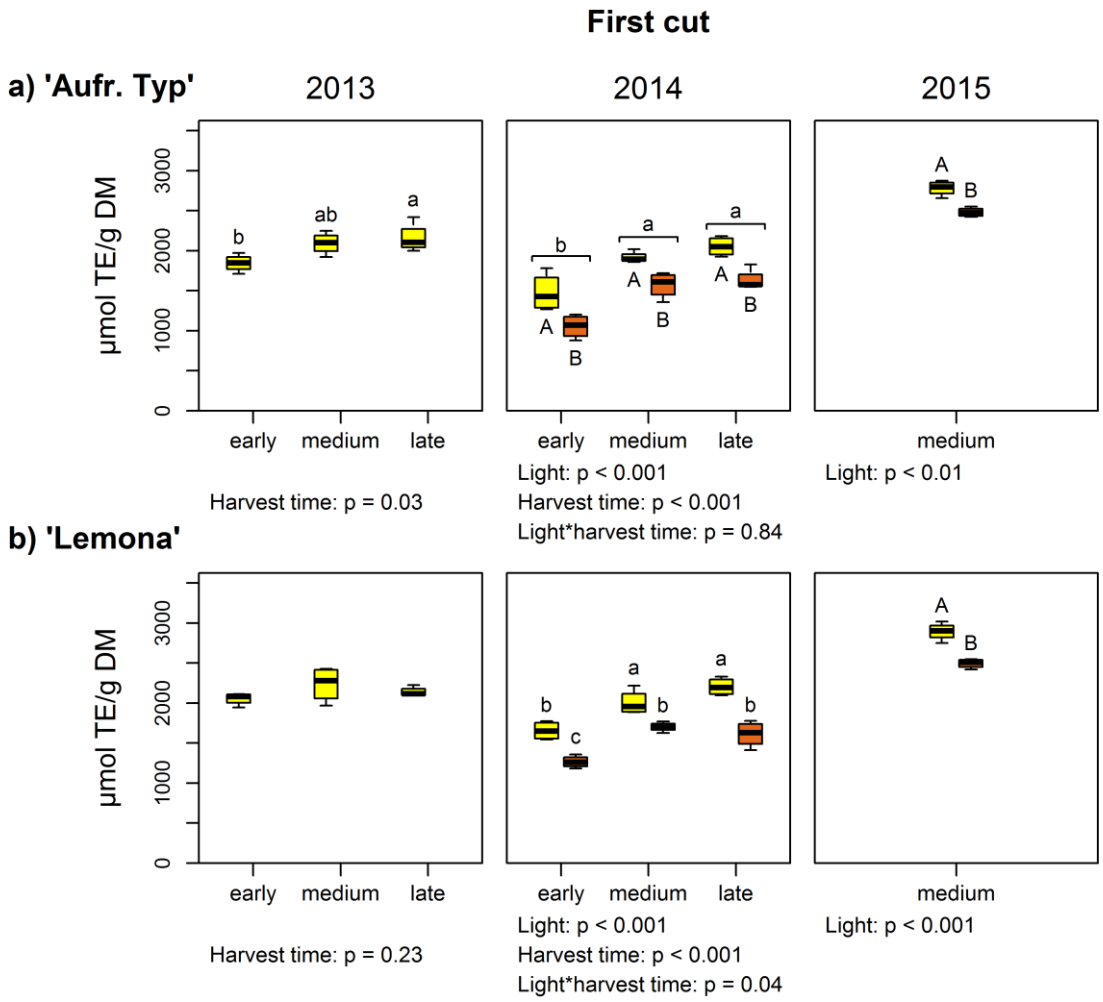

Fig. 75: Antioxidant capacity [µmol TE/g DM] of lemon balm. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

1678, 1617, and 1007 µmol TE/g DM for the shaded as well as 2179, 1658, and 1404 µmol TE/g DM for the non-shaded plants (**Fig. 76 b**) at early, medium, and late harvest stage, respectively. For the second cut in 2015, the light reduction reduced the antioxidant capacity significantly in 'Aufrechter Typ' from 2787 to 2012 µmol TE/g DM (**Fig. 76 a**), and in 'Lemona' from 2665 to 2161 µmol TE/g DM (**Fig. 76 b**).

Antioxidant capacity and TPC values were highly significantly correlated, calculated over all years and growth cycles ($r = 0.93$, $p < 0.001$; **Fig. A 16**).

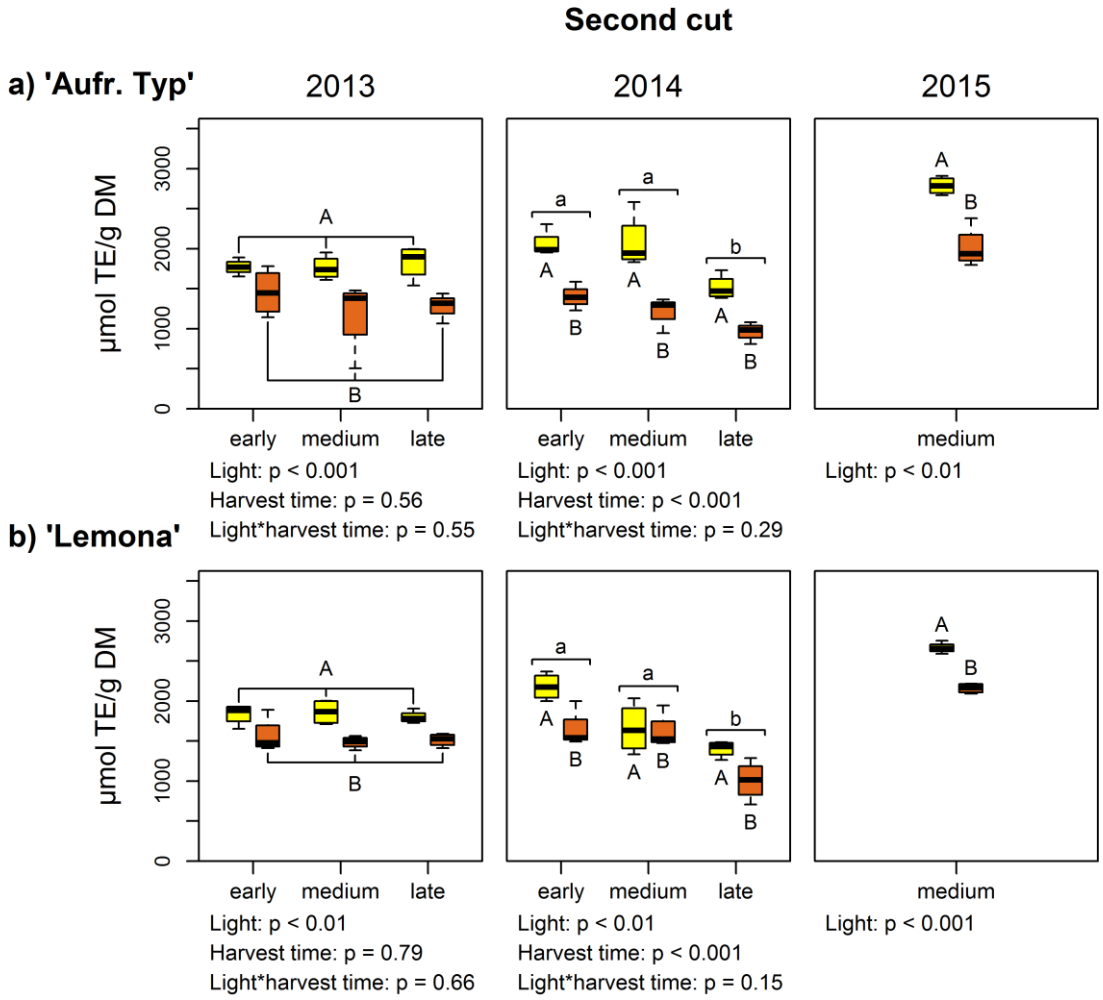

Fig. 76: Antioxidant capacity [µmol TE/g DM] of lemon balm. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

4.2.6 Rosmarinic acid content

Rosmarinic acid contents of the plants cultivated in Rauischholzhausen were ranging from 1.3 to 6.0%, depending on the experimental conditions, years, and cuts within a year (**Tab. A 28**). For the reference variants (natural light, medium harvest stage), RA content of the first cut of the harvested leaves was determined as 4.5%, 4.3% as well as 4.9% for 'Aufrechter Typ', and 4.7%, 4.1% as well as 6.0% for 'Lemona' (in the years 2013, 2014, and 2014). RA content of the second cut of the reference variants was determined as 4.0%, 4.9% as well as 5.5% for 'Aufrechter Typ', and 3.5%, 3.5% as well as 5.1% for 'Lemona' in the years 2013, 2014, and 2015, respectively. No significant differences regarding RA content were found between the two genotypes 'Aufrechter Typ' and 'Lemona' by the three-factorial analysis (**Tab. A 28**).

The results of the following two-factorial analysis are presented in **Fig. 77** (first cut) and **Fig. 78** (second cut). For the first cut in 2013, 'Aufrechter Typ' showed a significantly lower RA content at early harvest stage, compared to medium and late harvest stage (**Fig. 77 a**), whereas a similar pattern was not reaching significance for 'Lemona' (**Fig. 77 b**). In 2014, both genotypes exhibited a significant harvest stage effect, as well as a significant effect of the reduced light intensity, albeit for 'Lemona' in combination with an interaction effect light*harvest stage. The early harvest stage reached significantly lower RA contents than medium harvest stage in both genotypes and both light treatments. For 'Aufrechter Typ' as well as for the non-shaded 'Lemona' plants, RA contents of medium and late harvest stage were not significantly different from each other, whereas the shaded 'Lemona' plants showed a significant decrease from medium to late harvest stage (**Fig. 77 b**). RA contents were reaching 2.9%, 4.3%, and 4.2% for the non-shaded and 1.4%, 2.5%, and 2.6% for the shaded plants of the genotype 'Aufrechter Typ' (**Fig. 77 a**), as well as for 'Lemona' 3.0%, 4.1%, and 4.2% for the non-shaded and 1.3%, 3.2%, and 2.2% for the shaded plants (**Fig. 77 b**), for early, medium, and late harvest stage, respectively. For the first cut in 2015, RA contents of the shaded plants were significantly lower compared to the non-shaded plants in both 'Aufrechter Typ' (4.3% vs. 5.6%, **Fig. 77 a**) and 'Lemona (4.4% vs. 6.0%; **Fig. 77 b**).

For the second cut, 'Aufrechter Typ' showed a significant reduction of the RA content by the reduced light intensity in 2013 and 2014, but no significant influence of the harvest stage (**Fig. 78 a**). 'Lemona', however, exhibited both a light effect and a harvest stage effect in for the second cut in 2013, with the shaded plants having significantly lower RA contents than the non-shaded plants, and the late harvest stage having a significantly higher RA content than early and medium harvest stage (**Fig. 78 b**). In 2014, the situation was more complex due to an interaction effect light*harvest stage for 'Lemona':

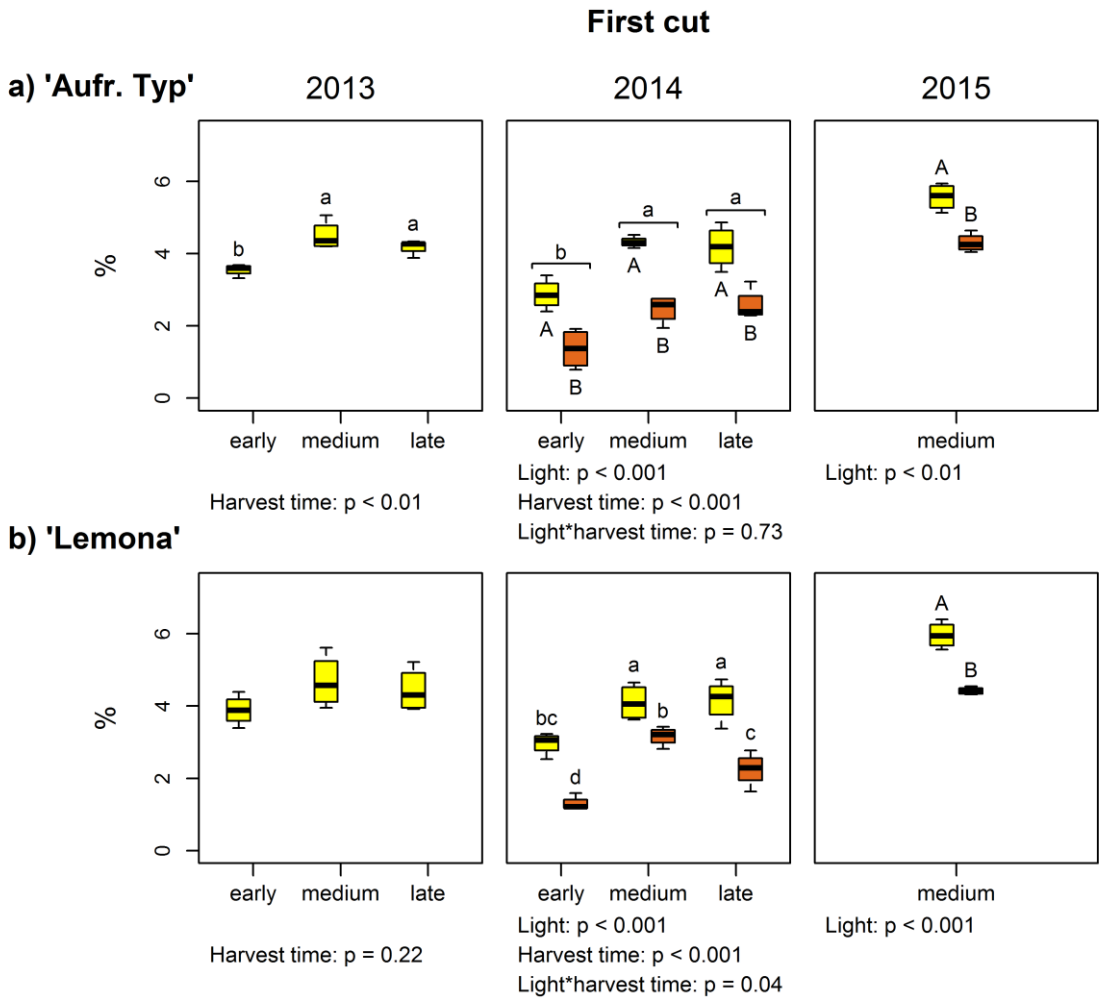

Fig. 77: Rosmarinic acid content [%] of dried lemon balm leaves. First cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (□ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

Non-shaded plants had the highest RA content at early harvest stage, while medium and late harvest stage did not differ significantly from each other. For the shaded plants, late harvest stage had the lowest RA content, while early and medium harvest stage were not significantly different from each other. Additionally, a significant difference between the two light treatments occurred only at early harvest stage, with the shaded plants having significantly lower RA values than the non-shaded plants (**Fig. 78 b**). For the second cut in 2015, RA values of the shaded plants were again significantly lower than those of the non-shaded plants in both 'Aufrechter Typ' (2.9% vs. 5.5%, **Fig. 78 a**) and 'Lemona (3.4% vs. 5.1%; **Fig. 78 b**).

RA content was highly positively correlated with TPC values ($r = 0.87$, $p < 0.001$; **Fig. A 17**) and antioxidant capacity ($r = 0.85$, $p < 0.001$; **Fig. A 18**).

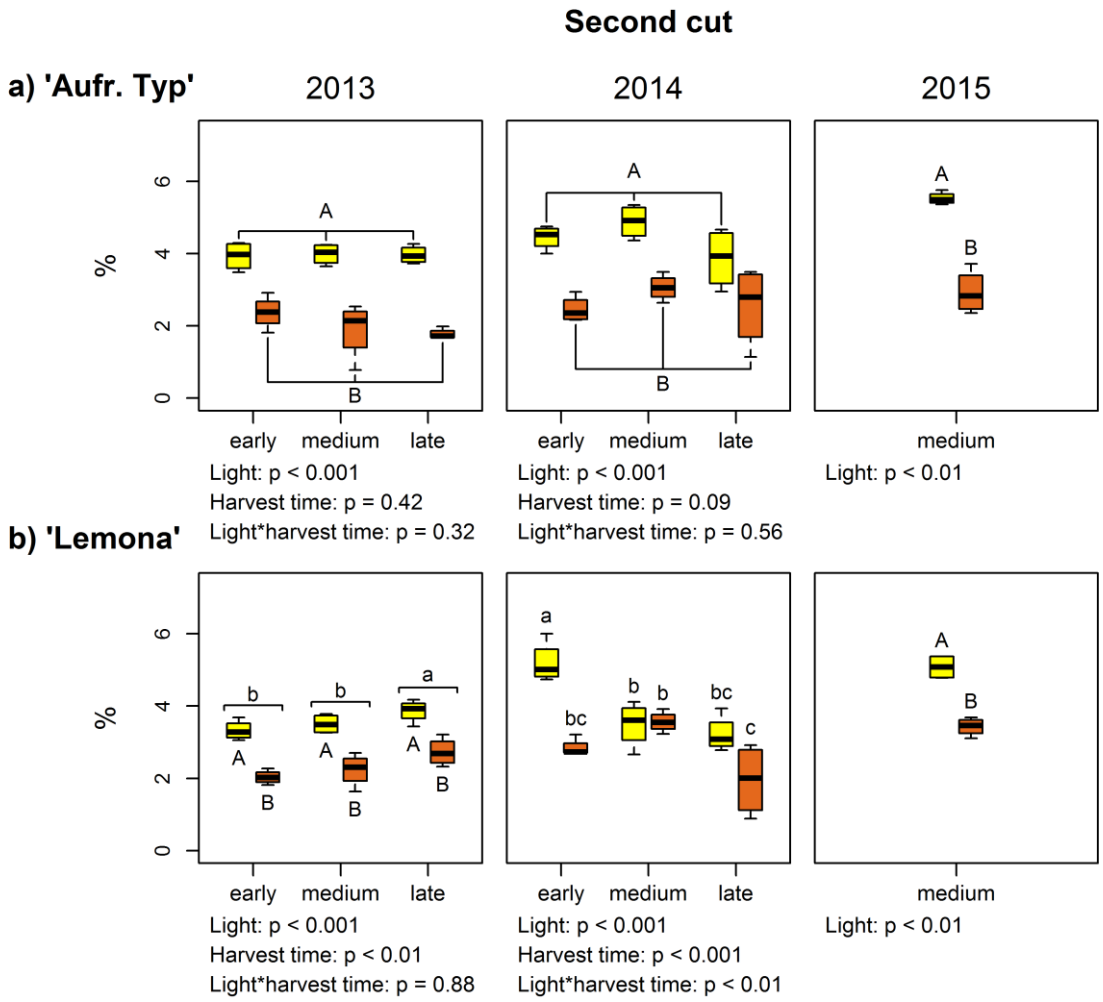

Fig. 78: Rosmarinic acid content [%] of dried lemon balm leaves. Second cut in Rauischholzhausen, 2013–2015. Two lemon balm genotypes (a + b) cultivated under different light conditions (■ = natural light; ■ = strong shading) and harvested at different harvest stages (early, medium, late). Boxes with different letters are significantly different ($p < 0.05$). Uppercase letters for light effect, lowercase letters for harvest time effect or interaction.

5 Discussion

5.1 Plant parameters

5.1.1 Plant height

Plant height can be influenced by several parameters. The genetic background determines the potential plant height range. However, environmental factors like soil quality, climatic conditions, nutrient and water supply as well as light quantity and quality can influence the actual plant height.

For lemon balm plant stands, Bomme et al. (2013) state a range of about 50 to 90 cm. The results of our experiment in RH are perfectly in this range for the first cut, while the plants of the second cut were on average lower. Also for the field experiment in GG, plant heights of the second cuts were lower than those of the first cuts. Lower plant heights can be explained by shorter internodes of the second cut (Bomme et al., 2013). Changes in internode length can be induced by factors like light intensity, photoperiod, water availability, as well as temperature. The mechanisms by which these changes can appear involve the mediation of phytohormones, like gibberellins (Bresinsky et al., 2013). The shorter internodes of the second cut might probably be caused by an increased plant development and thus an earlier shift from vegetative to generative development stage. This shift could be accelerated by the combination of a changed photoperiod, lower water availability, as well as higher temperatures in the period after the first cut.

For the experiment in GG, the plants were on average lower than in RH, which might be related to the sandy soil conditions with a low water and nutrient holding capacity. This is in accordance with findings in Iran for lemon balm plants cultivated on a sandy soil, where plant height was in a range of about 30 to 60 cm, depending on nitrogen fertilization (Abbaszadeh et al., 2009b).

The effect of the genotype on plant height was not so clear in our experiment. Especially in RH, where only two genotypes ('Aufrechter Typ' and 'Lemona') were cultivated, a clear genotype effect could not be observed. On the other hand, in GG, the genotype 'NLC' grew in many cases higher than the other two genotypes. The pattern that only some lemon balm genotypes grow higher, while several others do not differ clearly in plant height, is in accordance with a Hungarian investigation with lemon balm cultivated in pots. In this case, only one out of five genotype showed a significantly higher plant height than the other tested genotypes, and 'Lemona' was among those genotypes that did not differ significantly in their plant height (Szabó et al., 2016).

Light intensity is another factor that can have an influence on plant height. Generally, the shading of heliophyte plants can lead to plant elongation, a typical sign of shade

avoidance (Kadereit et al., 2014). This increase in plant height as a result of the shade avoidance syndrome is typically caused by the elongation of the internodes, an effect which is induced by a lower red:far-red ratio (R:FR) (Smith and Whitelam, 1997). In investigations in *Helianthus annuus* shoots, it has been shown that both lower PAR as well as a reduced R:FR led to an increase in internode lengths (Kurepin et al., 2007). In the process of plant elongation, several plant hormones play a role, like auxins, gibberellins, ethylene, or brassinolides (Kadereit et al., 2014). In *Lactuca sativa*, the gene expression of enzymes involved in gibberellin biosynthesis has been linked to the regulation by the photoreceptor phytochrome (Toyomasu et al., 1998). Additionally, it has been shown that brassinosteroids are involved in the light-dependent plant development in the model plant *Arabidopsis* (Li et al., 1996). Thus, it can be expected that light reduction may influence plant height of lemon balm plants in a phytohormone controlled manner.

In accordance with the explanation of the shade avoidance syndrome leading to plant elongation, plant height of *Salvia officinalis*, like lemon balm a member of the Lamiaceae family, was significantly increased with decreasing light intensity from 100% to 70% to 30% light intensity (Mapes and Xu, 2014). In experiments with another Lamiaceae plant, *Ocimum basilicum*, it was found that a light reduction of about 50% by differently colored nets led to increased plant heights (Paulus et al., 2016). The same effect was found for *Ocimum selloi* (Costa et al., 2010b). On the other hand, Chang et al. (2008) investigated the effect of different shading intensities (0%, 25%, 50%, and 75%) on several parameters in experiments with *Ocimum basilicum*, and found that strong shading (75%) significantly reduced plant height, whereas a moderate light reduction did not show an effect. In this case, the light reduction might already have been too strong, thus inhibiting the photosynthetic processes in the plant. In our experiments, plants were not shaded as strongly, thus no reduction in plant height was observed. On the other hand, plant height was also not increased by the moderate shading in GG for the first cut. In RH, the effect of the strong shading on plant height was also not visible in the first cuts for the two years investigated, except for the late cut in the genotype 'Lemona'. Therefore it could be suggested that lemon balm can cope with shading for a certain period of time without increasing plant height.

For the second cut, however, results were slightly different. Under the moderate light reduction in GG, one genotype ('Aufrechter Typ') did not show any effect on plant height, indicating a potentially genotypically determined different susceptibility to light reduction. On the other hand, the other two genotypes showed a significantly increased plant height under the shade treatment only in one year. It seems as if not only the genetic background, but also environmental factors might influence the susceptibility to light

reduction. A strong shading, as applied in RH, showed a clear increase in plant height for the second cuts, which is in accordance with findings of Oliveira et al. (2016) with lemon balm plants cultivated under differently colored nets with a light reduction of about 50%. This reaction is a clear sign of the shade avoidance syndrome. Interestingly, the second cut seems to be more susceptible to a strong light reduction. Presumably the timing of the applied shading during plant development plays a role, as the shading nets were installed every year only some time after plants had started growing in spring, whereas the second flush of growth after the first cut started already on the shaded plots. This is in accordance with investigations in maize that showed that plants were especially susceptible to a change in R:FR at an early development stage (Page et al., 2009; Rajcan et al., 2004).

Generally, the reduction of light intensity as well as the color of the shading net may have an influence on plant growth. This is underlined by findings of Mortensen and Strømme (1987), who found that plant height of chrysanthemum and tomato growing under green filters was significantly increased. The use of green nets, as applied in our experiments with lemon balm, changes the light quality by absorption of red light, therefore showing a reduced R:FR (Oren-Shamir et al., 2001). This changed R:FR might therefore be an explanation for the changes in plant height of lemon balm in some of the investigated cases in this study.

An increased plant height can be regarded as ambivalent for the production of medicinal plants. On the one hand, a greater distance of the leaves from the soil surface makes them easier to harvest and leads to less contamination from the soil. On the other hand, taller plants tend to be less stable, so that lodging might appear more easily, which in turn leads to contamination and harvest problems. Additionally, if only plant height is increased, but not the amount of leaves, a lower leaf:stem ratio means a lower percentage of marketable end product from the harvested biomass. Thus, higher effort and expenses for drying and separation of the leaves from the stems can be expected.

The significance of a changed plant height has therefore to be regarded in a combination with other factors, like the leaf:stem ratio. It can be regarded as positive that lemon balm plants were not etiolated, even under the strong shading as applied in RH. As no significant increases in plant height occurred for the first cut with generally taller plants, the negative sides of plant elongation were not relevant. For the second cuts with generally lower plant heights, however, the increase in plant height that occurred in some cases can be regarded as positive regarding a facilitated harvestability. However, the effect of a light reduction on plant height of lemon balm seems to be dependent on the intensity of light reduction and change of light quality, as well as on the timing at which it is applied, or possibly further environmental factors still to be elucidated.

5.1.2 Leaf area index (LAI)

LAI is a dimensionless measurement (actually it would be m^2 m^{-2}) of the total leaf area on a given area of soil (Bresinsky et al., 2013). The measurement of LAI is used as a non-destructive method to estimate the biomass production, as a relationship between LAI and crop growth rate exists (Larcher, 2003). It has been observed in several crops like winter wheat (Gebbers et al., 2011), rice (Sone et al., 2009), and corn (Wilhelm et al., 2000) that a close correlation exists between LAI on the one hand and the canopy structure and aboveground biomass on the other hand. Generally, LAI tends to increase with plant height. However, in our experiments with lemon balm plants, only a weak correlation between both parameters was observed. This finding might be unexpected at first. However, it can be explained by the fact that lemon balm plants tend to lose the basal leaves during their growth, induced by light deficiency and senescence processes, at a certain point. The younger, newly developed leaves have a different shape and are generally smaller, which can explain why the leaf area, as quantified by LAI measurements, did not increase with plant growth. The effect of differently shaped, smaller leaves occurs especially at the point where the plants shift from the vegetative to the generative stage (Bomme et al., 2013). This shift is even accelerated in situations of water shortage, as senescence is accelerated under increasing dehydration (Larcher, 2003). A temporary water shortage can appear more easily under sandy soil conditions, as in our experiment in GG.

Generally, LAI can be expected to increase under conditions of light reduction, as plants will increase the photosynthetically active leaf area to compensate for the light reduction. In the Lamiaceae plant *Pogostemon cablin*, leaf area was significantly increased under 50% shading with black, blue, and red nets, with the red nets showing a significantly higher effect than the black nets (Ribeiro et al., 2018). The Asteraceae plant *Mikania glomerata* showed an increased leaf area when cultivated under differently colored nets (grey, blue, and red), with the highest effect under grey and blue nets (Souza et al., 2011). However, in our experiments, no effect of the moderate light reduction applied in GG on LAI could be observed in both years and both cuts where the measurements were taken. Presumably, the light reduction was not strong enough to induce a morphological change towards an increased leaf area.

On the other hand, even for the strong light reduction of about 50% in our experiment in RH, no effect of the light reduction on LAI could be observed for the first cuts in both investigated years. This is in accordance with findings of Costa et al. (2012) on another Lamiaceae plant, *Mentha piperita*, cultivated in Brazil under a light reduction of about 50%, where leaf area was not increased under blue nets. However, they stated it was

significantly higher under black and red nets (Costa et al., 2012). For the second cuts, however, significantly higher LAI values of the shaded plants compared to the non-shaded plants were observed, at least for the medium and/or late harvest stage. Probably the duration and/or the timing of the applied shading during plant development is important for changes of the leaf area. In our case, the shading nets were installed every year in spring when plants had already started growing, whereas the second growth after the first cut started already under the shading net. The observation that an increase in leaf area occurs only after a longer period under a shading treatment, or in the second cut, respectively, is in accordance with findings of Oliveira et al. (2016). They observed that lemon balm plants did not show an increased leaf area for the first cut after 50 days under black, blue, or red nets, whereas it was significantly increased for the second cut (70 days after the first harvest, or 120 days after the beginning of the shading treatment, respectively) (Oliveira et al., 2016).

However, the significant differences in LAI between shaded and non-shaded plants found in our experiment at a later stage were not caused by an increased LAI of the shaded plants whose LAI remained stable, but rather by a decreased LAI of the non-shaded plants over time. This is probably due to the fact that shading delays the ontogenetic development of plants, and lemon balm plants develop more smaller leaves when the shift from vegetative to generative growth occurs. Additionally, they tend to lose the lower leaves by senescence processes at later stages of plant development. However, in the end a combination of both processes (increased leaf area as a compensation under light reduction as well as a delayed ontogenetic development) will have occurred in parallel.

If shading of plants is too strong, however, it will not increase, but could rather lead to a decrease in leaf area. This has been found in experiments of Chang et al. (2008) on *Ocimum basilicum*, where total leaf area was significantly decreased with increasing shading intensity (50% and 75%), but no difference were observed between control and 25% shading. In this case, the strong shading was connected to a strongly reduced rate of photosynthesis, leading to an impaired energy supply of the plants (Chang et al., 2008). In our experiments, plants were not shaded unto the point where a light reduction impairs the development of leaves, as it would be indicated by a reduced leaf area. It can therefore be suggested that the light reduction applied in our experiments was still tolerable for lemon balm plants.

5.1.3 SPAD values / Chlorophyll content

SPAD values are a common estimator for the chlorophyll content of the plants and, because of the importance of nitrogen for chlorophyll biosynthesis, as an indirect indicator of the nitrogen status of the plants (Papasavvas et al., 2008; Wakiyama, 2016). Chlorophyll is essential for photosynthesis and therefore for the energy supply of the plants. Its content in the photosynthetic organs of the plants can be modified by ontogenetic processes as well as by environmental factors. Generally, it is known that the chlorophyll content of plants decreases with senescence (Hörtensteiner, 2006), and under drought stress (Munné-Bosch and Alegre, 2000b). In accordance with expectations, SPAD values of the first cuts in our experiment in GG decreased with the ontogenetic development of the plants from early to medium and late harvest stage. However, for the second cut, the opposite was true in 2014, at least for two out of three genotypes. The finding that SPAD values were lower in summer, while they were higher earlier or later in the year, is in accordance with experiments with lemon balm plants under Mediterranean field conditions, where decreased chlorophyll contents were observed during the summer (Munné-Bosch and Alegre, 2000a). Especially the probably unexpectedly higher SPAD values for the late development stage of the second cut in 2014 might be explained by the weather conditions, as an unusually high precipitation occurred in August 2014 between the harvest of the medium and the late development stage. This might have led to a retarded ageing of the plants. It has also been shown in investigations on rosemary (*Rosmarinus officinalis*) (Munné-Bosch and Alegre, 2000b) as well as on lemon balm plants (Munné-Bosch and Alegre, 2000a) that a reduced chlorophyll content under drought conditions was restored after rainfalls.

The on average higher SPAD values in RH, as compared to GG, are presumably caused by the better nutrient holding capacity of the soil. On the sandy soil in GG, nutrient leaching occurs more easily, leading to a lower nitrogen supply of the plants. This can also explain the comparably stable SPAD values in RH, while they were decreasing for the first cuts in GG. Unexpectedly, SPAD values for the experiment in RH increased from early to medium harvest stage in both cuts in 2014. However, rather than showing very high SPAD values for medium and late harvest stage, the SPAD readings of the early harvest stages showed exceptionally low values. This state can be interpreted as a possible nitrogen deficiency of the plants. A possible explanation is an inhibited nitrogen mineralization and thus an impaired nitrogen supply of the plants. Nitrogen mineralization in the soil can be influenced by environmental conditions, like temperature, water status and pH of the soil (Schubert, 2018). It can be inhibited by drought and waterlogging conditions (Amberger, 1996; Schubert, 2018) – conditions that will have occurred in 2014. March and April had an exceptionally low precipitation, combined with above-average

temperature. On the other hand, May and July (the months directly before the harvest of the early development stages for the first and the second cut, respectively) experienced exceptionally high precipitation. Thus, it can be suggested that the soil underwent waterlogging conditions, leading to an inhibited nitrogen mineralization, and consequently an impaired nitrogen supply of the plants for the early harvest stages of both cuts.

Besides an influence of development stage or nitrogen supply, light conditions can also influence the chlorophyll content of plants. Chlorophyll biosynthesis is regulated in a light-dependent manner by the phytochrome system via phytochrome-interacting factors (Huq et al., 2004). If plants suffer from severe light deficiency, they produce less chlorophyll in a process called etiolation. However, plants in our experiments were not shaded as strongly. The moderate light reduction in our experiment in GG did not reduce the SPAD values. The non-significant tendency of slightly higher SPAD values of the shaded plants can be explained as an adaptation mechanism. The effect of increased SPAD values was, however, significant under the strong light reduction applied in RH for both cuts in 2014. This is in accordance with findings of an investigation on tea plants (*Camellia sinensis*) showing a strong increase in chlorophyll content under shading nets with a light reduction of around 80% (Wang et al., 2012). This could be expected, as shade leaves are known to have a higher chlorophyll content per area (Bresinsky et al., 2013).

Our observations that a moderate light reduction did not have a significant effect on SPAD values while a stronger light reduction did have such an effect is in accordance with investigations on several plants (albeit with species-dependent sensitivity) that have shown a shade intensity related change in chlorophyll contents. This was shown, for instance, in *Salvia officinalis*, where chlorophyll content was not significantly different between 100% and 70% light, but significantly increased under 50% light conditions (Mapes and Xu, 2014). In *Tetrastigma hemsleyanum*, chlorophyll content of plants receiving full sunlight was not significantly different from those plants cultivated under 50% shade, whereas chlorophyll content was significantly higher under 67% and 75% shade, and increased significantly even under 90% shade (Dai et al., 2009). The chlorophyll content of *Aloysia gratissima* was only non-significantly higher under 40% shading, compared to control, while it was significantly higher under 80% shading (Pinto et al., 2007). In *Myrica rubra*, total chlorophyll content increased significantly under 25% and 50% shading compared to non-shaded control plants, while at 75% shading, total chlorophyll content was significantly reduced (Zeng et al., 2017). This shows that a shading that is too strong impairs plant development. However, the lemon balm plants in our experiment were not shaded until this point. It can thus be suggested that lemon balm plants can compensate a certain shading level, as applied in our experiments, without impairing the photosynthetic capacity of the plants.

5.1.4 Shoots per plant

The number of shoots per plant can be influenced by the genetics of the plant as well as by nitrogen supply and environmental factors. A notable variation of shoot number in different lemon balm genotypes has been presented by Seidler-Łożykowska et al. (2013). In a screening of 22 genotypes from botanical gardens throughout Europe, they found a range from 16.6 to 38.5 shoots/plant (Seidler-Łożykowska et al., 2013). In a Hungarian investigation on five lemon balm genotypes, a range from 28.6 to 54.6 shoots/plant was reported, with 49.0 shoots/plant for 'Lemona' (Szabó et al., 2016). In our investigation, the variability regarding the number of shoots per plant between the different genotypes was quite low. This might on the one hand be due to the low number of tested genotypes (only two in RH, and three in GG), and on the other hand due to the choice of the genotypes, as they have already been selected over time for their suitability for commercial cultivation. However, the differences of the same genotypes between the two experimental sites were quite notable, which indicates a stronger influence of the environmental conditions. This is in accordance with findings in literature: It has been shown that, in addition to the genetic background, also environmental factors like nutrient supply (Abbaszadeh et al., 2009b) or drought stress (Abbaszadeh et al., 2009b; Ozturk et al., 2004; Radácsi et al., 2016) can have an influence on the morphological traits of lemon balm plants, including the number of shoots per plant. Depending on the nitrogen supply of the plants, Abbaszadeh et al. (2009a) found a range from 19.8 to 32.6 shoots/plant. Drought stress reduced the number of shoots in a Hungarian investigation from 26.5 in the control to 15.0 under strong water deficit (Radácsi et al., 2016). However, in a field experiment in Iran, the induced drought stress led only to a non-significant variation of this parameter, covering a range from 40.9 to 50.4 shoots/plant (Abbaszadeh et al., 2009a). In a lysimeter experiment in Turkey, only slight, but significant, differences between the investigated degrees of water deficit were found. In this case, however, the number of shoots per plant was generally quite low, ranging from 13.7 to 18.3 (Ozturk et al., 2004). Generally, the differences in the water and nutrient holding capacities between the two experimental sites in our experiment seem to explain the differences in the number of shoots per plant, even for the same genotypes.

A lowered shoot number induced by a reduction of the light intensity and/or a decreased R:FR could be expected as a result of the shade avoidance syndrome, especially under a green net, as in our experiment. In wheat, a lower R:FR reduced the numbers of tillers in different cultivars (Ugarte et al., 2010). A reduced branching under shading or reduced R:FR conditions has been observed in different dicotyledon plants as well, like soybean (Green-Tracewicz et al., 2011), sweet pepper (Rylski and Spigelman, 1986), or white clover (Lötscher and Nösberger, 1997). Also in the Lamiaceae plant *Ocimum basilicum*,

shading reduced the number of shoots (Chang et al., 2008). In our experiment with lemon balm, no reduction of the number of shoots/plant could be observed under the moderate light reduction in GG. Under a stronger light reduction, however, as applied in RH, the number of shoots/plant decreased either significantly, or showed at least a tendency of lower values. This is in accordance with findings in *Ocimum basilicum*, where only stronger shading reduced the number of shoots, while a moderate light reduction of 25% did not lead to a significant reduction in shoot number (Chang et al., 2008).

Unexpectedly, the lemon balm plants under strong light reduction in our experiment did not show a reduced shoot number for the second cut in 2013, although such a reduction was observed in 2014 and 2015 (for 'Lemona' at least in tendency). It seems as if not only the intensity of the shading, but also the timing of the shade application is important. In 2013, the plants were not shaded at all for the first cut. The shading net was installed around two weeks after the last harvest of the first cut. Although the plants were growing for six, seven, and eight weeks (for early, medium, and late harvest stage, respectively) under the shading net, they received natural light intensity in an earlier development stage of the second growth cycle. In wheat it was suggested that a low R:FR at an early development stage suppressed the outgrowth of the buds to form tillers (Evers et al., 2006). It seems as if also lemon balm plants are more susceptible regarding a light reduction in their early development stage.

In summary, the lemon balm plants showed a reduced shoot number, as a symptom of the shade avoidance syndrome, only under the stronger light reduction in RH, whereas the plants seem to well tolerate the moderate light reduction, as applied in GG. The number of shoots in our experiments was on the upper end of, or even above, the values found in literature. This might indicate good genetic material, adequate N supply, and no strong drought stress in our experiment.

5.2 Yield parameters

In addition to the quality of the harvested plants, the yield of the product is an important parameter for the producer of agricultural goods. The biomass and leaf yields found in our experiment for the reference variants (natural light, medium harvest stage) are in the range reported in the literature.

The biomass yield of 55.6 dt/ha dried plant material found in an investigation in a three-year-old plant stand under Slovak climate conditions (Mrlianová et al., 2002) is comparable to the results obtained for the reference variants in our experiment in RH. In investigations under Polish climate conditions, biomass yields of 93 dt FM/ha in the first year and 73 dt FM/ha in the second year were described (Dzida et al., 2015). In another Polish study, biomass yields at four organic and two conventional farms were investigated over three consecutive years. Averaged annual biomass yields were stated as 44.9 to 241.6 dt FM/ha (9.3 to 55.1 dt/ha dried biomass) at the organic farms, and 81.9 to 149.6 dt FM/ha (29.1 to 13.8 dt/ha dried biomass) at the conventional farms (Seidler-Łożykowska et al., 2015). In both Polish studies, only one cut was harvested. Comparing these results therefore only to the first cut of the reference variants, comparable yields were found in GG, whereas the biomass yields obtained in our experiment in RH were much higher. In an investigation on lemon balm cultivated in Turkey, biomass yields of 340.1 dt FM/ha in the first year and 457.9 dt FM/ha in the second year were achieved for the florescence stage, which was higher than biomass yields at pre-florescence and post-florescence stage (Avci and Giachino, 2016). However, also the biomass yields of the pre-florescence stage were quite remarkable, with 245.0 dt FM/ha in the first year, and 327.9 dt FM/ha in the second year. These yields are comparable to the first cut in RH, but higher than in GG, in some cases even higher than the sum of two cuts. Compared to the results from another Turkish investigation with yields of 48.2 and 75.8 dt/ha dried biomass (sum of two cuts) in the first and second year, respectively (Saglam et al., 2004), the reference variants in GG were at a similar yield level in the first year, but slightly lower in the second year, whereas the DM biomass yields in RH were higher. Under the climate conditions of Northern India, biomass yields of 216.6 dt FM/ha (47.7 dt/ha dried plant material) were obtained 160 days after planting (Singh et al., 2014), which is less than the reference variants in our experiment in RH.

Compared to the data reported by Bomme et al. (2013) for lemon balm grown in Germany, who described biomass yields of 160–350 dt FM/ha in the first year and 180–450 dt FM/ha for the following years for field trials under Bavarian conditions, our results of the biomass yields in RH are mostly on the upper end of the reported ranges. This, as well as the comparisons with the data from other countries presented above,

indicates the suitability of the region for the commercial production of lemon balm. The slightly lower biomass yields for the year 2015, most probably caused by the lower precipitation in that year, were still in the middle of the range reported by Bomme et al. (2013). Due to the lack of an irrigation possibility, the plants could not reach their full yield potential. Although, except for 2015, the precipitation in GG was not lower than in RH, the biomass yields in GG were generally lower than in RH, found rather in the lower third of the ranges reported by Bomme et al. (2013). Not only the water balance, but also the soil conditions play an important role regarding the biomass yield. The field in GG is characterized by sandy soil conditions, leading to a low water holding and cation exchange capacity, and thus to an impaired constant supply of the plants with water and nutrients. Although the experiment in GG could, regarding the temperature, be considered as to some extent closer to the Turkish conditions than RH, the soil conditions in GG are poorer than described in the experiment of Avci and Giachino (2016) in Turkey. The combination of the Mediterranean climate and the soil conditions of a sandy loam in the Turkish experiment underlines the importance of not only the temperature, but also the soil conditions with a good water holding capacity, for the biomass production of lemon balm.

Regarding the leaf yields, the results of the reference variants in our experiment in RH are comparable to or even slightly higher than the values presented from a Slovak investigation, with 21.1 dt/ha dried leaves in one cut (Mrlianová et al., 2002), as well as a Turkish investigation with yields of 30.0 and 46.0 dt/ha dried leaves as the sum of two cuts obtained in the first and second year, respectively (Saglam et al., 2004). For the leaf yields obtained under German climate conditions, Bomme et al. (2013) described 90–230 dt FM/ha (19–40 dt DM/ha) in the first year and 100–250 dt FM/ha (20–45 dt DM/ha) in the following years. The leaf yields of the reference variants in our experiment in RH were on the upper end of the reported ranges. The slightly lower values in 2015 were most probably caused by the dryer than average early summer months in that year. Like for the biomass yield, the leaf yield of the reference variants in GG was in the lower half of the ranges reported by Bomme et al. (2013).

Although lemon balm is typically cultivated for two to three years in Germany (Bomme et al., 2013), the advanced age of the plants in 2015 (fourth growth year) did not seem to have a negative effect on yield performance of lemon balm in the current study. The slight decrease of the yield parameters in that year in RH seems to be more related to the weather conditions, as such a decrease was not observed for the plants of the same age in the experiment in GG.

Differences in the yield of different cuts within a year have been described in literature. It has been reported that the first cut gives higher biomass and DM leaf yields than the second cut (Bomme et al., 2013). In an investigation on the yield parameters of different lemon balm accessions with two to three harvests per year, Özgüven et al. (1999) reported that the highest biomass and leaf yields were almost always reached for the first harvest of the year. Also for the case of three cuts under German conditions, a tendency of decreasing leaf yields from harvest to harvest has been reported (Bomme, 2001). In line with these findings, biomass and leaf yields (FM and DM) of the reference variants in RH were higher for the first cut, compared to the second cut. The same was true for most cases in GG, but in some cases the yields of the second cuts were on the level of the first cuts, or even higher. However, the general yield level on the sandy soil in GG was lower than in RH. For the cases in which no yield drop (or even a yield increase) for the second cut occurred in GG in the year 2015, this might have been caused by the favorable temperature conditions in connection with an adequate water supply by irrigation. Generally, these cases should not be interpreted as the lack of a yield drop from a high level to a low level, but rather be regarded as keeping a medium or low yield level between the cuts under less favorable soil conditions.

It could be expected that biomass and leaf yield would linearly increase over time, from early to late harvest stage. This has been observed in the Lamiaceae plants *Mentha* × *piperita* with increasing biomass yield (DM) as well as leaf yield (DM) from early bloom to late bloom (Rohloff et al., 2005), and *Satureja montana*, where the herb yield increased from the earliest, vegetative stage to the beginning of flowering and full flowering, and further to the stage after flowering (Nurzyńska-Wierdak et al., 2017).

However, such increasing yields were not necessarily observed in our experiments. This observation is in line with reports from Naghdi Badi et al. (2004) on thyme, as well as Singh et al. (2014) and Avci and Giachino (2016) on lemon balm.

In *Thymus vulgaris*, where three stages were investigated (beginning of blooming, full blooming and fruit set), the FM biomass yield of the first stage (beginning blooming) was significantly higher than for full blooming and fruit set, whereas DM biomass yield was not significantly different between the three stages (Naghdi Badi et al., 2004). In an investigation on lemon balm in Northern India, biomass yields (FM and DM) were increasing from first to second to third harvest (120, 140 and 160 days after planting, respectively), whereas a yield drop was observed for the fourth harvest stage 180 days after planting (Singh et al., 2014). In an investigation in Turkey, the florescence stage of lemon balm showed the highest FM biomass yields among the three tested harvest stages (pre-florescence, florescence, post-florescence) (Avci and Giachino, 2016). For the

dry leaf yield, however, no significant differences between the three stages were found (Avci and Giachino, 2016). In our experiments, especially for the first cut, FM biomass yields were not increasing over time, neither in GG nor in RH. This may be explained by the fact that lemon balm plants tend to lose their lower leaves during plant development, whereas the newly formed leaves are smaller in size during the development towards the generative stage. This leads rather to a reallocation than an accumulation of biomass. Contrary to the FM biomass yields of the first cut, however, DM biomass yields were increasing over time in most of the cases. Obviously, over time some changes within the plants have occurred. During plant development, a process of lignification takes place (Barros et al., 2015), which can also be seen in the increasing DM content of the plants. This is in accordance with findings in *Hyssopus officinalis*, where the DM content of the herb increased with development, being lowest in vegetative stage, and highest after flowering (Zawiślak, 2011). The lack of generally increasing biomass yields over time for the second cut for both FM and DM, as well as the possibly unexpected cases in which even a decrease was observed, may be due to the different environmental conditions. Especially under the influence of hot and dry weather conditions, as well as with decreasing day lengths, plants tend to shift faster from the vegetative to the generative development stage. This earlier shift also explains the generally lower yields of the second cut, as compared to the first cut. Regarding the yields over the different harvest stages, it can be concluded that a later harvest is not necessarily equivalent to a higher yield. However, a higher DM content, equivalent to a lower water content, could be beneficial regarding the drying costs, making it more economically feasible if the general quality of the plant material meets the expectations.

Yield differences between different lemon balm genotypes have been described for a pot experiment carried out with five genotypes in Hungary (Szabó et al., 2016). Among the tested genotypes, 'Lemona' was found in the middle regarding the DM biomass yield. Although a genotype effect was statistically significant in many cases regarding biomass or leaf yield in our experiments, the genotype effect did not show a clear pattern. It seems as if the different genotypes reacted in a different way on the environmental conditions of the different years, but more or less leveling out over several years in a row. The exact reason for the genotype-specific reactions in the individual years remains unclear.

As photosynthesis is a light dependent process, it could be expected that a light reduction could impair photosynthesis and therefore the production of biomass. On the other hand, as plants receive more sunlight than needed even under temperate climate conditions (Wilhelm and Selmar, 2011), a light reduction could be interpreted as less light stress, which might give the plants the possibility to allocate more photosynthates for the

production of biomass, rather than for light protection. For instance, the leaf yield (DM) of the Asteraceae plant *Mikania glomerata* was significantly increased under differently colored shading nets with a light reduction of 36–46% (blue, grey, and red nets), with the highest yield under blue nets (Souza et al., 2011). In *Mahonia breviracema*, a positive influence of a light reduction of 50% and 70% on DM leaf yield was observed, and the strong light reduction of 70% also showed an increased DM biomass yield (Y. Li et al., 2018). Total biomass yield (DM) as well as leaf yield (DM) of *Pogostemon cablin*, a member of the Lamiaceae family, was significantly increased under 50% shading with black, blue, and red nets, with the highest values under red nets (Ribeiro et al., 2018). Also in *Salvia officinalis*, both FM and DM biomass yields were higher under the shading nets (Abd El Azim and Badawy, 2015).

As the moderate light reduction in GG did not influence the biomass or leaf yield significantly, neither a yield reduction occurred due to an impaired photosynthesis, nor was a positive effect due to a reduced light stress visible. Under the stronger light reduction in RH, however, significant effects could be observed. The effect of reduced biomass or leaf yields was more obvious in DM than in FM. These differences between FM and DM yields can be explained by the significantly lower DM contents of the leaves under the strong shading, a finding that has also been described for grapevine (*Vitis vinifera* 'Chardonnay'), where a light reduction of 54%, 90%, and 99% with shade cloths resulted in a decrease in leaf and total plant dry matter (Vanden Heuvel et al., 2004). Althought leaf dry matter content of *Aloysia gratissima* was not significantly different under full sunlight and 40% shading, it was significantly decreased under 80% shading (Pinto et al., 2007). The significantly lower DM content of shaded plants in RH is also in accordance with findings in shaded leaves of *Fagus sylvatica*, *Acer pseudoplatanus*, and *Tilia cordata*, as well as shaded needles of *Abies alba*, where the relative water content was significantly higher compared to sun-exposed leaves or needles (Lichtenthaler et al., 2007). Obviously, the strongly shaded plants in our experiment contained more water than the plants under full sunlight, most probably by a reduced water loss through evaporation under these conditions.

The findings of our experiment that a moderate light reduction, as applied in GG, did not reduce the biomass or leaf yields, whereas a stronger light reduction of about 50%, as in RH, did at least in many cases influence the yield parameters negatively, is in accordance with findings on other Lamiaceae plants reported in literature. For instance, the plant weight of *Ocimum basilicum* was significantly reduced under 50% and 75% shading, but not under 25% shading (Chang et al., 2008). The biomass of *Mosla chinensis* and *Mosla scabra* was not significantly reduced under 30%, but under 75% light reduction (Liao et

al., 2005). Aerial biomass of *Salvia officinalis* was significantly decreased under 70%, 50%, and 30% light intensity, compared to full light conditions. However, the biomass among the three shading intensities did not differ significantly (Mapes and Xu, 2014). The leaf yield of *Mentha arvensis* was significantly reduced under differently colored nets with a shading intensity of 50%, compared to full sunlight (Chagas et al., 2013).

The negative effect of the strong shading on the DM biomass and leaf yields was significant for the first cut in the investigated years 2014 and 2015 (the first cut in 2013 was not yet shaded). The same was true for the second cut in 2013, where the setup of the shading net took place only after the first cut, and thus the second cut was the first harvest of the year grown under shade conditions. It thus seems as if especially the plants that did not have enough time to adapt to the shading conditions are more susceptible to the strong light reduction. However, this finding differs from reports from Brazil. DM leaf yield of lemon balm plants cultivated under differently colored nets with a shading intensity of about 50% was not significantly different after 50 days (first cut), whereas it was even increased after another 70 days (second cut) under blue nets, and showed a non-significant tendency of increase under black and red nets (Oliveira et al., 2016). The finding that a shading of about 50% did not affect DM leaf yield of the lemon balm plants for the first cut in this study might be attributed to the different environmental conditions. Under a strong sunlight intensity as in Brazil, a shading in the early phase is not interpreted as negative. The differences in light intensity between Brazil and Germany also explain why the strong shading in our experiments led to decreased DM biomass and leaf yields in most cases, whereas it even had a positive influence on the leaf yield in Brazil, at least for the second cut, most probably due to the reduction of light stress. Another point to mention is the different color of the nets used in the experiments of Oliveira et al., compared to our green nets. A green net changes the light quality in a different way by the absorption of red light, which not only leads to a changed R:FR (Oren-Shamir et al., 2001), but also impairs photosynthesis due to the importance of red light for the photosynthetic processes. This also explains the contradictory results in the experiment with the 50% shading on the Lamiaceae plant *Pogostemon cablin* with increased DM biomass and leaf yields under black, blue, and especially under red nets (Ribeiro et al., 2018).

The importance of the color of the shading nets on yield parameters can also be seen in the results of experiments with other Lamiaceae plants. Under 50% shade, DM leaf yields of *Ocimum selloi* were reduced under both red and blue shading, but to a greater degree under blue shading nets (Costa et al., 2010a). In *Ocimum basilicum* var. *citriodorum*, 50% shading reduced the FM and DM biomass yield of plants cultivated under black and red

nets significantly, and the FM biomass yield under the black nets even more strongly than under the red nets (Paulus et al., 2016). On the other hand, investigations in Brazil with *Mentha* × *piperita* cultivated under different nets with a light reduction of about 50% showed that biomass and leaf yields (DM) were not reduced under black and red nets, whereas they were reduced under blue nets (Costa et al., 2012). The partly different reactions regarding biomass or leaf yield on differently colored nets seem to indicate species-specifically different reactions, which leaves room for further experiments.

It might be suggested that the shading of plants, especially a strong shading, might influence the yield characteristics in the following years. It is conceivable that the light reduction leads to a lower accumulation of photosynthates due to an impaired photosynthesis, and thus to a lower amount of energy stored in the roots for the following year. Such a finding has been observed in *Vitis vinifera* (McArtney and Ferree, 1999). In this case, a very strong shading with a light reduction of 80% has reduced the total dry weight of shoots, as well as inhibited the leaf area development, in the year following the shade treatment. This was observed in connection with a reduced concentration of soluble carbohydrates as well as amino nitrogen in the xylem sap, indicating a lower mobilization of stored nutrients from the roots (McArtney and Ferree, 1999). However, the shading intensity in this investigation was much stronger than even the strong light reduction of about 50% in our experiment in RH, which might explain why such a negative effect of the shading in the previous year has not been observed in our experiments, in addition to possible species-specifically different susceptibilities.

The finding of significantly lower dry matter contents of the plants cultivated under strong shading conditions has to be regarded as negative from a commercial point of view, as a higher water content leads to higher drying costs. The observation in our experiment that a moderate light reduction did not reduce the biomass production indicates that lemon balm plants could tolerate these conditions. The potential use of plant protection nets with a similar light reduction could therefore be possible for lemon balm production. On the other hand, no improvements of biomass or leaf yield by a possible reduction of light stress were observed. Thus, a use of shading nets to improve lemon balm yields cannot be recommended under the temperate climate conditions in Germany, especially for nets with a stronger light reduction.

5.3 Essential oil content

Although lemon balm plants exert a typical, distinct fragrance, their EO content is quite low compared to other aromatic plants. The EO contents of the reference variants in our field experiment varied from approximately 0.1 to 0.7% within the investigated years and stations. It can be stated, that the EO contents of lemon balm from our experiments were in the same range reported in literature for lemon balm cultivated under different climate and field conditions (Carnat et al., 1998; Kowalska et al., 2014; Mrlianová et al., 2002; Seidler-Łożykowska et al., 2015).

Although it might be expected that lemon balm plants would give higher EO contents in warmer countries, the comparison of the EO content of our plants cultivated under temperate climate conditions in Germany can be regarded as competitive for the markets. The results are also in accordance with results from other investigations on lemon balm cultivated under comparable climate conditions, like 0.15−0.21% EO in different accessions (Bomme, 1996), or 0.45 to 0.52%, depending on the drying method employed (Argyropoulos and Müller, 2014). The EO content of 28 lemon balm accessions (16 ssp. *officinalis*, 12 ssp. *altissima*) cultivated under field conditions in Germany were reported to range between 0.01 and 0.78%, with differences between the genotypes, the first and second cut within a year, the trial sites, as well as between the experimental years (Kittler et al., 2018). This indicates the dependence of EO content in lemon balm on both genotype and environmental conditions. EO contents of ssp. *officinalis* were ranging from 0.02 to 0.16% for the first and 0.03 to 0.72% for the second cut in 2009 (Kittler et al., 2018, supplementary material).

Our finding that EO contents were generally higher for the second cut are in accordance with findings in literature from experiments also carried out in Germany (Böttcher et al., 2000; Chizzola et al., 2018; Kittler et al., 2018). It has been observed in our experiments that the second cut was characterized by a higher leaf:stem ratio, on average a higher number of shoots per plant, and smaller plant height. These parameters indicate a relatively higher number of terminal leaves, and a lower number of middle or basal leaves. It has been shown by other scientists that EO content of the Lamiaceae plant *Satureja douglasii* was higher in the tips than in lower leaves (Lincoln and Langenheim, 1978). As terminal lemon balm leaves contain higher amounts of essential oil than middle or basal leaves (Adzet et al., 1992a; Hefendehl, 1970; Mrlianová et al., 2002), this might be an explanation for the higher EO content of the second cut. The main sites for EO secretion are glandular trichomes, and it has been reported that the young leaves and organs of different Lamiaceae plants show a higher density of these trichomes (Werker, 1993). In addition, higher EO contents in lemon balm have been described under a certain degree

of drought stress (Abbaszadeh et al., 2009a; Farahani et al., 2009; Manukyan, 2011; Ozturk et al., 2004). It is suggested that a certain degree of drought stress has occurred for the second cut because of the weather conditions, yielding a higher EO content. However, under the climate conditions of Egypt, the opposite pattern with lower EO contents for the second cut was found (Rashed, 2012). Most probably, under a climate with higher temperatures, as in Egypt, the loss of EO due to evaporation played a major role, whereas under temperate climate conditions, this was not the case.

Some differences in the EO content between different lemon balm genotypes have been reported in literature. In an investigation on 15 *Melissa officinalis* accessions from the gene bank in Gatersleben, a high variability between the accessions was found regarding the EO content (Chizzola et al., 2018). Also in an investigation with three lemon balm lines in Turkey, some differences were observed, albeit on a quite low level (Cosge et al., 2009).

In a pot experiment with five genotypes covering a range from 0.067 to 0.298 ml EO/100 g, 'Lemona' gave the highest EO content, whereas the other four genotypes were not statistically significantly different (Szabó et al., 2016). Also in our investigation, 'Lemona' exceeded the other genotypes regarding EO content. Although a higher EO content in the upper leaves of lemon balm plants has been shown (Mrlianová et al., 2002), and thus a plant with more shoots per plant, having more terminal leaves, might be expected to show a higher EO content, this does not seem to be the reason for the higher values of 'Lemona'. In this case, it rather seems to be directly genetically determined, not indirectly by morphological traits, as a higher number of stems and terminal leaves.

A genotype specific reaction on different light conditions has been observed in *Mentha spicata*. In one genotype, an additional UV-B radiation led to an increased EO content, while there was no significant difference for a second genotype (Karousou et al., 1998). However, a strong genotype specific reaction was not observed in our experiments, although 'Aufrechter Typ' showed in some cases a stronger reaction on differing light conditions than the other genotypes, at least for the second cut.

In a screening of 11 accessions cultivated under Turkish conditions in two different locations, differences both between genotypes and between the locations were observed. EO contents were covering a range from 0.029 to 0.097% in one location, and from 0.010 to 0.063% in the second location (Sari and Ceylan, 2002). Interestingly, some accessions were similarly on the upper or lower part of the range in both locations, while others were closer to the upper end of the range in one location, but only average or even on the lower

end of the range in the other location. This could indicate a different adaptation to diverse locations because of differences in the genetic background. However, in the two genotypes investigated in our experiments in two different locations, such genotype specific different reactions in the different locations were not observed.

Generally, essential oil content of plants is influenced by the development stage (Sangwan et al., 2001). Since it has been shown in different plants that EO content was decreased after flowering or at fruiting stage (Ben Farhat et al., 2016; Chapman, 2009; Mirjalili et al., 2006; Naghdi Badi et al., 2004; Nurzyńska-Wierdak et al., 2017; Şenkal et al., 2015), this stage has not been investigated in our experiments.

As it has been described that younger leaves and organs of different Lamiaceae species exhibit a higher density of glandular trichomes (Ascensão et al., 1995; Werker, 1993), and EO content in several Lamiaceae species was found to be higher in the terminal leaves (Adzet et al., 1992a; Hefendehl, 1970; Lincoln and Langenheim, 1978; Mrlianová et al., 2002), it might be expected that EO content should be highest for the earlier development stages, and thus for an early harvest. However, that was not generally true in our investigation. Rather, especially for the first cut of the year, the early harvest stage was in most cases either on a similar level or even lower than for medium and/or late harvest stage. This finding might be explained by the fact that on the young leaves the trichomes are not yet fully developed (Ascensão et al., 1995), and thus EO production is not yet reaching its maximum.

In many cases, it has been reported that the flowering stage of different plants is characterized by a high EO content. In *Artemisia annua*, for instance, the EO content at full flowering was significantly higher than before flowering (Şenkal et al., 2015). Also for different plants from the Lamiaceae family, differences in EO content between different development stages have been described: In *Origanum vulgare* and *Origanum majorana*, EO content determined for five development stages from the beginning of the vegetative growth until full flowering was highest at full flowering stage (Tahmasebi et al., 2016). In the closely related plant *Origanum onites*, EO content was lowest in the pre-flowering stage, and highest at the end of flowering/ beginning of fruit set (Ozkan et al., 2010). In *Hyssopus officinalis*, EO content was highest at full flowering, while it was lowest at vegetative stage (Zawiślak, 2011). Chapman (2009) reported that the concentration of mono- and sesquiterpenes in *Aloysia citriodora* increased during the growing period until flowering in August (Chapman, 2009). In *Thymus kotschyanus*, EO content increased from the stage before flowering over beginning of flowering to complete flowering (Sefidkon et al., 1999). In *Thymus vulgaris*, however, EO content at the beginning of blooming was higher than at full bloom (Naghdi Badi et al., 2004). For *Salvia officinalis*,

slightly contradicting results have been published, with EO content being higher at flowering stage than at vegetative stage in one study (Ben Farhat et al., 2016), but in another study higher at vegetative stage than at flowering stage, with the budding stage having the highest EO content (Mirjalili et al., 2006). Also for *Satureja montana*, different patterns have been described. In one case, EO content increased from vegetative stage to full flowering stage (Nurzyńska-Wierdak et al., 2017), whereas in another study, EO content was highest at an earlier development stage in June (prior to flowering), compared to a later development stage in August (still prior to flowering), or for flowering stage (Mastelić and Jerković, 2003).

Despite some contradictory results, it can be seen from literature that for a high EO content the flowering stage is often regarded as a good harvesting stage. However, for the cultivation of lemon balm in Germany, it has been suggested to harvest at an early development stage, before flower buds are formed, as the EO content of freshly grown plants was said to be high (Bomme et al., 2013). This is also in agreement with the results of a study where EO content of *Melissa officinalis* grown in Turkey was highest before flowering, and decreased significantly at beginning of flowering, and again at full flowering (Uyanik and Gürbüz, 2015).

However, such a pattern could not be found in our investigation, at least for the first cut. Instead, in RH an increasing EO content over time was observed, which is in accordance with findings in some Lamiaceae plants, as mentioned above. In GG, however, EO content was quite stable over time, a pattern that agrees with reports in other plants of the Lamiaceae family. Angioni et al. (2006) stated that EO content of leaves and stems of *Lavandula stoechas* ssp. *stoechas* was relatively constant throughout the year (Angioni et al., 2006). Also in a Norwegian investigation with peppermint over three years, EO content did not differ significantly between three development stages (Rohloff et al., 2005).

Our finding that significant differences between the harvest stages could not be observed in all of the investigated years is in accordance with findings of Avci and Giachino (2016). As a result of their experiment with lemon balm plants, they stated that EO content was on average over the two investigated years significantly higher at florescence stage, compared to pre- and post-florescence stage. However, regarding only the first year of their investigation, no significant difference between the three harvest stages were observed. Instead, the pre-florescence stage showed a non-significant tendency of a higher EO content, compared to the later harvested plants (Avci and Giachino, 2016).

Different results obtained in the same plant, like mentioned above for *Salvia officinalis* (Ben Farhat et al., 2016; Mirjalili et al., 2006) and *Satureja montana* (Mastelić and

Jerković, 2003; Nurzyńska-Wierdak et al., 2017) may indicate that also additional external factors might interact with the development stage, which would explain the differences found in our experiments between the two experimental sites.

The effect of different reactions regarding the harvest stage at the two different investigated locations in RH and GG is in accordance with findings from Turkey. It was reported that EO content did not differ significantly between the three harvest stages (before flowering, flowering, after flowering) in one location, whereas a significant decrease over the harvest stages was observed in the second location (Ayanoglu et al., 2005). The second location was characterized by lower mean temperatures, which makes the results comparable with our experiments. In our case, an increase of EO content over time for the first cut could be observed in RH, whereas in GG, EO contents of the first cut were in most cases not significantly different between the harvest stages. This could on the one hand be explained by an initially higher EO production of the plants under higher temperatures, and on the other hand by a higher loss of EO by evaporation under these conditions.

EO content of plants can be increased under a certain degree of drought stress, as has also been shown for lemon balm (Abbaszadeh et al., 2009a; Farahani et al., 2009; Manukyan, 2011; Ozturk et al., 2004). Under the sandy soil conditions in GG, especially combined with the on average higher temperatures, the lemon balm plants will have faced some extent of drought stress, especially more or less constant over the growing period, compared to RH. In RH, the loamy soil conditions with a better water holding capacity lead to a later onset of possible drought stress conditions during the growing period, especially for the first cut. These conditions might explain the differences between GG with quite stable EO contents and RH with an increasing pattern of EO contents over the three harvest stages during the first cut. Additionally, due to the on average higher temperatures in GG, a possible EO loss by volatilization is more probable, as EO is a mixture of highly volatile substances. Although a loss of monoterpenes by volatilization was shown to be quite low under greenhouse conditions (Gershenzon et al., 2000), it can be expected to be substantially higher under field conditions, as radiation is higher and the wind conditions play an additional role. Thus, a possible increase in EO production over time might be counteracted by a higher loss of EO by volatilization in field-cultivated plants, and thus not be visible in the analysis of the harvested plants.

The different patterns between the two experimental sites regarding EO content for the harvest stages was especially distinct for the first cut in the year 2014. The observation of an increasing pattern in both genotypes in RH was connected with an extraordinarily low EO content for the first harvest stage, as it was also observed in GG. In GG, however,

such a low EO content was observed also for the medium and late harvest stage. Those low values can be explained by the above-average temperatures and below-average precipitation during the first half of the year in GG. Even in RH, with the better water holding capacity of the soil, the early spring of 2014 was characterized by very low precipitation and above-average temperatures, and thus drought stress might have appeared as well in that location, explaining the extraordinarily low EO content of the first harvest stage. Although a certain degree of drought stress can increase EO contents in plants, as described above, a very strong drought stress and the higher temperatures impair the accumulation of EO and might lead to a higher loss by volatilization. The increase of EO content in RH, however, can be explained by higher precipitation and thus less drought stress before the last harvest stage in that year. However, this increase cannot be interpreted as an above-average EO content. Rather, EO content of the later harvest stage can be seen as a normal level, not excessively high in the comparison of the years. Especially the plants in GG exhibited on average in many cases higher EO contents than the plants in RH. The elevated levels of EO of the shaded plants for the late harvest stage in RH may be interpreted as a combination of a positive effect of the shading during a period of quite high radiation and a protection from evaporation, rather than an effect of the harvest stage.

It is generally accepted that light conditions can influence the EO content of plants (Sangwan et al., 2001). It has been shown that a light reduction reduces the expression of enzymes involved in the biosynthesis of terpenes (Friedel et al., 2016; Sasaki et al., 2016). As the metabolic cost of terpenoid accumulation is quite high for the plants (Gershenzon, 1994), and the light-dependent process of photosynthesis is the basis for the energy supply of the plants, it might be expected that the reduction of the light intensity might impair the EO production of the plants. This would be in accordance with findings in *Mahonia breviracema*, where EO content decreased linearly under 50%, 70%, and 90% light reduction (Y. Li et al., 2018), *Lippia sidoides*, with highest EO content under full sunlight and reduced EO contents under 25%, 50%, and 75% shading (Souza et al., 2007), as well as *Ocimum basilicum*, where EO content was reduced by strong shading (75%) (Chang et al., 2008).

However, such an effect was almost not observed in our experiments. Obviously, the light supply under the given field conditions is high enough so that even a shading of around 10–15% (as in GG), or even around 50% (as in RH), does not necessarily negatively influence the production of EO in lemon balm plants. As different plants react also differently on changes in light conditions, it seems that lemon balm plants are quite tolerable to moderate reductions in light intensity.

Opposite to the expectation that a light reduction might impair EO accumulation in plants, it might be suggested that a shading might even be beneficial for the EO content. As it has been stated that even under temperate climate conditions the sunlight exceeds the needs of the plants for photosynthesis (Wilhelm and Selmar, 2011), it might be possible that a plant under light stress has to invest energy in photoprotection, for instance by photorespiration (Bresinsky et al., 2013; Peterhansel et al., 2010). Another factor might be the loss of EO by volatilization through the higher temperature. These reasons might explain the findings that EO content in sage was higher under shade in Egypt (Abd El Azim and Badawy, 2015), or in *Satureja douglasii* (a plant found in natural habitats with strongly differing light intensities) with higher EO content under low light (Lincoln and Langenheim, 1978). Also in a Turkish investigation with lemon balm cultivated in two different locations, lower EO contents were found in the place where plants grew under full sunlight, whereas EO contents were higher for the plants cultivated under partially disturbed forest canopy (Ayanoglu et al., 2005).

However, such a positive effect was, apart from some cases, not systematically found in our investigations. The measured air temperature was comparable between shaded and non-shaded plots in our experiments. To ensure that a higher radiation on the non-shaded plots did not influence the measured temperature, the sensors themselves were placed in a shaded position for both light variants, and thus registering the air temperature. Because of the comparable temperatures on the shaded and non-shaded plots, it could be expected that an EO loss by volatilization should not have occurred. However, the leaf temperature was not registered in this experiment. It might have happened that the leaf temperature of the non-shaded plants became higher due to the higher degree of direct radiation, and thus a higher EO volatilization has occurred in the few cases were EO contents were lower for the plants grown in full sunlight.

The case stated in literature where lemon balm plants grown under an agrotextile cover in Switzerland had significantly higher EO content than unprotected plants (Carron et al., 2008) is not comparable to our experiment. In that case the cover did also reduce the light intensity, but it also changed the microclimate remarkably under quite unfavorable conditions. Thus the plants had both a warmer micro-environment in a colder area, and were protected from wind which could have led to EO losses.

Apart from a possibly higher volatilization of EO induced by higher leaf temperatures, terpene emission of the plants might also depend on other exogenous factors. Even under the same temperature conditions, *Rosmarinus officinalis* has been shown to emit more volatile terpenes in June than in November (Peñuelas and Llusià, 1997), indicating the presence of further influencing factors, as may be the light intensity.

On average, the light reduction did not have a systematic positive or negative effect on the content of EO of the lemon balm plants in our experiments. This is in accordance with findings in investigations carried out with other plants of the Lamiaceae family, like those of Shafiee-Hajiabad et al. (2016), who reported no significant influence of a light reduction of about 26% on the EO content in the leaves of four accessions of *Origanum vulgare*, and Gonçalves et al. (2003), who stated that a light reduction of about 50% did not influence the EO content of *Ocimum selloi*. Also in *Aloysia gratissima*, the EO content did not differ between full sunlight, 40% shade, and 80% shade (Pinto et al., 2007).

Apart from the light intensity, the color of the shading nets might influence the EO content of plants, with possibly different reactions of different plants. The effect of a 50% light reduction, comparable to our experiment in RH, of differently colored nets has been investigated in several plants. Souza et al. (2011) investigated this effect in *Mikania glomerata* (Asteraceae). EO content was significantly increased under all tested nets (red, grey, and blue), with the blue nets showing the strongest increase in EO content among the tested colors (Souza et al., 2011). Even among different genera and species in the Lamiaceae family, different reactions were observed. A 50% light reduction with black and red nets, for instance, increased the EO content of *Ocimum gratissimum* (Martins et al., 2008) and *Ocimum basilicum* (Paulus et al., 2016), whereas the EO content of *Mentha arvensis* was significantly reduced (Chagas et al., 2013). No significant effect, however, was found in *Mentha* × *piperita* (Costa et al., 2012), *Ocimum selloi* (Costa et al., 2010a), and *Pogostemon cablin* (Ribeiro et al., 2018). For the use of a blue shading net with a light reduction of about 50%, however, results were slightly different. While EO content was increased under these conditions in *Ocimum gratissimum* (Martins et al., 2008) and *Pogostemon cablin* (Ribeiro et al., 2018), it was decreased in *Mentha* × *piperita* (Costa et al., 2012) and *Mentha arvensis* (Chagas et al., 2013), and again no significant effect was found in *Ocimum selloi* (Costa et al., 2010a).

Because of crop specific reactions, it seems difficult to transfer these results to lemon balm plants. However, information about the reaction of lemon balm plants on light reduction is relatively limited in literature. In a pot experiment in Brazil, EO content of lemon balm plants was not significantly affected under 50% light reduction with black and blue nets, but it was significantly reduced under red nets with the same light reduction (Brant et al., 2009). In another experiment, EO content of *Melissa officinalis* was significantly reduced under black and especially under red shading - at least for the second cut (Oliveira et al., 2016). For the first cut in the same experiment, however, no significant differences in EO content were reported (Oliveira et al., 2016). Possibly, these differing reactions are related to the time period of the applied shading. Plants in the

experiment of Oliveira et al. (2016) were shaded for 50 days for the first cut and an additional 70 days (i.e. 120 days in total) for the second cut, and those in the experiment of Brant et al. (2009) for around 90 days. It seems as if the reaction of lemon balm plants on a shading, regarding EO content, takes some time. This is in line with the statement of Friedel et al. (2016) who described that changes in the expression of terpene biosynthesis related enzymes after changes in light intensity were quite slow, and that the maximum accumulation of linalool occurred only with some delay after the maximum gene expression of terpene synthases involved in linalool biosynthesis. This might explain why in our experiments differences between the two light intensities, if any, could only be seen for the later harvest stages of the first cut (under the strong light reduction in RH), or even just for the second cut.

Contrary to the aforementioned results with a possible reduction of EO content in lemon balm plants, the EO content in our experiments was even increased in some cases. This might also have to do with the differently colored nets. While Oliveira et al. (2016) and Brant et al. (2009) found significant reductions in EO content under red nets, the color of the nets used in our experiments was green, which is the complementary color to red. By the absorption of red light, a green net leads to a change in the R:FR (Oren-Shamir et al., 2001), and thus to different signaling processes in the plant. It seems as if lemon balm plants are quite tolerable regarding the use of a green-colored shading net, at least at a moderate light reduction, as in GG, or for a certain period of time even under a stronger light reduction, as applied in RH in our experiments.

5.4 Phenolic compounds and antioxidant properties

Among 70 investigated medicinal plants, lemon balm exhibited the highest antioxidant capacity and total phenolic content (Katalinic et al., 2006). The quality of lemon balm leaves according to the European Pharmacopoeia is dependent on the content of rosmarinic acid (RA), for which a minimal content of 1.0% is required (Ph. Eur. 7, 2011). RA is also the most prevalent phenolic compound accumulated in lemon balm (Weitzel and Petersen, 2010). Because of its antioxidant properties (Adomako-Bonsu et al., 2017; Fadel et al., 2011; Nakamura et al., 1998; Soobrattee et al., 2005) and its abundance in lemon balm leaves, it can be regarded as an important factor for their antioxidant capacity, as measured with the ORAC assay. Also for the TPC values, RA thus plays an important role, as in plant extracts with a high content of phenolic substances, the reducing capacity measured by the Folin-Ciocalteu assay can be regarded as a rough approximation of the content of phenolic substances (Everette et al., 2010). This view is supported by the findings of RA percentages in the range of 73.8−85.8% of the measured TPC values (Chizzola et al., 2018). Although it was not clear at the beginning of our investigations to which degree these parameters would correlate under the conditions of our experiments, the results showed a connection and quite similar patterns of TPC, ORAC, as well as RA content of lemon balm. Thus the three parameters will be discussed together in the following.

Generally, **TPC** values found in our investigation are in the range stated in literature. However, TPC values of lemon balm found in literature vary in a very broad range, depending on plant material and extraction procedure. Additionally, a comparison is further complicated due to the different units used, as well as the different reference points (dry matter, air-dried plant material, fresh matter, plant extract). Furthermore, different reference substances (e.g. gallic acid, (+)-catechin, quercetin) are employed. However, most of the literature results are given in gallic acid equivalents (GAE), as in our investigations.

TPC values of aqueous lemon balm extracts found in literature (if necessary converted to the unit mg GAE/g, as in our investigation) start from less than 1 mg GAE/g (Ašimović et al., 2013; Vinha et al., 2012), have been presented as 27.2 mg GAE/g for an infusion as well as 43.5 mg GAE/g for a decoction (Popova et al., 2016), 46.5 mg GAE/g (Moraes-de-Souza et al., 2008), 54.4 mg GAE/g and 64.2 mg GAE/g for two different green leaf samples, but lower values if stalks and stems were included in the plant material (Petkova et al., 2017), 75.2 mg GAE/g (Jiménez-Zamora et al., 2016), in a comparison of two different extraction times as 79.1 mg GAE/g after 5 min and 92.9 mg GAE/g after 15 min extraction (Komes et al., 2011), 82.4 mg GAE/g (Kratchanova et al., 2010), or

91.8 mg GAE/g (Ho et al., 2010). However, some investigations also reached values of more than 100 mg GAE/g, like 119.5 mg GAE/g for a conventional to 145.8 mg GAE/g for a microwave assisted extraction (İnce et al., 2013), 105.9 to 178.1 mg GAE/g for plants exposed to different degrees of drought stress (Radácsi et al., 2016), 169.1 mg GAE/g (Chervenkov et al., 2018), or 209 mg GAE/g (Rusaczonek et al., 2010). Even higher values were found after a longer extraction time of 24 h, with values ranging from 359 to 478 mg GAE/g (Németh-Zámbori et al., 2016; Szabó et al., 2016). It has been hypothesized that during an aqueous extraction, especially a longer one or a decoction, not only phenolic substances, but also other water-soluble organic acids with reducing properties might have been extracted, contributing to the measured TPC values (Sentkowska et al., 2015).

That might also be the reason why TPC values of methanolic or ethanolic extracts found in literature do on average not reach very high values, but might be closer to the real phenolic content of the plant material. TPC values of ethanolic extracts (Ašimović et al., 2013; Cocan et al., 2018; Dastmalchi et al., 2008; Duda et al., 2015; Mabrouki et al., 2017; Moacă et al., 2018; Şahin et al., 2017; Vinha et al., 2013) cover a similar range as methanolic extracts. TPC values of extractions with different concentrations of methanol have been stated (if necessary converted to the unit mg GAE/g, as in our investigation) as 0.5 mg GAE/g (Atanassova et al., 2011), 1.7 mg GAE/g (Sytar et al., 2018), 15.1 mg GAE/g (Proestos et al., 2013), 17.0 mg GAE/g (Proestos et al., 2005), 69.2 mg GAE/g (Kırca and Arslan, 2008), 70.4 mg GAE/g (Skendi et al., 2017), or 70.9 mg GAE/g (Tusevski et al., 2014). Interestingly, in an investigation on different genotypes, values of the lowest and highest measurements varied almost fivefold, from 5.5 to 26.7 mg GAE/g (Boneza and Niemeyer, 2018), indicating the importance of the genotype for this parameter. However, these results are much lower than the TPC values found in our experiment. This might be explained by the chosen cultivars (different from the genotypes in our experiment), and the cultivation conditions (pot experiment under greenhouse conditions). The findings of Ulewicz-Magulska and Wesolowski (2019) point out variations in the quality of commercially available lemon balm. In this case, TPC values of eight lemon balm samples obtained from different distributors were differing more than fivefold, covering a wide range from 54.9 to 299.5 mg GAE/g, with a median value of 88.71 mg GAE/g (Ulewicz-Magulska and Wesolowski, 2019). As in this case material from different sources was measured in the same work group, it can be concluded that the different TPC values presented in literature are not necessarily due to differences in the measuring procedure between different laboratories, but also due to differences in the quality of the used plant material. The plant material used in our experiments was perfectly in the range, and for the reference variant always above the

median, of the values presented by Ulewicz-Magulska and Wesolowski (2019). Their finding of the great differences between commercial samples underlines the importance of investigations on the parameters influencing the quality of lemon balm.

ORAC values of lemon balm in literature are scarce, and a comparability is difficult due to the different extraction methods, or whether measured in fresh or dried samples. However, ORAC values from our investigations fit into the range of results stated in literature. Published ORAC values of two investigations, determined in fresh lemon balm, seem quite low. Zheng and Wang (2001), for instance, presented a value of 9.54 µmol TE/g fresh weight, and Ninfali et al. (2005) an ORAC value of 5996.5 µmol TE/100 g fresh weight, corresponding to 59.97 µmol TE/g fresh weight. However, in both cases not only was fresh plant material used, but also the extraction protocols were quite different from our extraction protocol. On the other extreme of the range, very high ORAC values (about ten times higher than in our investigation) of 14688.4 µmol TE/g DW were presented by Vasileva et al. (2018) in lemon balm plant material remaining as a residue after EO distillation (regarded as waste). Possibly, during the distillation process some chemical changes occurred in the plant material, and thus these results are not directly comparable to normal lemon balm leaves. However, a larger proportion of investigations found in literature perfectly covers the range of ORAC values found in our investigations, like (if necessary, own calculations to µmol TE/g by taking into account the extraction yield or the weight of the samples) 855.27 µmol TE/g herb material (Mabrouki et al., 2017), 1121 µmol TE/g for an acetone extract or 996 µmol TE/g for a hot water extract (Kratchanova et al., 2010), 1460 µmol TE/g (Masuda et al., 2015), or 2700 µmol TE/g sample (Ho et al., 2010). Generally, and in accordance with the findings from our investigations, these results indicate a high antioxidant capacity of the lemon balm material, making it especially interesting for the food industry.

RA contents of the plants in our experiments were perfectly within the range stated in literature (cf. **Tab. 1**), covering a range from less than 1%, like around 0.19% (Boneza and Niemeyer, 2018), 0.24% (Petkova et al., 2017), 0.78% (Benedec et al., 2015), or 0.83% (Skendi et al., 2017), to more than 6% (Engel et al., 2016) or in some cases up to, or even more than, 8% (Chizzola et al., 2018; Krüger et al., 2010), but in most cases lying somewhere between 2% and 6% (Argyropoulos and Müller, 2014; Boneza and Niemeyer, 2018; Carnat et al., 1998; Engel et al., 2016; Fialová et al., 2008; Ieri et al., 2017; Komes et al., 2011; Petkova et al., 2017; Radácsi et al., 2016; Shekarchi et al., 2012; Szabó et al., 2016; Tóth, et al., 2003; Wang et al., 2004). More importantly, all samples of our investigation met the requirements of the European Pharmacopoeia, with a minimal RA content of 1.0% in the dried drug (Ph. Eur. 7, 2011). The importance of good quality lemon

balm material for the market can be seen from the investigation of Arceusz and Wesolowski (2013) who investigated 19 commercial lemon balm samples from 12 suppliers, and found a wide range of 0.016−4.86% RA, with several samples not meeting the requirements of the European Pharmacopoeia. For the reference variants in our experiments, however, RA contents were always above 3%, indicating the general good suitability of the tested genotypes as well as the regions in which our experiments were conducted for the cultivation of lemon balm meeting the requirements of the European Pharmacopoeia.

The RA content of lemon balm plants might differ between the harvests within a year. In a Slovak investigation with the lemon balm cultivar 'Citra', the second cut in September had a slightly higher RA content than the first cut in July (Fialová et al., 2008). Kittler et al. (2018) conducted an investigation on several gene bank accessions of lemon balm (albeit containing not only ssp. *officinalis*, but also ssp. *altissima*) at two different sites in Germany. The median RA content was higher for the first cut, compared to the second cut. Additionally, differences between the median values of the RA content were observed between the two sites, as well as between two years (Kittler et al., 2018). This indicates that RA content of lemon balm is not only genetically determined, but to a certain degree depending on environmental conditions. Also Chizzola et al. (2018), who investigated the same gene bank accessions as Kittler et al. (2018), found that RA contents of lemon balm leaves were higher for the first cut than for those from the second cut (Chizzola et al., 2018), with RA values of 15 different accessions (ssp. *officinalis*) between 52.1 and 85.7 mg/g DM (on average 70.1 mg/g DM) for the first cut, and between 53.2 and 90.1 mg/g DM (on average 64.8 mg/g DM) for the second cut (Chizzola et al., 2018). However, a clear pattern between the two cuts was not observed in our investigation, possibly because of genotypes that are different from the ones used by the abovementioned researchers. However, also an earlier investigation on the same gene bank accessions did not find distinct differences between the two cuts (Krüger et al., 2010). Although in our investigations RA contents of the reference variants in GG were quite similar for the first and second cut in most cases, a different pattern occurred in 2015. In this case, the values of the first cut were higher than on average (above 5%). Probably this was due to an extremely dry spring, leading to a higher degree of drought stress. Drought stress can increase the phytohormone abscisic acid (ABA) in plants (Bresinsky et al., 2013). ABA has been shown to induce PAL activity and the production of the phenolic substances salvianolic acid A and B in hairy root cultures of *Salvia miltiorrhiza* (Hao et al., 2012), as well as PAL, TAT, and RAS activity, and TPC and RA content in *Melissa officinalis* shoot cultures (Mousavi and Shabani, 2019). Thus, probably phytohormone-mediated changes in the activity of enzymes involved in the biosynthesis of

phenolic substances play a certain role. Our findings and the different reports in literature indicate that observed differences of the RA content between different cuts within a year cannot be generalized, but are rather dependent on the actual environmental conditions.

Generally, different **genotypes** within a species can differ in the content of secondary metabolites. The screening of 23 Iranian accessions of *Ocimum basilicum*, like lemon balm a member of the Lamiaceae family, showed a range of TPC values from 22.9 to 65.5 mg GAE/g (Javanmardi et al., 2003). In another investigation on 15 *Ocimum basilicum* cultivars, significant differences in TPC were found, ranging fivefold from 3.47 to 17.58 mg GAE/g DW (Kwee and Niemeyer, 2011). In different *Origanum* accessions, a certain degree of variation in their TPC content was observed (Yan et al., 2016). Also in five genotypes of lemon balm, TPC values were varying almost fivefold, ranging from 5.5 to 26.7 mg GAE/g (Boneza and Niemeyer, 2018). These results indicate the importance of the genotype for this parameter. Regarding the RA content of the set of 15 basil cultivars mentioned above, even a hundredfold difference was observed, ranging from 0.06 to 6.09 mg/g DW (Kwee and Niemeyer, 2011). This shows a great genetically determined variability within a Lamiaceae species regarding this single phenolic substance, and indicates that TPC and RA do not necessarily correlate closely. In an investigation on five lemon balm genotypes, however, only slight differences in their RA content were found. Only the genotypes with the highest (2.72%; 'Quedlinburger Niederliegende') and the lowest RA content (2.43%; 'Lemona') were differing significantly in this case (Szabó et al., 2016).

In the case of our investigation, no clear, reproducible effect of the genotype was found regarding TPC, ORAC, as well as RA. Although in some years or cuts significant differences between the genotypes were found, these were not following a clear, unambiguous pattern. This does not necessarily mean that these traits are not genetically determined in lemon balm. Rather, it could be the case that the chosen genotypes do generally show good values for the tested parameters, as they have already been selected over a certain period of time to fit specific quality requirements. Additionally, only three genotypes have been tested in our experiments, which can not cover the range of a larger set of different genotypes. However, the results from other investigations mentioned above show that a variability in TPC and RA content of lemon balm exists, leading to the conclusions that a farmer should choose an appropriate genotype for producing high-quality lemon balm, and that the genotypes tested in our investigation are suitable for the production under the soil and climate conditions in Hesse to meet the requirements of the European Pharmacopoeia.

Generally, it is possible that different genotypes may react differently on changes of the environment. In two *Lactuca sativa* cultivars, for instance, a cultivar dependent reaction on different treatments with blue light regarding secondary metabolites, including phenolic substances, was found (Ouzounis et al., 2015). A genotype specific reaction in the content of several phenolic acids as well as total flavonoid content on different temperatures was found in the comparison of several wheat genotypes (Shamloo et al., 2017). A genotype specific reaction on environmental changes was also shown in lemon balm, where different genotypes reacted differently on water deficiency stress, regarding e.g. the content of essential oil or the content of phenolic compounds (Szabó et al., 2017). Although in our investigations an interaction effect of the genotype with the other investigated factors, i.e. a genotype specific reaction, occurred in some single cases regarding TPC, ORAC values, or RA content, no clear and reproducible pattern could be derived.

Besides the genetic background, also **ontogenetic** factors can influence the production of secondary metabolites in plants (Verma and Shukla, 2015). It might be hypothesized that the content of phenolic substances should be higher in the younger leaves, as they are more tender and thus more prone to damage, and phenolic substances might act as a protective agent, e.g. against UV light (Close and McArthur, 2002; Jordan, 2002; Li et al., 1993) or herbivores (Barbehenn and Peter Constabel, 2011; Bennett and Wallsgrove, 1994). On the other hand, it could also be expected that the content of phenolic substances could increase during the development of the plants, as the light intensity increases during the course of the year.

Changes in the content of phenolic substances during plant development have been investigated in several plant species. In an investigation with *Astragalus compactus*, TPC did not differ between vegetative and flowering stage, but was significantly increased at fructification stage (Naghiloo et al., 2012). Both TPC and RA content of *Origanum onites* were lowest in the pre-flowering stage (mid-June), and highest at the end of flowering / beginning of fruit set (mid-July) (Ozkan et al., 2010). In an experiment with *Ocimum basilicum*, TPC and RA content increased over time, but quite short intervals (0, 7, and 14 days) were tested in this case (Shiga et al., 2009). On the other hand, in an investigation with several *Thymus* species, where the content of various phenolic compounds, among others rosmarinic acid, varied significantly at different phenological stages, they were highest at the stage of budding and flowering, and decreasing during fruit maturation (Raudone et al., 2017).

Besides these different patterns in different plants, however, not all phenolic substances do necessarily follow the same pattern even in the same plant. During the leaf

development of tea plants (*Camellia sinensis*), for instance, the content of lignin, flavonols and some phenolic acids, like gallic acid, increased, whereas catechins and proanthocyanidins decreased (Wang et al., 2012). In *Hypericum origanifolium*, the contents of chlorogenic acid and hyperoside were significantly higher at vegetative stage, compared to floral budding and full flowering, whereas quercitrin content was significantly higher at full flowering, compared to vegetative stage and floral budding (Çirak et al., 2007). Even in an *in vitro* model, and thus independent from changing sunlight intensities over time, different patterns for different phenolic acids were observed. When hairy root cultures of *Echinacea purpurea* were grown under continuous light, compared to darkness, several caffeic acid derivatives were increased significantly, albeit to a different degree and at different time points. While chlorogenic acid was significantly increased already after five days, significantly higher values for caftaric acid were observed after 20 days, for cichoric acid after 30 days, and for caffeic acid only after 45 days (Abbasi et al., 2007). However, although the content of different phenolic substances could develop differently over time, there was a good correlation between TPC values and RA content in our experiments. Obviously, the high content of RA in the lemon balm leaves overlaid possibly different reactions of different phenolic substances, which were not specifically determined in our investigation.

Literature regarding changes in the content of phenolic substances during the plant development of lemon balm is quite scarce. Although some literature sources stated very low RA contents of lemon balm harvested in the flowering stage, even less than 1% (Benedec et al., 2015; Skendi et al., 2017), these findings cannot necessarily be generalized and attributed to the development stage itself. The majority of literature presents RA contents of lemon balm plants harvested before flowering, and these values are normally much higher than 1% (cf. literature cited above). However, also higher RA contents, like 3.65%, have been found in plants harvested at flowering stage (Shekarchi et al., 2012). Because these different results come from different literature sources with differences in the genetic background of the plants, growing conditions, as well as extraction procedures, it is interesting to determine the change of phenolic substances over time within one investigation, as in our experiment. In a Slovak investigation with the lemon balm cultivar 'Citra', no significant differences in RA content between the tested development stages (from flower calyx formation until full flowering) were found (Tóth, et al., 2003). However, in this case an earlier, vegetative stage was not determined.

Besides a general reaction over time, a possible effect of the development stage on secondary metabolites might also be specific for certain species or even genotypes. In *Ocimum basilicum*, RA contents were higher in the younger, upper leaves in one

investigation (Shiga et al., 2009), while in another study with a sweet Thai variety of basil, RA contents were higher when the leaves matured, albeit still on a quite low level (Vassão et al., 2006). In the latter case, however, the content of another secondary metabolite (methylchavicol) decreased in the older leaves (Vassão et al., 2006). In another study, three genotypes of *Ocimum basilicum* were investigated. RA content of the leaves increased in two genotypes from vegetative stage to flowering stage, albeit to a different degree, whereas it was not significantly changed in the third genotype (Kiferle et al., 2011). In the case of our experiments, however, no clear different patterns in TPC, RA or ORAC values over time were observed between the tested genotypes. Rather, these patterns seemed to be more dependent on the location and/or the year.

Interestingly, the patterns for the first cut of the year were quite different between the two locations. While in GG, some degree of decreasing values was observed, it was more of an increasing pattern in RH. Probably, the growing conditions in GG with higher light intensity, higher temperatures, and the soil conditions with a lower water holding capacity led to an earlier and stronger lignification of the plants. This can be seen from the results of the DM content of the leaves (cf. **Fig. 40**, **Fig. 41**, **Fig. 67**, **Fig. 68**), which increased more strongly during the time course of the first cut in GG, as compared to RH. Lignification is a process that occurs during plant development, in which the formation of lignin takes place (Barros et al., 2015). Lignin is biosynthesized from phenolic monomers (Bonawitz and Chapple, 2010; Rogers and Campbell, 2004), and can thus decrease the pool of soluble phenolic substances. This would explain why the content of phenolic substances showed more of a decreasing or at least stable pattern. In the cases where the content of phenolic substances did not decrease, but remained rather stable in GG, probably a higher production of phenolic substances over time was compensated by their use as precursors for the biosynthesis of lignin. In RH, however, a higher production of phenolic substances during the time course of the first cut, measured as TPC or RA content, was not completely used for the formation of lignin, as seen by a slower increase of the DM content of the lemon balm leaves. For the second cut, the content of phenolic substances remained either stable or showed more of a decreasing pattern in most of the cases in both locations, which is probably connected to the lower sunlight intensity during that time of the year.

It can be concluded that the optimal harvest stage for the RA content of lemon balm plants cannot easily be generalized. It is rather dependent on the local growing conditions, especially for the first cut of the year. Later during the year, the RA content seems on average to be more stable, or following more of a decreasing pattern. In these cases, an

earlier harvest seems to be a good option, but has to take into account the biomass yield and EO content as well.

Although **sunlight** is essential for the plants for photosynthesis, plants experience a certain degree of light stress even under temperate climate conditions (Wilhelm and Selmar, 2011). Especially UV radiation can be harmful, as the DNA is prone to damage due to its absorption maximum which lies close to the wavelengths of UV radiation (Britt, 2004). Especially epidermal flavonoids are regarded as an important sunscreen of the plants, to protect them from DNA damage. However, it has been shown that species of the genus *Melissa* did not accumulate external flavonoids (Tomás-Barberán and Wollenweber, 1990), and thus other phenolic substances seem to be important for the photoprotection of lemon balm plants. It has also been reported that UV absorbing substances within the leaves were increased under elevated UV radiation (Cuadra et al., 1997).

Because of the importance of several phenolic compounds for the protection of the plants from UV light, it can be expected that differences in the light intensity could influence their contents in lemon balm plants. Such an influence has been shown in several plants, albeit with some differences between the tested plants and/or different phenolic substances. Živanović et al. (2017) investigated the effect of polytunnels, reducing PAR and UV radiation, on tomato plants (*Solanum lycopersicum*). The contents of some phenolic substances, like caffeic acid or quercetin, in the tomato peel were higher under open field conditions, compared to the plants grown under the polytunnels. The same was true for epidermal flavonoids of the tomato leaves (Živanović et al., 2017). In an investigation on tea plants (*Camellia sinensis*), a 80% light reduction under shading nets lead to a decrease in flavonoid content (catechins by 16.76%, O-glycosylated flavonols by 43.26%, proanthocyanidins by 53.37%), as well as in lignin content (decreased by 9.53%) (Wang et al., 2012). On the other hand, however, the content of phenolic acids, like gallic acid, was increased (Wang et al., 2012). Interestingly, although the expression of the PAL gene was decreased 4.45-fold, and thus a general reduction of the biosynthesis of phenolic substances could be expected, the expression of further genes relevant for flavonoid biosynthesis were even more strongly decreased, explaining the different reactions of flavonoids and phenolic acids. Obviously, in this case, the direction of the biosynthetic pathways was changed more towards the biosynthesis of phenolic acids. In *Vitis vinifera*, a shade treatment led to a significant reduction in total phenolics and total flavonols (Friedel et al., 2015). However, TPC in the seeds of *Nigella sativa* was significantly increased under white nets, compared to the plants grown under direct sun light (Ali et al., 2018). In most cases, TPC was also increased under green nets, albeit mostly to a lower

extent than under white nets (Ali et al., 2018). In *Spinacia oleracea*, the situation was slightly more complex. While the use of shading nets reduced the total flavonoid content, the concentration of the major flavonoid was partly increased, partly decreased under the shading nets, depending on the time of the year and whether the first or the second cut was analyzed (Bergquist et al., 2007). This indicates that not only the light intensity, but also further environmental factors can have an influence on the content of phenolic substances, and may even interact with a light reduction.

However, in our investigations, the results regarding TPC or RA content were quite reproducible for the shade treatments in the different years and for the different cuts within a year. Possibly, this is due to the high content of phenolic substances, especially rosmarinic acid, in lemon balm, which overshadows possible fluctuations of minor phenolic compounds which might react more strongly on further environmental factors.

For the moderate light reduction, as applied in GG, no significant light effect on TPC and RA content was found at all. Also in investigations on lemon balm plants cultivated under plant protection nets to protect the plants from leafhoppers (unfortunately without stating the degree of light reduction), no changes in RA content were observed (Blum et al., 2011; Planer and Blum, 2009). This is in accordance with findings of Shiga et al. (2009) who found that RA production of *Ocimum basilicum* became saturated at a certain light integral. Presumably, this point has already been reached under the moderate shading applied in our experiment. Obviously, that degree of a light reduction was not yet decreasing the activity of the corresponding enzymes, like PAL. Whether a shift in the composition of different phenolic substances occurred has not been investigated in this study. However, even if it occurred, it did not have a noticeable effect, probably due to the high content of RA, the main phenolic compound in lemon balm leaves (Weitzel and Petersen, 2010). On the other hand, the strong light reduction, as applied in RH, led to a significant reduction of TPC, ORAC, as well as RA in almost all cases. Obviously, the plants were not forced to produce a high amount of photoprotecting phenolic substances. Rather, it can be concluded that a shift occurred, leading metabolites more towards primary metabolites, to secure plant survival under the quite unfavorable low-light conditions. Such a shift has been shown in *Vitis vinifera*, where a strong negative correlation between the accumulation of phenolics and amino acids was reported, with a significant increase in amino acids under a shading treatment (Friedel et al., 2015).

Not only the soluble phenolics can be influenced under a light reduction. The DM content of the leaves can be regarded as a parameter reflecting indirectly the lignification of the leaves. Under increasing irradiation, the leaf dry weight per area increased in *Betula pendula*, *Corylus avellana*, and *Lonicera xylosteum* (Kull and Niinemets, 1993). A higher

dry matter of non-shaded compared to shaded plants has also been shown in *Ocimum basilicum*, and it has been suggested to be possibly related to the close relationship of primary and secondary metabolism (Chang et al., 2008). The DM content of the lemon balm leaves in our experiment was lower under the strong light reduction, whereas it was not different from the non-shaded leaves under the moderate light reduction. Obviously, the light differences were not strong enough to lead to significant differences in the DM content of lemon balm plants.

However, a lower degree of lignification in the strongly shaded plants did not lead to an increase in the pool of soluble phenolic substances that were not needed as monomers for lignin biosynthesis. Apparently, a generally lower biosynthesis of phenolic substances was the consequence of the lower light intensity.

Generally, the biosynthesis of phenolic substances depends on the expression of the genes coding for the relevant enzymes, and can be influenced by phytohormones. Methyl jasmonate, a plant hormone, has been shown to increase the transcription of different enzymes of the phenylpropanoid pathway, like PAL, 4CL, and C4H, as well as the RA content, in cell suspension cultures of *Agastache rugosa*, another member of the Lamiaceae family (Kim et al., 2013). In *Arabidopsis*, the expression of TAT, another enzyme responsible for the formation of a precursor of RA, was induced by the application of methyl jasmonate (Lopukhina et al., 2001). As the sensitivity of a plant to jasmonates is reduced by a low R:FR (Moreno et al., 2009), most probably the reduction in phenolic substances under the strong shading has been the result of changes in the expression of the relevant genes coding for enzymes of phenylpropanoid biosynthesis, presumably by a light-quality dependent change of jasmonate signaling.

Apart from the light intensity, the spectral composition of the light might play a role regarding the accumulation of phenolic substances, which might explain some differences between different studies. In an investigation of Grbic et al. (2016) with *Perilla frutescens*, two differently colored plastic films were used to modify light intensity and spectral composition of the light that the plants received. Although one of the two films had a higher light transmission, reducing PAR only by about 22%, the TPC values were significantly reduced by 55.8%, whereas the film which reduced PAR more strongly, by about 46%, only led to a non-significant reduction of TPC by 36.5% (Grbic et al., 2016). In this case the spectral composition of the light had a higher influence on TPC than the light intensity. In another investigation, *Ocimum basilicum* plants were cultivated under red, white and blue light with the same PPFD of 100 µmol/m^2/s (Shiga et al., 2009). Red and white light induced RA accumulation more strongly than blue light (Shiga et al., 2009).

These findings indicate the importance of the spectral composition of the light. Especially ratios, like R:FR, seem to be important. R:FR is known to be connected to the shade avoidance syndrome and phytochrome signaling. Green nets, as they were employed in our investigation, absorb red light and therefore reduce R:FR (Oren-Shamir et al., 2001). Possibly, this shift in R:FR has induced a decreased accumulation of phenolic substances in our experiment with lemon balm plants.

However, in an investigation with lemon balm plants cultivated under different light sources (LED lights and fluorescent lights) with quite similar PPFD, but different spectral compositions and different R:FR, TPC was higher under the LED lights with a lower R:FR (Frąszczak et al., 2015). Obviously, also other parts of the light spectrum play a role. Interestingly, although TPC was significantly higher, the concentration of some single phenolic acids, like gallic, caffeic, and p-coumaric acid, were significantly reduced. It seems as if the biosynthesis of these phenolic substances has been influenced more strongly than some other phenolic substances, which were not further elucidated. It was also interesting that basil plants under the same treatment did not show a change in TPC, indicating a possible species-specific reaction on changes in the spectral composition of the light (Frąszczak et al., 2015). Thus it can be concluded that some need for research remains to further elucidate this topic.

It can be concluded that lemon balm plants can tolerate a certain degree of shading without compromising the quality in terms of phenolic substances and antioxidant capacity. Only a strong shading of the plants had a clear negative effect on these parameters of lemon balm plants. Thus, plant protection nets with only a moderate light reduction may be used, whereas the use of nets with a stronger light reduction cannot be recommended for the cultivation of high-quality lemon balm under German field conditions.

6 Summary

Lemon balm (*Melissa officinalis* L.) is an important medicinal and aromatic plant belonging to the Lamiaceae family. The lemon-like fragrance is caused by its content of essential oil (EO). Besides EO, lemon balm also contains several phenolic substances, with rosmarinic acid (RA) being the most important one. A minimal RA content of 1% is required by the European Pharmacopoeia.

The use of various kinds of plant protection nets is increasingly popular, and they have been proposed for the prevention of leafhopper infestations in the cultivation of medicinal and aromatic plants, like lemon balm, as well. However, they also lead to a light reduction. Therefore, this work wanted to determine how yield and quality of lemon balm plants are influenced by the use of nets with two different degrees of shading, and whether different genotypes and harvest stages react differently on the light reduction.

Thus, two field experiments have been conducted at two experimental sites with differing soil and climate conditions. A moderate shading with a light reduction of about 10–15% was tested at the experimental site in Gross-Gerau (GG) with three genotypes, and a stronger shading with a light reduction of about 50% in Rauischholzhausen (RH) with two genotypes. The perennial plants were harvested for three years, in two cuts per year, at three different harvest stages within each cut.

Regarding the plant parameters, no significant influence of the moderate light reduction was observed. The stronger light reduction, however, led in many cases to increased plant height, LAI, and SPAD values, as well as a decreased number of shoots per plant. The late onset of some of these reactions might indicate that the timing of the shade application is important. These changes are typical signs of the shade avoidance syndrome of the plants. However, the plants did not show reduced SPAD values as a sign of etiolation, or a reduction of plant height or LAI. Thus, the reactions on the strong shading do not seem to be detrimental, but rather show an adaptation of the plants after a longer period of light reduction, and thus their ability to cope with a certain degree of shading. Differences in the plant parameters between the tested genotypes did not show a reproducible, clear pattern. Differences were greater between the two experimental sites, indicating a greater importance of the environmental conditions than the genotypes for these parameters.

The yield level in RH was on average higher than in GG. No effect of the moderate shading in GG on biomass or leaf yield, dry matter (DM) content of the leaves, or leaf:stem-ratio was observed. The stronger light reduction in RH, however, decreased the

DM content of the leaves, and, partly, biomass and leaf yield. This effect was observed mainly for the freshly shaded plants, but scarcely for the plants grown under the shade for a longer period of time. No reproducible pattern regarding a genotype effect could be observed, indicating a general suitability of the chosen genotypes for an appropriate yield level in the regions were the field trials took place.

The measured quality parameters were not reproducibly affected by the moderate light reduction in GG. EO content was also not reproducibly influenced by the stronger light reduction. EO content was markedly higher in the second cut, and generally highest in the genotype 'Lemona'. There was no reproducible pattern regarding the harvest stage in GG, whereas in RH, EO contents were (at least in tendency) increasing over time for the first cut, and decreasing for the second cut. Thus, the optimal harvest stage regarding EO content seems to depend on the environmental conditions of the specific cultivation site. Total phenolic content (TPC), antioxidant capacity (ORAC), and RA content were significantly decreased under the stronger light reduction in RH. There was no reproducible genotype effect on these parameters. In GG, the quality parameters TPC, ORAC, and RA content were either higher at early harvest stage, compared to late harvest stage, or not significantly different. In RH, however, this pattern was true only for the second cut. For the first cut, TPC and ORAC values were either not significantly different between early and late harvest stage, or even showing an increasing pattern.

It can be concluded that the tested genotypes are generally suitable for the production of high-quality lemon balm leaves, meeting the requirements of the European Pharmacopoeia, under German climate conditions. An optimal harvest stage seems to be dependent on the actual environmental conditions of a specific cultivation site. A stronger light reduction induced some changes in plant morphology, partly reduced the yield parameters, and had a negative effect on the content of phenolic compounds. A moderate shade application seems to be well tolerated by the lemon balm plants. Thus, the use of plant protection nets with only moderate light reductions may be used in the cultivation of lemon balm plants in Germany.

7 Zusammenfassung

Die Zitronenmelisse (*Melissa officinalis* L.) ist eine wichtige Arznei- und Gewürzpflanze aus der Familie der Lamiaceae. Der zitronenartige Duft dieser Pflanze wird durch ihren Gehalt an ätherischem Öl hervorgerufen. Neben dem ätherischen Öl sind in der Zitronenmelisse auch verschiedene phenolische Verbindungen, darunter vor allem Rosmarinsäure (RA), enthalten. Im Europäischen Arzneibuch ist ein Mindestgehalt an RA von 1 % vorgeschrieben.

Der Einsatz verschiedenartiger Pflanzenschutznetze gewinnt zunehmend an Bedeutung. Ihr Einsatz wurde auch für den Anbau von Arznei- und Gewürzpflanzen, wie beispielsweise Zitronenmelisse, zum Schutz vor Zikadenbefall empfohlen. Allerdings führt ihr Einsatz auch zu einer Lichtreduktion. Daher sollte diese Arbeit den Einfluss von Netzen mit zwei verschiedenen Beschattungsintensitäten auf Ertrag und Qualität der Zitronenmelisse bei verschiedenen Genotypen bzw. Erntezeitpunkten untersuchen. Dafür wurden zwei Feldversuche, an zwei Versuchsstandorten mit unterschiedlichen Boden- und Klimabedingungen, durchgeführt. Eine moderate Lichtreduktion von ca. 10–15 % wurde am Versuchsstandort Groß-Gerau (GG) mit drei Genotypen untersucht. In Rauischholzhausen (RH) wurde dagegen eine stärkere Lichtreduktion von ca. 50 % mit zwei Genotypen getestet. Die Zitronenmelisse wurde drei Jahre mit zwei Aufwüchsen pro Jahr kultiviert und an drei Ernteterminen pro Aufwuchs untersucht.

Es wurde festgestellt, dass die Pflanzenparameter durch die moderate Lichtreduktion nicht signifikant beeinflusst wurden. Die stärkere Lichtreduktion hingegen führte in vielen Fällen zu einer Zunahme von Pflanzenhöhe, LAI- und SPAD-Werten, sowie zu einer Abnahme der Triebzahl. Das späte Auftreten einiger dieser Reaktionen deutet auf die Bedeutung des Zeitpunktes der Beschattung hin. Diese Veränderungen sind typische Zeichen einer Schattenvermeidungsreaktion der Pflanzen. Allerdings zeigten die Pflanzen keine Abnahme der SPAD-Werte (als Zeichen einer Etiolierung), der Pflanzenhöhe oder des LAI. Die Reaktionen deuten daher weniger auf eine Schädigung, sondern eher auf eine gewisse Schattentoleranz der Pflanzen durch eine Adaptation unter längerer Beschattung hin. Unterschiede der Pflanzenparameter zwischen den untersuchten Genotypen zeigten kein reproduzierbares Muster. Die Unterschiede zwischen den Versuchsstandorten waren größer, was auf eine stärkere Bedeutung der Umweltbedingungen im Vergleich zu den Genotypen hinweist.

Das Ertragsniveau der Zitronenmelisse war in RH höher als in GG. Die moderate Beschattung in GG hatte keinen signifikanten Einfluss auf Biomasse- bzw. Blattertrag, Blatt-Trockenmassegehalt (DM) sowie das Blatt-Stängel-Verhältnis. Die stärkere

Beschattung in RH dagegen führte zum verminderten DM sowie teilweise, vor allem bei frisch beschatteten Pflanzen, zur Reduktion der Biomasse- und Blatterträge. Eine abweichende Reaktion der Genotypen wurde nicht beobachtet.

Die gemessenen Qualitätsparameter wurden nicht reproduzierbar durch die moderate Lichtreduktion in GG beeinflusst. In RH hatte auch die stärkere Beschattung keinen gesicherten Einfluss auf den Gehalt an ätherischem Öl. Der Gehalt an ätherischem Öl war im zweiten Aufwuchs deutlich höher, und generell am höchsten beim Genotyp 'Lemona'. In GG zeigte sich kein reproduzierbares Muster bezüglich des Erntezeitpunktes, während der Ätherisch-Öl-Gehalt in RH für den ersten Aufwuchs über die Zeit tendenziell anstieg und für den zweiten Aufwuchs abnahm. Daher scheint der optimale Erntezeitpunkt von den Umweltbedingungen des jeweiligen Standortes abhängig zu sein. Gesamtphenol-Gehalt (TPC), antioxidative Kapazität (ORAC) und RA-Gehalt waren unter der stärkeren Lichtreduktion in RH signifikant reduziert. Ein reproduzierbarer Effekt des Genotyps auf diese Parameter wurde nicht beobachtet. In GG waren die Qualitätsparameter TPC, ORAC und RA-Gehalt entweder beim frühesten Erntezeitpunkt höher als beim späten Erntezeitpunkt oder nicht signifikant unterschiedlich. In RH zeigte sich dieses Muster hingegen nur beim zweiten Aufwuchs. Beim ersten Aufwuchs unterschieden sich TPC und ORAC entweder nicht zwischen frühem und spätem Erntezeitpunkt, oder zeigten sogar einen Anstieg.

Es lässt sich schlussfolgern, dass die untersuchten Genotypen unter den Klimabedingungen in Deutschland generell für die Produktion qualitativ hochwertiger Zitronenmelisse in Arzneibuchqualität geeignet sind. Ein optimaler Erntezeitpunkt scheint von den jeweiligen Umweltbedingungen des Standortes abhängig zu sein. Eine stärkere Beschattung beeinflusste in gewissem Maß die Pflanzenmorphologie, reduzierte teilweise den Ertrag, und hatte einen negativen Einfluss auf den Gehalt an phenolischen Verbindungen. Die moderate Beschattung der Pflanzen wird von der Zitronenmelisse aber gut toleriert. Daraus wird geschlussfolgert, dass der Anbau von Zitronenmelisse bei Verwendung von Pflanzenschutznetzen mit moderater Lichtreduktion bei vergleichbaren Standortbedingungen möglich ist.

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Appendix

Scatterplots of correlations

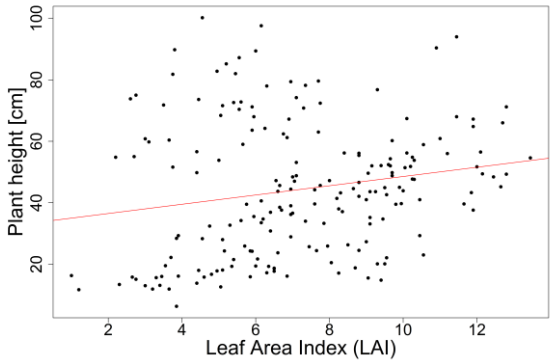

Fig. A 1: Correlation of Leaf Area Index (LAI) and plant height of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = 0.19, p < 0.01.

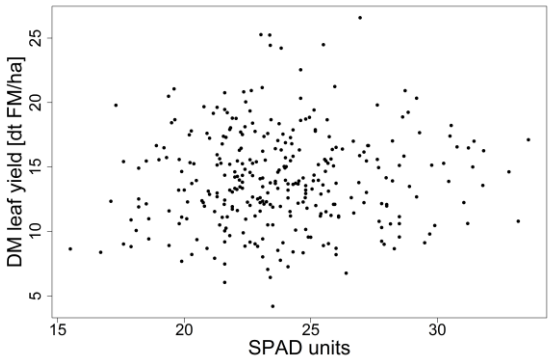

Fig. A 2: Correlation of SPAD values and DM leaf yield of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = 0.06, p = 0.26.

Fig. A 3: Correlation of SPAD values and essential oil content of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = 0.40, p < 0.001.

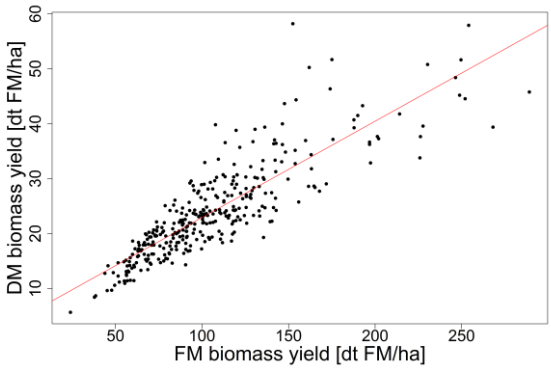

Fig. A 4: Correlation of FM and DM biomass yield of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = 0.86, p < 0.001.

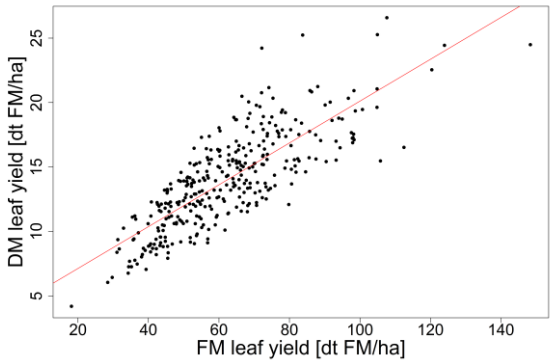

Fig. A 5: Correlation of FM and DM leaf yield of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = 0.80, p < 0.001.

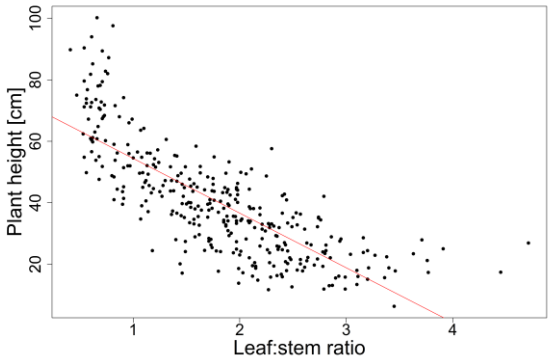

Fig. A 6: Correlation of leaf:stem ratio and plant height of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = -0.79, p < 0.001.

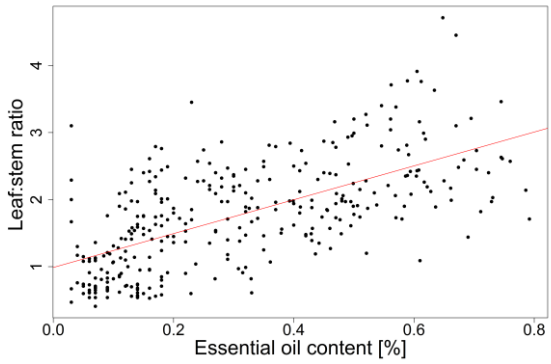

Fig. A 7: Correlation of essential oil content and leaf:stem ratio of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = 0.64, p < 0.001.

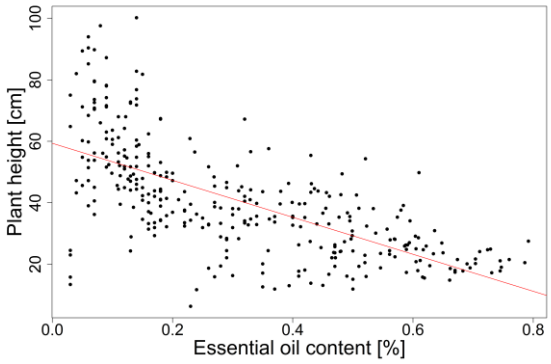

Fig. A 8: Correlation of essential oil content and plant height of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = -0.67, p < 0.001.

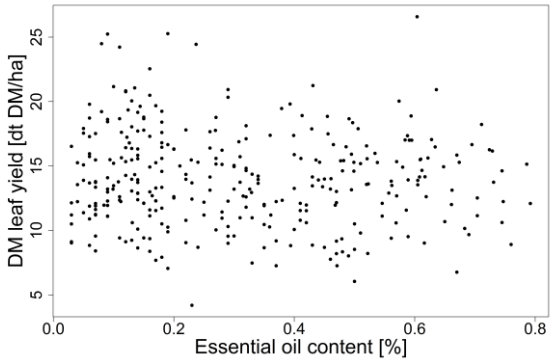

Fig. A 9: Correlation of essential oil content and DM leaf yield of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = -0.08, p = 0.15.

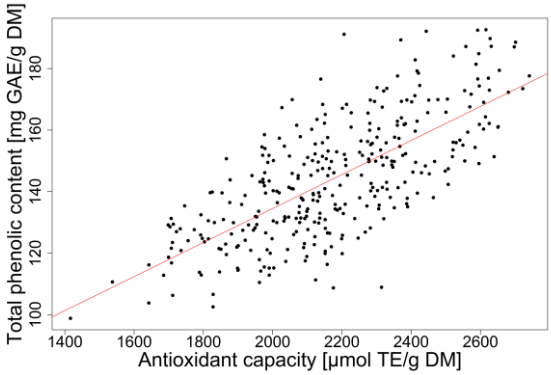

Fig. A 10: Correlation of antioxidant capacity and total phenolic content of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = 0.71, p < 0.001.

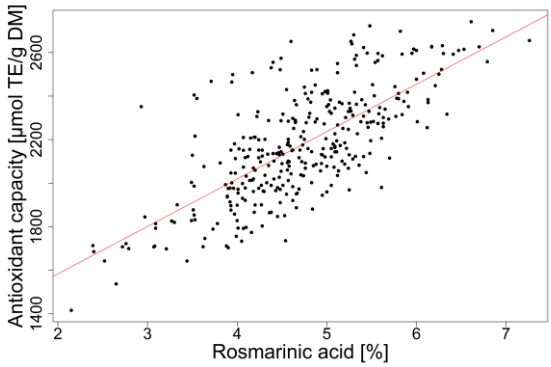

Fig. A 11: Correlation of rosmarinic acid and antioxidant capacity of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = 0.72, p < 0.001.

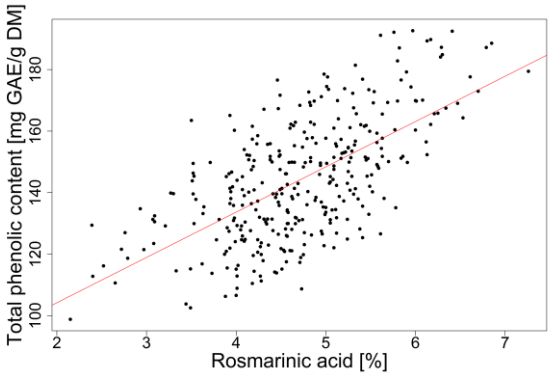

Fig. A 12: Correlation of rosmarinic acid and total phenolic content of lemon balm in Gross-Gerau, 2013–2015. Pearson's r = 0.63, p < 0.001.

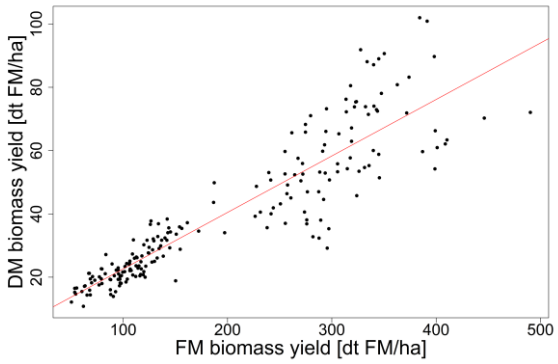

Fig. A 13: Correlation of FM and DM biomass yield of lemon balm in Rauischholzhausen, 2013–2015. Pearson's r = 0.90, p < 0.001.

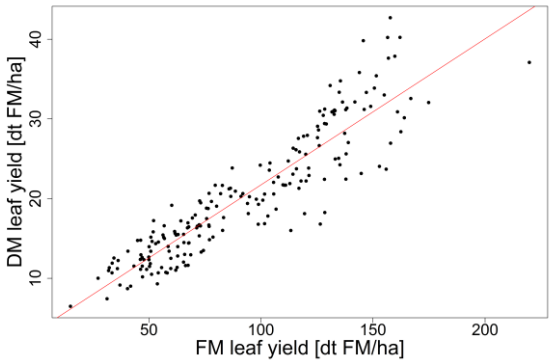

Fig. A 14: Correlation of FM and DM leaf yield of lemon balm in Rauischholzhausen, 2013–2015. Pearson's r = 0.91, p < 0.001.

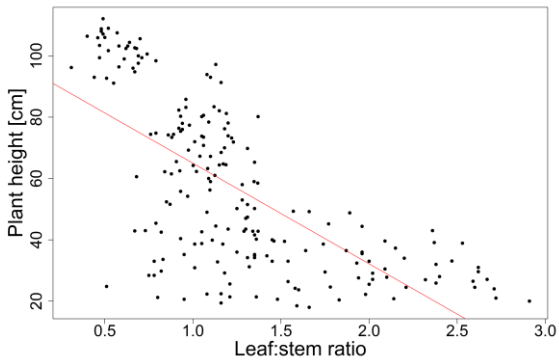

Fig. A 15: Correlation of leaf:stem ratio and plant height of lemon balm in Rauischholzhausen, 2013–2015.
Pearson's r = -0.68, p < 0.001.

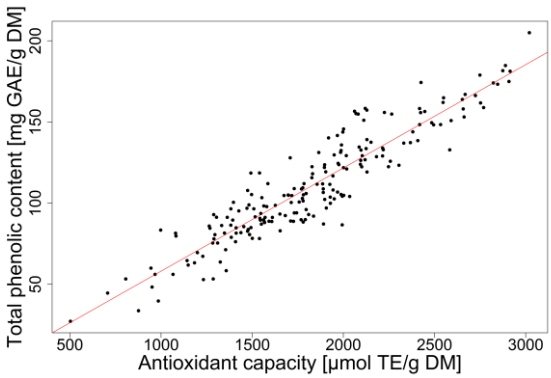

Fig. A 16: Correlation of antioxidant capacity and total phenolic content of lemon balm in Rauischholzhausen, 2013–2015.
Pearson's r = 0.93, p < 0.001.

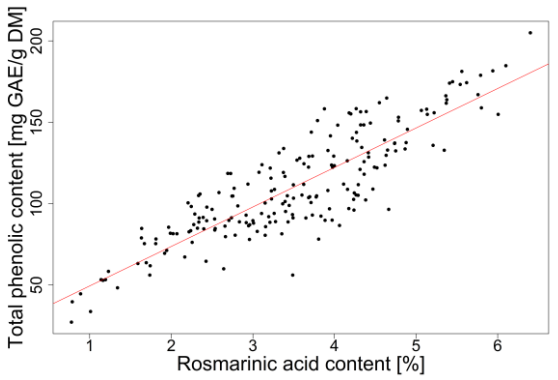

Fig. A 17: Correlation of RA content and total phenolic content of lemon balm in Rauischholzhausen, 2013–2015.
Pearson's r = 0.87, p < 0.001.

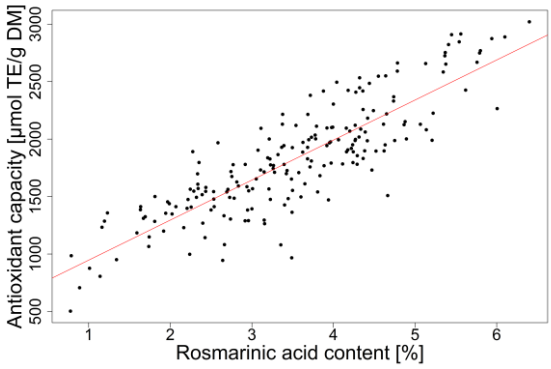

Fig. A 18: Correlation of RA content and antioxidant capacity of lemon balm in Rauischholzhausen, 2013–2015.
Pearson's r = 0.85, p < 0.001.

Tab. A 1: Plant height [cm], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Plant height [cm]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			40.85 a	1.95	0.761	25.79 b	0.74	0.002	61.99 a	3.58	0.337	27.65 a	3.05	0.896	46.13 a	3.25	0.477	26.92 a	3.02	0.302
Shading			41.73 a			30.99 a			67.28 a			28.24 a			49.62 a			31.74 a		
	'Aufr. Typ'		38.42 b	1.62	< 0,001	27.53 b	0.89	< 0,001	68.36 a	2.86	0.017	27.19 b	2.39	< 0,001	46.61 a	3.49	0.882	24.49 b	3.09	< 0,001
	'Lemona'		37.31 b			22.32 c			61.67 b			18.13 c			48.16 a			18.88 b		
	'NLC'		48.14 a			35.32 a			63.87 ab			38.52 a			48.85 a			44.63 a		
		early	38.29 b	1.62	0.003	27.63 a	0.89	0.449	49.71 c	2.86	< 0,001	28.00 b	2.39	< 0,001	n.d.			n.d.		
		medium	42.25 a			28.32 a			68.46 b			32.80 a								
		late	43.33 a			29.23 a			75.74 a			23.05 c								
Natural light	'Aufr. Typ'		37.93 c	2.29	0.828	26.38 bc	1.27	0.022	63.53 ab	4.04	0.264	27.09 cdf	3.38	0.673	43.08 a	4.93	0.582	22.10 c	4.38	0.768
	'Lemona'		37.35 c			20.30 d			60.10 ab			18.52 eg			49.18 a			17.88 c		
	'NLC'		47.28 ab			30.69 b			62.33 ab			37.35 ab			46.15 a			40.78 ab		
Shading	'Aufr. Typ'		38.91 bc			28.69 bc			73.18 a			27.28 bde			50.15 a			26.88 bc		
	'Lemona'		37.28 bc			24.34 cd			63.25 b			17.74 fg			47.15 a			19.88 c		
	'NLC'		49.01 a			39.95 a			65.42 ab			39.69 ac			51.55 a			48.48 a		
Natural light		early	37.59 a	2.29	0.872	25.19 c	1.27	0.933	47.48 c	4.04	0.705	28.54 ab	3.38	0.128	n.d.			n.d.		
		medium	42.25 a			25.82 bc			66.48 ab			30.38 ab								
		late	42.71 a			26.36 bc			72.00 a			24.03 ab								
Shading		early	38.99 a			30.07 abc			51.93 bc			27.45 b								
		medium	42.24 a			30.82 ab			70.43 a			35.21 a								
		late	43.96 a			32.10 a			79.48 a			22.06 b								
	'Aufr. Typ'	early	36.98 cd	2.19	0.365	27.66 bcde	1.54	0.017	55.55 cd	3.65	0.414	26.30 bc	2.98	0.100	n.d.			n.d.		
		medium	40.31 bcd			29.00 bcd			71.90 ab			36.03 ab								
		late	37.96 cd			25.94 cde			77.63 a			19.24 cd								
	'Lemona'	early	33.66 d			23.25 de			48.23 d			18.99 cd								
		medium	37.86 cd			21.46 e			64.22 bc			20.93 cd								
		late	40.41 bcd			22.25 de			72.57 ab			14.48 d								
	'NLC'	early	44.24 abc			31.98 bc			45.35 d			38.70 a								
		medium	48.56 ab			34.49 ab			69.25 ab			41.44 a								
		late	51.63 a			39.50 a			77.02 ab			35.43 ab								
Natural light	'Aufr. Typ'	early	36.73 cd	3.10	0.197	27.20 cd	2.18	0.366	51.55 cegh	5.17	0.146	26.10 abcdef	4.22	0.131	n.d.			n.d.		
		medium	40.20 abcd			28.23 cd			65.90 abcdef			36.00 abcdef								
		late	36.85 cd			23.70 d			73.15 abdf			19.18 hklm								
	'Lemona'	early	32.48 d			20.40 d			45.55 eh			22.38 deghjk								
		medium	40.88 abcd			21.13 d			68.15 abcdfg			19.28 hklm								
		late	38.70 cd			19.38 d			66.60 abcdfg			13.90 klm								
	'NLC'	early	43.58 abcd			27.98 cd			45.35 eh			37.15 abcdef								
		medium	45.68 abcd			28.10 cd			65.40 abcdef			35.88 abcdef								
		late	52.58 ab			36.00 abc			76.25 abd			39.02 abcfi								
Shading	'Aufr. Typ'	early	37.23 bd			28.12 cd			59.55 bcdefg			26.50 bcdefg								
		medium	40.42 abcd			29.78 bcd			77.90 abc			36.05 abcdef								
		late	39.08 abcd			28.18 cd			82.10 a			19.30 fgijlm								
	'Lemona'	early	34.85 d			26.10 cd			50.90 dfgh			15.60 ijm								
		medium	34.85 d			21.80 d			60.30 bcdefg			22.58 cefgijk								
		late	42.13 abcd			25.13 cd			78.55 abc			15.05 jm								
	'NLC'	early	44.90 abcd			35.98 abc			45.35 fgh			40.25 abdh								
		medium	51.45 ac			40.88 ab			73.10 abce			47.00 a								
		late	50.68 ac			43.00 a			77.80 abc			31.83 abcdef								

Tab. A 2: Leaf Area Index (LAI), Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

LAI			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			n.d.			6.47 a	0.32	0.117	6.85 a	1.09	0.707	10.39 a	0.79	0.383	8.44 a	0.84	0.226
Shading									7.29 a			6.24 a			9.33 a			6.83 a		
	'Aufr. Typ'								7.90 a	0.33	< 0,001	6.69 ab	0.81	0.003	10.04 ab	0.65	0.031	6.86 ab	0.90	0.039
	'Lemona'								6.48 b			5.66 b			10.64 a			6.44 b		
	'NLC'								6.27 b			7.28 a			8.90 b			9.59 a		
		early							8.96 a	0.33	< 0,001	6.39 a	0.81	0.801	n.d.			n.d.		
		medium							7.12 b			6.56 a								
		late							4.56 c			6.68 a								
Natural light	'Aufr. Typ'								7.12 ab	0.47	0.174	6.77 a	1.15	0.670	10.75 a	0.92	0.324	7.79 a	1.27	0.943
	'Lemona'								6.51 b			6.04 a			11.50 a			7.01 a		
	'NLC'								5.78 b			7.73 a			8.91 a			10.51 a		
Shading	'Aufr. Typ'								8.68 a			6.61 a			9.34 a			5.94 a		
	'Lemona'								6.44 b			5.28 a			9.78 a			5.86 a		
	'NLC'								6.75 b			6.82 a			8.89 a			8.68 a		
Natural light		early							8.22 ab	0.47	0.355	7.09 a	1.15	0.258	n.d.			n.d.		
		medium							7.00 b			6.52 a								
		late							4.20 c			6.93 a								
Shading		early							9.70 a			5.69 a								
		medium							7.24 b			6.59 a								
		late							4.93 c			6.44 a								
		early							10.47 a	0.55	0.005	6.03 a	0.92	0.139	n.d.			n.d.		
	'Aufr. Typ'	medium							8.43 ab			7.47 a								
		late							4.81 de			6.58 a								
	'Lemona'	early							8.89 ab			5.70 a								
		medium							7.06 bcd			5.77 a								
		late							3.49 e			5.52 a								
	'NLC'	early							7.53 bc			7.44 a								
		medium							5.88 cde			6.43 a								
		late							5.40 cde			7.96 a								
Natural light	'Aufr. Typ'	early							9.47 abc	0.77	0.124	6.34 a	1.31	0.549	n.d.			n.d.		
		medium							7.79 abcdef			7.40 a								
		late							4.11 fgh			6.56 a								
	'Lemona'	early							8.14 abcde			6.76 a								
		medium							8.29 abcde			6.03 a								
		late							3.11 h			5.34 a								
	'NLC'	early							7.04 bcdefg			8.16 a								
		medium							4.93 efgh			6.14 a								
		late							5.39 defgh			8.89 a								
Shading	'Aufr. Typ'	early							11.46 a			5.71 a								
		medium							9.08 abcd			7.54 a								
		late							5.50 cdefgh			6.59 a								
	'Lemona'	early							9.64 ab			4.64 a								
		medium							5.83 bcdefg			5.51 a								
		late							3.86 gh			5.70 a								
	'NLC'	early							8.01 abcdef			6.71 a								
		medium							6.83 bcdefg			6.72 a								
		late							5.41 defgh			7.02 a								

Tab. A 3: SPAD values, Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

SPAD			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			22.60 a	0.45	0.214	27.97 a	0.37	0.103	22.24 a	0.58	0.337	24.16 a	0.53	0.592	21.98 a	0.36	0.224	22.28 a	0.70	0.903
Shading			23.49 a			27.10 a			23.09 a			24.59 a			22.62 a			22.16 a		
	'Aufr. Typ'		23.29 a	0.36	0.093	27.89 b	0.45	< 0,001	22.63 a	0.51	0.129	23.90 ab	0.62	0.010	20.80 b	0.44	< 0,001	21.38 b	0.82	0.003
	'Lemona'		23.15 a			29.76 a			22.13 a			25.90 a			23.81 a			25.09 a		
	'NLC'		22.70 a			24.96 c			23.24 a			23.33 b			22.29 ab			20.20 b		
		early	25.17 a	0.36	< 0,001	n.d.	0.37	0.483	24.58 a	0.51	< 0,001	23.25 b	0.62	< 0,001	n.d.			n.d.		
		medium	22.01 b			27.72 a			22.53 b			23.03 b								
		late	21.95 b			27.35 a			20.89 c			26.85 a								
Natural light	'Aufr. Typ'		22.82 a	0.50	0.237	28.81 ab	0.64	0.426	21.98 a	0.72	0.761	24.06 a	0.88	0.755	20.85 b	0.62	0.584	21.73 ab	1.16	0.708
	'Lemona'		22.96 a			29.94 a			21.77 a			25.53 a			23.38 ab			24.60 ab		
	'NLC'		22.03 a			25.17 c			22.97 a			22.90 a			21.73 ab			20.53 ab		
Shading	'Aufr. Typ'		23.76 a			26.97 bc			23.28 a			23.75 a			20.75 b			21.03 ab		
	'Lemona'		23.34 a			29.58 ab			22.49 a			26.27 a			24.25 a			25.58 a		
	'NLC'		23.36 a			24.75 c			23.51 a			23.75 a			22.85 ab			19.88 b		
Natural light		early	24.89 ab	0.50	0.517	n.d.	0.52	0.484	24.14 ab	0.72	0.492	23.32 b	0.88	0.565	n.d.			n.d.		
		medium	21.56 c			28.34 a			22.43 ab			23.06 b								
		late	21.35 c			27.60 a			20.15 c			26.11 ab								
Shading		early	25.45 a			n.d.			25.02 a			23.18 b								
		medium	22.46 bc			27.10 a			22.63 bc			23.00 b								
		late	22.55 bc			27.10 a			21.63 bc			27.59 a								
	'Aufr. Typ'	early	25.93 a	0.45	0.021	n.d.	0.64	0.676	24.82 ab	0.74	0.785	22.68 bcd	1.05	0.013	n.d.			n.d.		
		medium	21.78 c			27.94 a			22.21 bcd			23.33 abcd								
		late	22.15 c			27.84 a			20.86 cd			25.71 abc								
	'Lemona'	early	25.23 ab			n.d.			23.52 abc			26.65 ab								
		medium	22.63 c			30.27 a			22.46 abcd			24.21 abcd								
		late	21.59 c			29.25 a			20.41 d			26.83 ab								
	'NLC'	early	24.35 b			n.d.			25.39 a			20.41 d								
		medium	21.62 c			24.96 b			22.93 abcd			21.55 cd								
		late	22.12 c			24.97 b			21.40 cd			28.01 a								
Natural light	'Aufr. Typ'	early	25.74 abce	0.64	0.944	n.d.	0.90	0.303	24.50 ab	1.05	0.845	22.78 ab	1.49	0.336	n.d.			n.d.		
		medium	21.27 hi			28.76 abc			21.65 abc			24.93 ab								
		late	21.45 ghi			28.87 abc			19.80 bc			24.48 ab								
	'Lemona'	early	25.20 abcef			n.d.			23.05 abc			27.43 ab								
		medium	22.54 dghi			31.21 a			22.80 abc			23.35 ab								
		late	21.14 i			28.67 abc			19.47 c			25.80 ab								
	'NLC'	early	23.73 abcdefgh			n.d.			24.87 a			19.75 b								
		medium	20.88 i			25.06 bc			22.85 abc			20.90 ab								
		late	21.47 ghi			25.28 bc			21.17 abc			28.05 a								
Shading	'Aufr. Typ'	early	26.12 a			n.d.			25.15 ab			22.58 ab								
		medium	22.30 efhi			27.12 abc			22.78 abc			21.73 ab								
		late	22.86 bcdefghi			26.82 abc			21.93 abc			26.95 ab								
	'Lemona'	early	25.27 abd			n.d.			24.00 abc			25.88 ab								
		medium	22.72 cefghi			29.33 ab			22.13 abc			25.08 ab								
		late	22.03 fhi			29.84 a			21.35 abc			27.85 a								
	'NLC'	early	24.96 abcdg			n.d.			25.90 a			21.08 ab								
		medium	22.35 efhi			24.86 c			23.00 abc			22.20 ab								
		late	22.77 bcdefghi			24.65 c			21.63 abc			27.98 a								

Tab. A 4: Shoots per plant, Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Shoots/plant			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			33.0 a	1.12	0.860	38.8 a	0.79	0.567	58.8 a	4.48	0.512	77.7 a	5.20	0.298	73.0 a	9.45	0.993	78.2 a	5.47	0.076
Shading			32.7 a			39.4 a			54.4 a			70.1 a			73.1 a			61.7 a		
	'Aufr. Typ'		29.6 b	1.34	0.009	35.4 b	0.97	< 0,001	60.8 a	4.22	< 0,001	80.5 a	6.26	0.001	76.9 a	11.57	0.208	76.3 a	6.05	0.196
	'Lemona'		35.4 a			41.1 a			69.0 a			86.9 a			85.9 a			72.4 a		
	'NLC'		33.6 ab			40.8 a			40.0 b			54.3 b			56.3 a			61.2 a		
		early	35.0 a	1.34	0.156	29.9 b	0.97	< 0,001	58.9 a	4.22	0.610	88.7 a	6.43	< 0,001	n.d.			n.d.		
		medium	32.0 a			42.1 a			56.9 a			79.8 a								
		late	31.7 a			45.2 a			54.1 a			53.2 b								
Natural light	'Aufr. Typ'		29.4 a	1.89	0.724	34.5 b	1.37	0.791	60.6 abc	5.97	0.444	78.5 ab	8.85	0.516	82.4 a	16.37	0.418	89.0 a	8.56	0.144
	'Lemona'		36.4 a			40.9 a			74.7 a			93.1 a			73.0 a			70.7 ab		
	'NLC'		33.3 a			40.9 a			41.2 bc			61.6 ab			63.4 a			75.0 ab		
Shading	'Aufr. Typ'		29.8 a			36.2 ab			61.1 ab			82.6 ab			71.4 a			63.5 ab		
	'Lemona'		34.4 a			41.3 a			63.3 ab			80.7 ab			98.7 a			74.0 ab		
	'NLC'		33.9 a			40.8 a			38.9 c			46.9 b			49.2 a			47.5 b		
Natural light		early	35.2 a	1.89	0.979	31.0 b	1.37	0.205	60.1 a	5.97	0.608	85.3 ab	9.33	0.312	n.d.			n.d.		
		medium	31.9 a			41.4 a			57.4 a			90.2 a								
		late	32.0 a			43.9 a			59.0 a			57.8 ab								
Shading		early	34.8 a			28.9 b			57.6 a			92.1 a								
		medium	32.0 a			42.9 a			56.5 a			69.4 ab								
		late	31.4 a			46.5 a			49.1 a			48.7 b								
	'Aufr. Typ'	early	31.5 a	2.29	0.617	27.5 e	1.68	0.174	66.9 ab	6.42	0.395	116.2 a	10.84	0.032	n.d.			n.d.		
		medium	30.0 a			39.8 bc			53.9 abc			70.8 abc								
		late	27.3 a			38.9 cd			61.6 abc			54.6 c								
	'Lemona'	early	37.3 a			31.0 e			66.2 ab			91.3 abc								
		medium	35.5 a			42.7 abc			74.9 a			109.2 ab								
		late	33.4 a			49.5 a			65.9 ab			60.2 bc								
	'NLC'	early	36.1 a			31.3 de			43.5 bc			58.6 bc								
		medium	30.4 a			44.0 abc			42.0 bc			59.2 c								
		late	34.3 a			47.2 ab			34.7 c			44.9 c								
Natural light	'Aufr. Typ'	early	30.5 a	3.24	0.617	28.3 ef	2.38	0.218	60.5 a	9.09	0.612	116.0 ab	15.33	0.160	n.d.			n.d.		
		medium	31.8 a			40.1 abcd			54.6 a			65.3 abc								
		late	25.8 a			35.2 bcde			66.5 a			54.1 bc								
	'Lemona'	early	39.7 a			31.8 cdef			74.4 a			77.9 abc								
		medium	34.4 a			42.8 abc			74.2 a			140.2 a								
		late	35.2 a			48.2 a			75.6 a			61.3 abc								
	'NLC'	early	35.4 a			33.0 cdef			45.4 a			62.0 abc								
		medium	29.5 a			41.3 abcd			43.3 a			64.9 abc								
		late	34.9 a			48.4 a			35.0 a			57.9 bc								
Shading	'Aufr. Typ'	early	32.5 a			26.8 f			73.3 a			116.3 ab								
		medium	28.3 a			39.4 abcd			53.2 a			76.4 abc								
		late	28.8 a			42.6 abc			56.6 a			55.2 bc								
	'Lemona'	early	34.9 a			30.3 def			58.0 a			104.7 abc								
		medium	36.6 a			42.6 abc			75.7 a			78.2 abc								
		late	31.7 a			50.9 a			56.3 a			59.1 bc								
	'NLC'	early	36.8 a			29.6 def			41.6 a			55.3 bc								
		medium	31.3 a			46.7 ab			40.7 a			53.5 bc								
		late	33.8 a			46.1 ab			34.4 a			31.8 c								

Tab. A 5: Biomass yield (FM) [dt FM/ha], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different (p < 0.05). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Biomass yield [dt FM/ha]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			89.96 a	7.38	0.801	87.28 a	3.84	0.293	137.73 a	11.45	0.506	88.95 a	10.99	0.706	155.09 a	14.45	0.519	109.48 a	11.36	0.889
Shading			92.71 a			93.55 a			149.19 a			82.79 a			141.64 a			107.13 a		
	'Aufr. Typ'		84.92 b	5.84	< 0,001	89.53 ab	4.43	0.020	178.76 a	9.86	< 0,001	84.35 b	8.56	< 0,001	153.81 a	17.70	0.127	94.58 b	10.69	0.050
	'Lemona'		83.20 b			82.02 b			129.73 b			71.93 b			172.19 a			102.99 ab		
	'NLC'		105.88 a			99.70 a			121.90 b			101.33 a			119.10 a			127.34 a		
		early	91.67 a	5.84	0.385	80.73 b	4.43	0.024	154.22 a	9.86	0.002	86.41 a	8.56	0.105	n.d.			n.d.		
		medium	94.32 a			93.20 ab			153.57 a			92.32 a								
		late	88.01 a			97.32 a			122.60 b			78.88 a								
Natural light	'Aufr. Typ'		79.81 bd	8.26	0.096	87.96 a	6.27	0.517	167.84 ab	13.95	0.468	87.06 abcd	12.10	0.888	152.30 a	25.04	0.568	96.98 a	15.12	0.976
	'Lemona'		87.57 abcd			81.33 a			130.67 abc			76.68 bd			194.53 a			102.71 a		
	'NLC'		102.50 ac			92.54 a			114.68 c			103.12 ac			118.44 a			128.74 a		
Shading	'Aufr. Typ'		90.03 cd			91.09 a			189.67 a			81.65 abcd			155.32 a			92.17 a		
	'Lemona'		78.84 cd			82.71 a			128.78 bc			67.19 cd			149.86 a			103.27 a		
	'NLC'		109.26 ab			106.85 a			129.11 bc			99.54 ab			119.75 a			125.94 a		
Natural light		early	86.02 a	8.26	0.247	75.76 a	6.27	0.636	136.57 ab	13.95	0.118	94.23 a	12.10	0.327	n.d.			n.d.		
		medium	96.14 a			93.40 a			153.77 ab			90.78 a								
		late	87.73 a			92.68 a			122.85 ab			81.85 a								
Shading		early	97.33 a			85.70 a			171.86 a			78.59 a								
		medium	92.51 a			92.99 a			153.36 ab			93.86 a								
		late	88.29 a			101.96 a			122.34 b			75.92 a								
	'Aufr. Typ'	early	90.26 abcd	7.40	0.096	79.84 ab	7.51	0.614	211.04 a	13.88	0.062	83.29 abcd	10.56	0.043	n.d.			n.d.		
		medium	90.76 abcd			99.22 ab			186.69 ab			104.71 ab								
		late	73.75 d			89.52 ab			138.53 bc			65.05 d								
	'Lemona'	early	84.95 bcd			73.61 b			129.76 c			78.57 abcd								
		medium	87.06 bcd			81.08 ab			146.19 bc			67.10 cd								
		late	77.61 cd			91.37 ab			113.23 c			70.12 bcd								
	'NLC'	early	99.82 abc			88.74 ab			121.85 c			97.38 abcd								
		medium	105.15 ab			99.29 ab			127.83 c			105.15 a								
		late	112.68 a			111.06 a			116.02 c			101.47 abc								
Natural light	'Aufr. Typ'	early	86.61 ab	10.46	0.143	76.08 a	10.63	0.619	178.46 ab	19.63	0.270	87.73 a	14.94	0.283	n.d.			n.d.		
		medium	85.72 ab			104.63 a			185.65 ab			108.28 a								
		late	67.10 b			83.18 a			139.42 ab			65.16 a								
	'Lemona'	early	76.98 ab			69.78 a			123.69 b			96.43 a								
		medium	102.91 ab			87.76 a			162.79 ab			65.78 a								
		late	82.82 ab			86.45 a			105.53 b			67.82 a								
	'NLC'	early	94.46 ab			81.41 a			107.56 b			98.53 a								
		medium	99.78 ab			87.81 a			112.89 b			98.26 a								
		late	113.27 a			108.41 a			123.61 b			112.56 a								
Shading	'Aufr. Typ'	early	93.90 ab			83.60 a			243.62 a			78.85 a								
		medium	95.80 ab			93.81 a			187.74 ab			101.14 a								
		late	80.39 ab			95.87 a			137.65 b			64.95 a								
	'Lemona'	early	92.92 ab			77.44 a			135.83 b			60.71 a								
		medium	71.21 ab			74.40 a			129.58 b			68.42 a								
		late	72.39 ab			96.29 a			120.93 b			72.42 a								
	'NLC'	early	105.17 ab			96.07 a			136.14 b			96.22 a								
		medium	110.52 ab			110.76 a			142.77 b			112.03 a								
		late	112.10 ab			113.72 a			108.43 b			90.37 a								

Tab. A 6: Biomass yield (DM) [dt DM/ha], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different (p < 0.05). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Biomass yield [dt DM/ha]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			23.59 a	1.51	0.916	21.37 a	0.86	0.567	30.96 a	2.07	0.717	18.10 a	1.59	0.861	31.90 a	2.28	0.362	23.47 a	2.76	0.437
Shading			23.83 a			22.06 a			32.07 a			17.69 a			28.89 a			20.22 a		
	'Aufr. Typ'		22.49 b	1.22	< 0,001	21.93 ab	1.05	0.039	39.51 a	1.84	< 0,001	17.82 b	1.30	< 0,001	31.61 a	2.80	0.152	17.09 b	2.98	0.020
	'Lemona'		21.36 b			19.67 b			27.72 b			15.06 c			33.69 a			19.24 ab		
	'NLC'		27.27 a			23.55 a			27.31 b			20.81 a			25.88 a			29.20 a		
		early	21.43 b	1.22	< 0,001	19.47 a	1.05	0.039	25.81 b	1.84	< 0,001	16.82 b	1.30	0.042	n.d.			n.d.		
		medium	24.01 a			22.84 a			33.77 a			19.56 a								
		late	25.69 a			22.84 a			34.95 a			17.32 ab								
Natural light	'Aufr. Typ'		21.56 bd	1.72	0.095	22.59 a	1.48	0.229	38.21 ab	2.60	0.648	18.11 abcd	1.83	0.857	32.46 a	3.95	0.526	18.18 a	4.22	0.719
	'Lemona'		22.51 abcd			19.76 a			28.17 c			15.52 bd			37.74 a			19.59 a		
	'NLC'		26.70 ac			21.75 a			26.50 c			20.67 ac			25.51 a			32.63 a		
Shading	'Aufr. Typ'		23.43 cd			21.26 a			40.81 a			17.52 abcd			30.77 a			16.00 a		
	'Lemona'		20.21 cd			19.58 a			27.28 bc			14.60 cd			29.65 a			18.89 a		
	'NLC'		27.84 ab			25.35 a			28.12 bc			20.96 ab			26.25 a			25.77 a		
Natural light		early	20.54 b	1.72	0.322	19.27 a	1.48	0.794	23.31 b	2.60	0.201	18.15 a	1.83	0.213	n.d.			n.d.		
		medium	24.66 ab			22.90 a			34.67 a			18.95 a								
		late	25.57 a			21.93 a			34.89 a			17.21 a								
Shading		early	22.31 ab			19.67 a			28.31 ab			15.50 a								
		medium	23.37 ab			22.77 a			32.87 ab			20.16 a								
		late	25.80 ab			23.76 a			35.02 ab			17.42 a								
	'Aufr. Typ'	early	21.94 bc	1.58	0.001	19.05 a	1.82	0.803	34.67 ab	2.66	0.690	16.26 bcd	1.71	0.023	n.d.			n.d.		
		medium	23.44 bc			24.52 a			41.47 a			21.84 ab								
		late	22.10 bc			22.20 a			42.39 a			15.35 cd								
	'Lemona'	early	19.95 c			17.95 a			21.46 c			15.03 cd								
		medium	22.11 bc			19.68 a			31.84 abc			13.75 d								
		late	22.03 bc			21.38 a			29.87 bc			16.40 bcd								
	'NLC'	early	22.39 bc			21.40 a			21.31 c			19.17 abcd								
		medium	26.49 b			24.31 a			28.01 bc			23.08 a								
		late	32.94 a			24.96 a			32.61 ab			20.19 abc								
Natural light	'Aufr. Typ'	early	21.84 cde	2.23	0.074	19.29 a	2.57	0.589	29.82 abcdef	3.76	0.312	17.07 ab	2.42	0.359	n.d.			n.d.		
		medium	22.70 cde			26.92 a			43.11 a			22.11 ab								
		late	20.13 de			21.57 a			41.69 abcd			15.16 ab								
	'Lemona'	early	18.46 de			18.39 a			20.77 efg			17.94 ab								
		medium	25.89 abcde			20.73 a			35.23 abcdef			13.49 ab								
		late	23.19 cde			20.15 a			28.50 abcdef			15.15 ab								
	'NLC'	early	21.33 cde			20.14 a			19.35 g			19.43 ab								
		medium	25.38 abcde			21.05 a			25.68 bcdefg			21.27 ab								
		late	33.39 ab			24.08 a			34.47 abcdef			21.31 ab								
Shading	'Aufr. Typ'	early	22.05 bde			18.81 a			39.51 abcdef			15.44 ab								
		medium	24.18 abcde			22.13 a			39.83 abce			21.58 ab								
		late	24.07 abcde			22.84 a			43.09 ab			15.55 ab								
	'Lemona'	early	21.44 bde			17.52 a			22.16 dfg			12.13 b								
		medium	18.32 e			18.63 a			28.44 abcdef			14.02 b								
		late	20.86 de			22.60 a			31.24 abcdef			17.65 ab								
	'NLC'	early	23.45 abcde			22.66 a			23.27 cdefg			18.91 ab								
		medium	27.61 abcd			27.57 a			30.35 abcdef			24.88 a								
		late	32.48 ac			25.84 a			30.75 abcdef			19.07 ab								

Tab. A 7: Leaf fresh matter yield [dt FM/ha], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different (p < 0.05). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Leaf yield [dt FM/ha]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			58.07 a	4.34	0.880	62.66 a	2.57	0.482	59.65 a	3.93	0.311	57.41 a	5.54	0.703	80.87 a	6.39	0.237	77.60 a	5.87	0.420
Shading			59.03 a			65.38 a			65.79 a			54.28 a			69.82 a			70.42 a		
	'Aufr. Typ'		57.28 ab	3.59	0.018	65.89 a	2.93	0.152	78.44 a	3.53	< 0,001	56.36 a	4.40	< 0,001	79.08 a	7.82	0.314	65.54 a	5.63	0.073
	'Lemona'		54.52 b			59.49 a			52.32 b			47.96 b			81.55 a			74.01 a		
	'NLC'		63.85 a			66.67 a			57.40 b			63.22 a			65.40 a			82.48 a		
		early	58.35 a	3.59	0.474	57.23 b	2.93	0.011	73.19 a	3.53	< 0,001	56.56 ab	4.40	0.019	n.d.			n.d.		
		medium	60.63 a			65.30 ab			63.41 b			60.51 a								
		late	56.66 a			69.52 a			51.56 c			50.47 b								
Natural light	'Aufr. Typ'		54.81 a	5.08	0.299	64.80 a	4.14	0.800	72.75 ab	5.00	0.303	57.27 ab	6.22	0.870	81.46 a	11.07	0.652	69.92 a	7.97	0.882
	'Lemona'		56.91 a			59.30 a			52.46 c			50.56 ab			93.06 a			75.69 a		
	'NLC'		62.47 a			63.86 a			53.74 c			64.41 ab			68.09 a			87.19 a		
Shading	'Aufr. Typ'		59.74 a			66.97 a			84.13 a			55.46 ab			76.70 a			61.16 a		
	'Lemona'		52.13 a			59.68 a			52.18 bc			45.35 b			70.05 a			72.33 a		
	'NLC'		65.22 a			69.48 a			61.06 bc			62.02 a			62.71 a			77.77 a		
Natural light		early	55.35 a	5.08	0.256	53.81 a	4.14	0.479	64.49 ab	5.00	0.045	61.16 a	6.22	0.261	n.d.			n.d.		
		medium	63.02 a			66.62 a			63.28 ab			59.41 a								
		late	55.83 a			67.54 a			51.17 b			51.67 a								
Shading		early	61.35 a			60.66 a			81.88 a			51.96 a								
		medium	58.25 a			63.98 a			63.54 b			61.61 a								
		late	57.50 a			71.50 a			51.94 b			49.27 a								
		early	59.13 a	4.84	0.195	58.98 a	4.94	0.370	101.33 a	5.18	0.003	55.13 ab	5.59	0.051	n.d.			n.d.		
	'Aufr. Typ'	medium	62.20 a			72.91 a			76.43 b			68.21 a								
		late	50.50 a			65.77 a			57.55 bc			45.75 b								
		early	55.91 a			54.31 a			55.30 bc			53.18 ab								
	'Lemona'	medium	56.36 a			57.16 a			56.15 bc			45.00 b								
		late	51.30 a			67.01 a			45.51 c			45.69 b								
		early	60.01 a			58.41 a			62.93 bc			61.37 ab								
	'NLC'	medium	63.35 a			65.83 a			57.65 bc			68.32 a								
		late	68.19 a			75.78 a			51.62 c			59.96 ab								
Natural light		early	57.98 a	6.84	0.354	55.13 a	6.98	0.585	87.21 ab	7.32	0.262	57.38 a	7.90	0.331	n.d.			n.d.		
	'Aufr. Typ'	medium	60.26 a			77.26 a			73.97 bc			68.98 a								
		late	46.20 a			62.03 a			57.05 bc			45.44 a								
		early	51.44 a			50.63 a			51.37 c			63.98 a								
	'Lemona'	medium	66.22 a			62.32 a			63.89 bc			43.36 a								
		late	53.07 a			64.96 a			42.13 c			44.35 a								
		early	56.63 a			55.68 a			54.89 bc			62.12 a								
	'NLC'	medium	62.58 a			60.26 a			51.98 c			65.90 a								
		late	68.20 a			75.64 a			54.34 bc			65.21 a								
Shading		early	60.28 a			62.84 a			115.45 a			52.88 a								
	'Aufr. Typ'	medium	64.14 a			68.55 a			78.88 bc			67.45 a								
		late	54.81 a			69.52 a			58.04 bc			46.06 a								
		early	60.38 a			58.00 a			59.23 bc			42.38 a								
	'Lemona'	medium	46.49 a			51.99 a			48.41 c			46.64 a								
		late	49.53 a			69.06 a			48.90 bc			47.04 a								
		early	63.39 a			61.14 a			70.97 bc			60.61 a								
	'NLC'	medium	64.12 a			71.39 a			63.33 bc			70.75 a								
		late	68.17 a			75.92 a			48.90 bc			54.71 a								

Tab. A 8: DM leaf yield [dt DM/ha], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Leaf yield [dt DM/ha]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			15.63 a	0.86	0.757	15.00 a	0.52	0.680	12.75 a	0.65	0.390	11.53 a	0.72	0.817	16.98 a	1.01	0.072	14.41 a	0.83	0.304
Shading			16.03 a			15.30 a			13.60 a			11.29 a			14.26 a			13.09 a		
	'Aufr. Typ'		15.62 ab	0.75	0.001	15.52 a	0.64	0.460	15.87 a	0.63	< 0,001	11.36 b	0.63	< 0,001	15.97 a	1.24	0.491	11.92 b	0.89	0.014
	'Lemona'		14.46 b			14.50 a			10.92 c			9.68 c			16.46 a			13.35 ab		
	'NLC'		17.41 a			15.43 a			12.73 b			13.19 a			14.42 a			15.96 a		
		early	14.47 b	0.75	0.009	13.28 b	0.64	0.002	12.70 a	0.63	0.544	10.96 ab	0.63	0.032	n.d.			n.d.		
		medium	16.23 ab			15.57 a			13.34 a			12.43 a								
		late	16.79 a			16.59 a			13.48 a			10.85 b								
Natural light	'Aufr. Typ'		14.94 ab	1.06	0.203	15.74 a	0.90	0.558	14.89 ab	0.89	0.338	11.37 abcd	0.89	0.795	16.66 a	1.75	0.620	12.76 ab	1.26	0.789
	'Lemona'		15.04 ab			14.52 a			11.06 c			10.06 bd			18.80 a			13.55 ab		
	'NLC'		16.91 ab			14.73 a			12.30 bc			13.17 ac			15.48 a			16.91 a		
Shading	'Aufr. Typ'		16.29 ab			15.29 a			16.85 a			11.36 abcd			15.29 a			11.08 b		
	'Lemona'		13.87 b			14.48 a			10.79 c			9.31 cd			14.12 a			13.16 ab		
	'NLC'		17.91 a			16.13 a			13.17 bc			13.20 ab			13.37 a			15.02 ab		
Natural light		early	13.83 a	1.06	0.219	12.85 b	0.90	0.758	11.25 a	0.89	0.063	11.63 a	0.89	0.283	n.d.			n.d.		
		medium	16.79 a			15.79 ab			13.58 a			12.05 a								
		late	16.27 a			16.35 ab			13.43 a			10.92 a								
Shading		early	15.11 a			13.72 ab			14.16 a			10.29 a								
		medium	15.66 a			15.35 ab			13.10 a			12.81 a								
		late	17.32 a			16.83 a			13.54 a			10.77 a								
		early	15.03 b	1.06	0.019	13.59 a	1.10	0.308	16.78 a	0.98	0.189	10.35 bc	0.91	0.011	n.d.			n.d.		
	'Aufr. Typ'	medium	16.71 ab			17.21 a			15.71 ab			13.59 ab								
		late	15.10 b			15.75 a			15.12 ab			10.15 bc								
	'Lemona'	early	13.69 b			13.20 a			9.52 c			10.30 bc								
		medium	14.92 b			13.67 a			11.72 bc			8.68 c								
		late	14.76 b			16.63 a			11.53 bc			10.07 bc								
	'NLC'	early	14.68 b			13.07 a			11.81 bc			12.23 abc								
		medium	17.04 ab			15.83 a			12.59 abc			15.02 a								
		late	20.52 a			17.39 a			13.80 ab			12.31 abc								
Natural light	'Aufr. Typ'	early	14.85 abc	1.50	0.292	12.93 a	1.56	0.341	14.25 ab	1.38	0.301	10.74 ab	1.28	0.295	n.d.			n.d.		
		medium	16.29 abc			18.72 a			15.56 ab			13.41 ab								
		late	13.67 abc			15.58 a			14.88 ab			9.96 ab								
	'Lemona'	early	12.72 c			12.93 a			8.89 b			12.15 ab								
		medium	17.37 abc			14.68 a			13.38 ab			8.45 b								
		late	15.03 abc			15.96 a			10.91 b			9.59 ab								
	'NLC'	early	13.92 abc			12.70 a			10.60 b			12.00 ab								
		medium	16.72 abc			13.97 a			11.80 b			14.30 ab								
		late	20.11 ab			17.51 a			14.50 ab			13.22 ab								
Shading	'Aufr. Typ'	early	15.22 abc			14.25 a			19.31 a			9.96 ab								
		medium	17.13 abc			15.71 a			15.87 ab			13.77 ab								
		late	16.53 abc			15.92 a			15.36 ab			10.34 ab								
	'Lemona'	early	14.66 abc			13.47 a			10.15 b			8.45 b								
		medium	12.47 bc			12.65 a			10.06 b			8.92 b								
		late	14.50 abc			17.31 a			12.15 b			10.55 ab								
	'NLC'	early	15.45 abc			13.44 a			13.03 ab			12.47 ab								
		medium	17.37 abc			17.70 a			13.38 ab			15.74 a								
		late	20.92 a			17.26 a			13.10 ab			11.41 ab								

Tab. A 9: DM content of the leaves [%], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Leaf DM [%]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			27.18 a	0.70	0.827	24.05 a	0.72	0.624	21.96 a	0.36	0.443	20.53 a	0.84	0.746	21.43 a	0.82	0.751	18.62 a	0.44	0.857
Shading			27.40 a			23.52 a			21.53 a			20.93 a			21.05 a			18.74 a		
	'Aufr. Typ'		27.79 a	0.55	0.084	23.74 a	0.60	0.139	21.35 b	0.38	0.006	20.67 a	0.62	0.118	21.03 a	0.97	0.500	18.61 a	0.51	0.160
	'Lemona'		26.81 a			24.35 a			21.23 b			20.42 a			20.55 a			18.01 a		
	'NLC'		27.26 a			23.26 a			22.66 a			21.10 a			22.15 a			19.43 a		
		early	25.11 c	0.55	< 0,001	23.37 a	0.60	0.403	17.58 c	0.38	< 0,001	19.75 c	0.62	< 0,001	n.d.			n.d.		
		medium	27.14 b			24.08 a			21.26 b			20.71 b								
		late	29.62 a			23.90 a			26.40 a			21.73 a								
Natural light	'Aufr. Typ'		27.82 a	0.78	0.845	24.31 a	0.84	0.614	21.21 a	0.53	0.382	20.45 a	0.88	0.993	21.31 a	1.38	0.743	18.51 a	0.71	0.915
	'Lemona'		26.60 a			24.51 a			21.48 a			20.24 a			20.18 a			17.83 a		
	'NLC'		27.11 a			23.33 a			23.18 a			20.90 a			22.82 a			19.54 a		
Shading	'Aufr. Typ'		27.76 a			23.17 a			21.48 a			20.89 a			20.74 a			18.71 a		
	'Lemona'		27.02 a			24.19 a			20.98 a			20.61 a			20.93 a			18.19 a		
	'NLC'		27.42 a			23.20 a			22.14 a			21.30 a			21.48 a			19.33 a		
Natural light		early	25.27 de	0.78	0.443	24.01 a	0.84	0.245	17.77 c	0.53	0.819	19.40 cd	0.88	0.704	n.d.			n.d.		
		medium	27.03 cde			23.83 a			21.63 b			20.58 abcd								
		late	29.23 ab			24.30 a			26.48 a			21.61 ab								
Shading		early	24.95 e			22.74 a			17.38 c			20.11 bd								
		medium	27.25 bd			24.32 a			20.89 b			20.83 abcd								
		late	30.01 ac			23.51 a			26.33 a			21.85 ac								
		early	25.79 de	0.70	0.728	23.06 a	0.80	0.532	16.60 d	0.60	0.530	19.49 c	0.70	< 0,001	n.d.			n.d.		
	'Aufr. Typ'	medium	27.62 bcd			24.26 a			20.86 bc			20.39 bc								
		late	29.97 ab			23.90 a			26.58 a			22.12 ab								
	'Lemona'	early	24.82 e			24.29 a			17.22 d			19.59 c								
		medium	26.79 cde			23.91 a			20.90 bc			19.48 c								
		late	28.83 abc			24.86 a			25.58 a			22.21 a								
	'NLC'	early	24.72 e			22.78 a			18.91 cd			20.18 c								
		medium	27.00 cde			24.07 a			22.01 b			22.26 a								
		late	30.06 a			22.95 a			27.06 a			20.86 abc								
Natural light	'Aufr. Typ'	early	25.97 abcdef	0.99	0.995	23.13 a	1.14	0.375	16.48 g	0.85	0.768	19.46 abcdef	0.99	0.571	n.d.			n.d.		
		medium	27.76 abcdef			24.77 a			21.18 cde			19.89 abcdef								
		late	29.73 abe			25.01 a			25.97 ab			21.99 abc								
	'Lemona'	early	24.93 cdf			25.37 a			17.38 efg			19.04 def								
		medium	26.47 abcdef			23.47 a			20.90 cdef			19.71 abcdef								
		late	28.41 abcdef			24.70 a			26.15 ab			21.97 abc								
	'NLC'	early	24.92 cdf			23.54 a			19.44 defg			19.69 abcdef								
		medium	26.86 abcdef			23.25 a			22.80 bcd			22.14 abc								
		late	29.55 abe			23.20 a			27.31 a			20.88 abcdef								
Shading	'Aufr. Typ'	early	25.61 bdef			22.98 a			16.72 fg			19.53 bcef								
		medium	27.48 abcdef			23.74 a			20.54 defg			20.89 abcdef								
		late	30.21 ac			22.80 a			27.19 ab			22.25 abde								
	'Lemona'	early	24.71 ef			23.21 a			17.05 efg			20.14 abcdef								
		medium	27.11 abcdef			24.35 a			20.89 cdefg			19.24 cf								
		late	29.25 abcd			25.02 a			25.00 abc			22.45 ad								
	'NLC'	early	24.53 ef			22.02 a			18.38 defg			20.67 abcdef								
		medium	27.15 abcdef			24.88 a			21.23 cde			22.37 abde								
		late	30.58 a			22.70 a			26.81 ab			20.85 abcdef								

Tab. A 10: Leaf:stem ratio, Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Leaf:stem ratio			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			1.98 a	0.08	0.989	2.60 a	0.11	0.149	0.77 a	0.03	0.868	2.06 a	0.18	0.935	1.17 a	0.07	0.241	2.31 a	0.22	0.359
Shading			1.98 a			2.34 a			0.78 a			2.04 a			1.04 a			2.00 a		
	'Aufr. Typ'		2.19 a	0.08	< 0,001	2.67 a	0.13	< 0,001	0.73 b	0.03	< 0,001	2.16 a	0.14	< 0,001	1.11 ab	0.08	0.010	2.46 a	0.19	0.005
	'Lemona'		2.07 a			2.82 a			0.69 b			2.32 a			0.92 b			2.34 a		
	'NLC'		1.70 b			1.93 b			0.92 a			1.68 b			1.29 a			1.66 b		
		early	2.02 a	0.08	0.142	2.44 a	0.13	0.269	0.97 a	0.03	< 0,001	2.17 a	0.14	0.094	n.d.			n.d.		
		medium	2.06 a			2.34 a			0.71 b			1.92 a								
		late	1.87 a			2.63 a			0.66 b			2.06 a								
Natural light	'Aufr. Typ'		2.26 a	0.11	0.135	2.75 ab	0.18	0.817	0.72 c	0.04	0.991	2.14 abcd	0.20	0.396	1.16 ab	0.11	0.862	2.53 a	0.28	0.702
	'Lemona'		1.95 abc			2.93 a			0.69 c			2.27 ac			0.97 ab			2.48 a		
	'NLC'		1.74 bc			2.12 bc			0.92 ab			1.78 bd			1.39 a			1.91 a		
Shading	'Aufr. Typ'		2.11 ab			2.58 ab			0.73 bc			2.17 ab			1.06 ab			2.39 a		
	'Lemona'		2.18 ab			2.70 ab			0.69 c			2.37 ab			0.87 b			2.20 a		
	'NLC'		1.66 c			1.73 c			0.93 a			1.59 cd			1.19 ab			1.42 a		
Natural light		early	2.04 a	0.11	0.651	2.30 ab	0.18	0.033	0.96 a	0.04	0.668	2.11 a	0.20	0.542	n.d.			n.d.		
		medium	2.09 a			2.57 ab			0.72 b			1.96 a								
		late	1.82 a			2.93 a			0.64 b			2.11 a								
Shading		early	2.01 a			2.58 ab			0.99 a			2.23 a								
		medium	2.02 a			2.12 b			0.69 b			1.88 a								
		late	1.92 a			2.32 ab			0.67 b			2.01 a								
	'Aufr. Typ'	early	2.09 abc	0.13	0.398	2.62 abc	0.22	0.360	0.91 b	0.05	0.081	2.38 ab	0.18	0.068	n.d.			n.d.		
		medium	2.38 a			2.73 abc			0.68 cde			1.77 bcde								
		late	2.10 abc			2.65 abc			0.59 e			2.32 abc								
	'Lemona'	early	2.22 ab			2.90 ab			0.83 bcd			2.46 a								
		medium	2.07 abc			2.41 abc			0.59 e			2.25 abcd								
		late	1.91 abc			3.14 a			0.64 de			2.23 abcde								
	'NLC'	early	1.76 bc			1.80 c			1.17 a			1.68 de								
		medium	1.72 bc			1.89 c			0.85 bc			1.75 cde								
		late	1.61 c			2.09 bc			0.74 bcde			1.62 e								
Natural light	'Aufr. Typ'	early	2.15 ab	0.18	0.654	2.39 ab	0.31	0.843	0.95 abc	0.07	0.293	2.44 ab	0.26	0.361	n.d.			n.d.		
		medium	2.52 a			2.97 ab			0.64 cde			1.71 ab								
		late	2.11 ab			2.90 ab			0.58 e			2.26 ab								
	'Lemona'	early	2.24 ab			2.65 ab			0.81 bcde			2.24 ab								
		medium	1.93 ab			2.60 ab			0.62 de			2.19 ab								
		late	1.70 ab			3.55 a			0.63 cde			2.36 ab								
	'NLC'	early	1.73 ab			1.87 b			1.12 ab			1.65 ab								
		medium	1.84 ab			2.16 ab			0.91 abcd			1.98 ab								
		late	1.66 ab			2.33 ab			0.72 cde			1.70 ab								
Shading	'Aufr. Typ'	early	2.03 ab			2.85 ab			0.88 bcde			2.31 ab								
		medium	2.24 ab			2.50 ab			0.72 cde			1.82 ab								
		late	2.08 ab			2.39 ab			0.60 de			2.39 ab								
	'Lemona'	early	2.21 ab			3.15 ab			0.85 bcde			2.68 a								
		medium	2.21 ab			2.23 ab			0.57 de			2.32 ab								
		late	2.12 ab			2.74 ab			0.65 cde			2.10 ab								
	'NLC'	early	1.80 ab			1.74 b			1.23 a			1.71 ab								
		medium	1.61 ab			1.62 b			0.79 bcde			1.51 b								
		late	1.57 b			1.85 b			0.76 cde			1.54 b								

Tab. A 11: Essential oil content [%], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Essential oil [%]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			0.22 a	0.01	0.251	0.55 b	0.01	0.037	0.10 a	0.01	0.748	0.33 a	0.02	0.516	0.17 a	0.01	0.446	0.53 a	0.02	0.133
Shading			0.24 a			0.58 a			0.10 a			0.35 a			0.18 a			0.57 a		
	'Aufr. Typ'		0.19 b	0.01	< 0,001	0.55 b	0.01	< 0,001	0.07 b	0.01	< 0,001	0.31 b	0.02	< 0,001	0.14 b	0.01	< 0,001	0.53 b	0.02	< 0,001
	'Lemona'		0.34 a			0.67 a			0.16 a			0.43 a			0.29 a			0.66 a		
	'NLC'		0.15 c			0.47 c			0.07 b			0.28 b			0.11 b			0.45 c		
		early	0.25 a	0.01	0.007	0.53 b	0.01	< 0,001	0.10 a	0.01	0.047	0.42 a	0.02	< 0,001	n.d.			n.d.		
		medium	0.21 b			0.61 a			0.12 a			0.32 b								
		late	0.21 b			0.56 b			0.09 a			0.27 b								
Natural light	'Aufr. Typ'		0.18 b	0.02	0.234	0.53 bc	0.01	0.138	0.07 b	0.01	0.474	0.26 b	0.03	0.046	0.13 b	0.01	0.220	0.48 bc	0.02	0.090
Natural light	'Lemona'		0.32 a			0.65 a			0.16 a			0.43 a			0.28 a			0.65 a		
Natural light	'NLC'		0.15 b			0.47 c			0.08 b			0.31 ab			0.12 b			0.45 c		
Shading	'Aufr. Typ'		0.20 b			0.58 b			0.08 b			0.36 ab			0.15 b			0.58 ab		
Shading	'Lemona'		0.37 a			0.69 a			0.16 a			0.44 a			0.31 a			0.67 a		
Shading	'NLC'		0.15 b			0.47 c			0.07 b			0.25 b			0.10 b			0.45 c		
Natural light		early	0.24 a	0.02	0.735	0.50 c	0.02	0.100	0.10 a	0.01	0.689	0.43 a	0.03	0.130	n.d.			n.d.		
Natural light		medium	0.21 a			0.61 a			0.11 a			0.33 ab								
Natural light		late	0.20 a			0.54 bc			0.09 a			0.23 b								
Shading		early	0.27 a			0.55 abc			0.09 a			0.42 a								
Shading		medium	0.22 a			0.60 a			0.12 a			0.31 ab								
Shading		late	0.23 a			0.58 ab			0.09 a			0.32 ab								
	'Aufr. Typ'	early	0.22 b	0.02	0.351	0.50 de	0.02	0.006	0.07 c	0.01	0.489	0.46 ab	0.04	< 0,001	n.d.			n.d.		
	'Aufr. Typ'	medium	0.18 bc			0.60 bc			0.08 c			0.30 bcd								
	'Aufr. Typ'	late	0.17 bc			0.56 cd			0.06 c			0.17 d								
	'Lemona'	early	0.38 a			0.60 bc			0.16 a			0.44 abc								
	'Lemona'	medium	0.31 a			0.74 a			0.18 a			0.49 a								
	'Lemona'	late	0.34 a			0.67 ab			0.14 ab			0.37 abc								
	'NLC'	early	0.16 bc			0.49 de			0.06 c			0.37 abc								
	'NLC'	medium	0.15 bc			0.49 de			0.09 bc			0.18 d								
	'NLC'	late	0.13 c			0.44 e			0.08 c			0.29 cd								
Natural light	'Aufr. Typ'	early	0.21 cde	0.03	0.492	0.45 gh	0.02	0.323	0.06 de	0.02	0.447	0.46 abc	0.06	0.122	n.d.			n.d.		
Natural light	'Aufr. Typ'	medium	0.17 e			0.60 bcde			0.08 cde			0.29 abcd								
Natural light	'Aufr. Typ'	late	0.16 e			0.53 defgh			0.06 e			0.03 d								
Natural light	'Lemona'	early	0.34 ab			0.59 bcde			0.17 ab			0.41 abc								
Natural light	'Lemona'	medium	0.31 abcd			0.72 ab			0.15 abc			0.53 a								
Natural light	'Lemona'	late	0.31 abc			0.65 abcd			0.15 abcd			0.34 abc								
Natural light	'NLC'	early	0.17 e			0.47 efgh			0.06 de			0.42 abc								
Natural light	'NLC'	medium	0.15 e			0.52 defgh			0.10 bcde			0.19 cd								
Natural light	'NLC'	late	0.13 e			0.43 h			0.08 cde			0.32 abc								
Shading	'Aufr. Typ'	early	0.22 bcde			0.56 cdefg			0.08 cde			0.47 ab								
Shading	'Aufr. Typ'	medium	0.19 cde			0.60 bcde			0.08 cde			0.31 abc								
Shading	'Aufr. Typ'	late	0.18 de			0.58 bcde			0.07 de			0.30 abc								
Shading	'Lemona'	early	0.42 a			0.61 bcde			0.15 abc			0.46 ab								
Shading	'Lemona'	medium	0.31 abc			0.76 a			0.20 a			0.44 abc								
Shading	'Lemona'	late	0.37 a			0.70 abc			0.14 abcd			0.40 abc								
Shading	'NLC'	early	0.16 e			0.50 efgh			0.05 e			0.33 abc								
Shading	'NLC'	medium	0.16 e			0.45 fgh			0.09 cde			0.18 cd								
Shading	'NLC'	late	0.14 e			0.45 efgh			0.07 cde			0.25 bcd								

Tab. A 12: Total phenolic content [mg GAE/g DM], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Total phenolic content [mg GAE/g DM]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			150.1 a	4.4	0.862	122.9 b	1.4	0.012	161.9 a	2.0	0.158	141.3 a	1.7	0.107	157.8 a	3.1	0.985	137.2 a	3.0	0.853
Shading			149.0 a			128.3 a			157.8 a			137.4 a			157.9 a			138.0 a		
	'Aufr. Typ'		140.6 b	3.7	< 0,001	129.2 a	1.8	< 0,001	149.3 b	2.5	< 0,001	140.9 a	2.1	0.515	146.9 b	3.7	0.008	129.0 b	3.7	0.031
	'Lemona'		151.1 a			119.4 b			155.9 b			139.6 a			161.2 a			140.4 ab		
	'NLC'		157.0 a			128.3 a			174.3 a			137.5 a			165.4 a			143.3 a		
		early	166.4 a	3.7	< 0,001	124.1 a	1.8	0.267	161.0 a	2.5	0.156	145.8 a	2.1	< 0,001	n.d.			n.d.		
		medium	144.5 b			128.0 a			162.5 a			142.6 a								
		late	137.8 b			124.8 a			156.0 a			129.7 b								
Natural light	'Aufr. Typ'		138.9 b	5.2	0.472	128.9 ab	2.5	0.208	150.1 c	3.5	0.810	142.5 a	3.0	0.196	153.1 ab	5.2	0.130	125.8 a	5.2	0.315
	'Lemona'		153.7 a			116.5 c			158.0 bc			144.5 a			159.2 ab			138.1 a		
	'NLC'		157.7 a			123.4 abc			177.4 a			137.0 a			161.1 ab			147.6 a		
Shading	'Aufr. Typ'		142.2 ab			129.5 ab			148.4 c			139.3 a			140.7 b			132.1 a		
	'Lemona'		148.5 ab			122.2 bc			153.7 c			134.7 a			163.2 ab			142.7 a		
	'NLC'		156.2 ab			133.1 a			171.2 ab			138.0 a			169.8 a			139.1 a		
Natural light		early	166.7 ac	5.2	0.593	124.8 ab	2.5	0.076	167.3 a	3.5	0.065	153.4 a	3.0	0.001	n.d.			n.d.		
		medium	146.9 bd			123.7 ab			160.6 a			138.7 bc								
		late	136.8 d			120.4 b			157.7 a			131.9 c								
Shading		early	166.1 ab			123.4 ab			154.6 a			138.2 bc								
		medium	142.0 cd			132.2 a			164.5 a			146.4 ab								
		late	138.9 d			129.2 ab			154.3 a			127.4 c								
	'Aufr. Typ'	early	155.2 ab	5.1	0.043	127.2 ab	3.1	0.714	149.1 c	4.3	0.086	150.4 a	3.6	0.045	n.d.			n.d.		
		medium	140.8 bc			130.5 ab			152.5 c			140.8 abc								
		late	125.6 c			129.9 ab			146.3 c			131.6 bc								
	'Lemona'	early	170.4 a			120.2 ab			157.5 bc			143.9 ab								
		medium	137.2 bc			120.8 ab			152.2 c			150.0 a								
		late	145.9 b			117.1 b			157.9 bc			124.9 c								
	'NLC'	early	173.6 a			124.9 ab			176.3 ab			143.0 ab								
		medium	155.4 ab			132.6 a			182.9 a			137.0 abc								
		late	142.0 bc			127.3 ab			163.8 abc			132.5 bc								
Natural light	'Aufr. Typ'	early	156.2 abcde	7.1	0.145	133.5 ab	4.3	0.246	150.6 bc	6.1	0.364	155.0 ab	5.2	0.108	n.d.			n.d.		
		medium	143.0 bdef			127.6 ab			149.1 bc			134.5 abcd								
		late	117.5 f			125.5 ab			150.7 bc			138.1 abcd								
	'Lemona'	early	169.3 abcd			122.0 ab			168.0 abc			160.1 a								
		medium	145.7 abcdef			116.1 ab			147.0 c			147.7 abcd								
		late	146.2 abcdef			111.5 b			159.0 abc			125.7 cd								
	'NLC'	early	174.5 ac			118.9 ab			183.4 a			145.0 abcd								
		medium	152.1 abcde			127.4 ab			185.6 a			134.0 abcd								
		late	146.6 abcdef			124.0 ab			163.3 abc			131.9 bcd								
Shading	'Aufr. Typ'	early	154.3 abcdef			121.0 ab			147.6 c			145.8 abcd								
		medium	138.7 cdef			133.4 ab			155.9 abc			147.1 abcd								
		late	133.8 def			134.3 a			141.8 c			125.0 d								
	'Lemona'	early	171.4 ab			118.4 ab			147.0 c			127.7 cd								
		medium	128.6 ef			125.5 ab			157.4 abc			152.3 abc								
		late	145.6 abcdef			122.7 ab			156.8 abc			124.1 d								
	'NLC'	early	172.8 ab			130.9 ab			169.1 abc			140.9 abcd								
		medium	158.6 abcde			137.8 a			180.2 ab			140.0 abcd								
		late	137.3 cdef			130.6 ab			164.2 abc			133.2 bcd								

Tab. A 13: ORAC [µmol TE/g DM], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

ORAC [µmol TE/g DM]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			2056.7 a	66.95	0.987	1991.4 a	25.46	0.065	2450.6 a	28.77	0.694	2236.5 a	23.23	0.089	2319.9 a	52.38	0.152	2033.1 a	28.50	0.026
Shading			2055.1 a			2072.4 a			2434.5 a			2169.8 a			2198.3 a			1935.4 b		
	'Aufr. Typ'		2003.9 a	56.01	0.224	2081.2 a	29.20	0.037	2357.4 b	35.23	< 0,001	2237.4 a	27.97	0.008	2178.1 a	54.35	0.081	1946.2 a	34.90	0.084
	'Lemona'		2088.8 a			2038.6 ab			2377.2 b			2242.7 a			2249.5 a			2052.1 a		
	'NLC'		2075.0 a			1975.9 b			2593.2 a			2129.4 b			2349.6 a			1954.4 a		
		early	2251.9 a	56.01	< 0,001	1993.2 a	29.20	0.239	2486.5 a	35.23	0.083	2219.7 a	27.97	0.586	n.d.			n.d.		
		medium	1964.2 b			2044.7 a			2378.3 a			2209.3 a								
		late	1951.6 b			2057.8 a			2463.0 a			2180.4 a								
Natural light	'Aufr. Typ'		1925.0 b	79.21	0.002	2043.7 a	41.30	0.919	2339.1 c	49.83	0.523	2276.0 ab	39.56	0.267	2307.7 a	76.86	0.251	1954.5 ab	49.36	0.379
	'Lemona'		2196.7 a			1988.8 a			2381.3 bc			2305.3 a			2263.9 a			2124.8 a		
	'NLC'		2048.4 ab			1941.7 a			2631.5 a			2128.0 b			2388.3 a			2020.0 ab		
Shading	'Aufr. Typ'		2082.8 ab			2118.8 a			2375.7 bc			2198.8 ab			2048.6 a			1937.9 ab		
	'Lemona'		1980.8 ab			2088.4 a			2373.1 bc			2180.0 ab			2235.2 a			1979.4 ab		
	'NLC'		2101.7 ab			2010.1 a			2554.8 ab			2130.7 b			2311.0 a			1888.8 b		
Natural light		early	2182.4 ab	79.21	0.013	1979.1 a	41.30	0.497	2514.8 a	49.83	0.267	2247.2 a	39.56	0.492	n.d.			n.d.		
		medium	2052.2 abc			1984.6 a			2339.1 a			2222.6 a								
		late	1935.6 c			2010.5 a			2497.9 a			2239.6 a								
Shading		early	2321.5 a			2007.3 a			2458.1 a			2192.2 a								
		medium	1876.2 bc			2104.8 a			2417.4 a			2196.1 a								
		late	1967.5 bc			2105.1 a			2428.1 a			2121.1 a								
	'Aufr. Typ'	early	2253.6 ab	76.33	0.399	2026.3 a	49.38	0.615	2473.0 abc	61.02	0.073	2249.2 ab	48.17	0.011	n.d.			n.d.		
		medium	1933.6 c			2105.8 a			2236.0 c			2170.0 ab								
		late	1824.5 c			2111.6 a			2363.2 abc			2293.1 ab								
	'Lemona'	early	2232.1 ab			2028.9 a			2383.1 abc			2287.2 ab								
		medium	1981.9 abc			2001.7 a			2270.1 bc			2332.5 a								
		late	2052.3 abc			2085.1 a			2478.4 abc			2108.3 b								
	'NLC'	early	2270.1 a			1924.3 a			2603.3 a			2122.8 ab								
		medium	1977.1 bc			2026.6 a			2628.7 a			2125.5 ab								
		late	1977.9 bc			1976.8 a			2547.4 ab			2139.7 ab								
Natural light	'Aufr. Typ'	early	2126.9 abcd	107.95	0.120	2099.6 a	69.83	0.082	2487.8 abc	86.30	0.840	2355.4 ab	68.13	0.176	n.d.			n.d.		
		medium	1984.5 abcd			2031.4 a			2156.0 c			2121.8 ab								
		late	1663.7 d			2000.1 a			2373.5 abc			2351.0 ab								
	'Lemona'	early	2193.1 abc			1947.4 a			2434.2 abc			2314.5 ab								
		medium	2234.6 abc			1924.9 a			2199.0 bc			2413.1 a								
		late	2162.5 abc			2094.1 a			2510.6 abc			2188.3 ab								
	'NLC'	early	2227.1 abc			1890.1 a			2622.4 ab			2071.7 ab								
		medium	1937.4 abcd			1997.6 a			2662.5 a			2132.9 ab								
		late	1980.7 abcd			1937.3 a			2609.7 ab			2179.5 ab								
Shading	'Aufr. Typ'	early	2380.4 a			1953.0 a			2458.1 abc			2143.0 ab								
		medium	1882.8 bcd			2180.3 a			2316.1 abc			2218.2 ab								
		late	1985.2 abcd			2223.0 a			2352.9 abc			2235.2 ab								
	'Lemona'	early	2271.1 ab			2110.4 a			2332.0 abc			2259.8 ab								
		medium	1729.1 cd			2078.6 a			2341.2 abc			2251.9 ab								
		late	1942.2 abcd			2076.1 a			2446.2 abc			2028.3 b								
	'NLC'	early	2313.1 ab			1958.5 a			2584.2 abc			2173.9 ab								
		medium	2016.8 abcd			2055.5 a			2595.0 abc			2118.2 ab								
		late	1975.1 abcd			2016.2 a			2485.2 abc			2100.0 ab								

Tab. A 14: Rosmarinic acid content [%], Gross-Gerau 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different (p < 0.05). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Rosmarinic acid [%]			2013						2014						2015					
			First cut			Second cut			First cut			Second cut			First cut			Second cut		
Light intensity	Genotype	Harvest stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			4.25 a	0.18	0.348	4.39 a	0.10	0.084	5.12 a	0.12	0.252	4.79 a	0.08	0.517	5.52 a	0.15	0.386	4.80 a	0.16	0.364
Shading			3.98 a			4.69 a			5.32 a			4.72 a			5.34 a			4.57 a		
	'Aufr. Typ'		3.80 c	0.15	< 0,001	4.65 a	0.10	0.210	4.71 b	0.14	< 0,001	4.94 a	0.10	0.001	4.93 b	0.18	0.011	4.61 a	0.18	0.060
	'Lemona'		4.43 a			4.44 a			5.19 b			4.88 a			5.64 a			5.04 a		
	'NLC'		4.11 b			4.53 a			5.76 a			4.44 b			5.71 a			4.41 a		
		early	4.94 a	0.15	< 0,001	4.37 a	0.10	0.054	5.77 a	0.14	< 0,001	5.24 a	0.10	< 0,001	n.d.			n.d.		
		medium	3.85 b			4.64 a			5.16 b			4.36 b								
		late	3.55 c			4.61 a			4.73 b			4.66 b								
Natural light	'Aufr. Typ'		3.77 b	0.21	0.083	4.61 a	0.14	0.235	4.52 c	0.20	0.449	5.03 a	0.14	0.720	5.24 ab	0.25	0.137	4.75 a	0.26	0.885
	'Lemona'		4.63 a			4.18 a			5.05 abc			4.92 ab			5.44 ab			5.19 a		
	'NLC'		4.33 a			4.38 a			5.81 a			4.42 b			5.89 a			4.45 a		
Shading	'Aufr. Typ'		3.83 ab			4.70 a			4.91 bc			4.85 ab			4.62 b			4.47 a		
	'Lemona'		4.23 ab			4.69 a			5.33 abc			4.83 ab			5.85 a			4.89 a		
	'NLC'		3.88 ab			4.68 a			5.71 ab			4.47 ab			5.54 ab			4.36 a		
Natural light		early	4.93 a	0.21	0.133	4.22 a	0.14	0.995	5.79 a	0.20	0.386	5.28 a	0.14	0.732	n.d.			n.d.		
		medium	4.10 bc			4.50 a			4.90 b			4.44 c								
		late	3.71 c			4.45 a			4.68 b			4.64 bc								
Shading		early	4.94 ab			4.51 a			5.76 a			5.20 ab								
		medium	3.61 c			4.79 a			5.41 ab			4.27 c								
		late	3.39 c			4.76 a			4.78 b			4.68 bc								
	'Aufr. Typ'	early	4.48 bc	0.19	0.030	4.33 a	0.16	0.479	5.31 abcd	0.25	0.274	5.36 a	0.17	0.041	n.d.			n.d.		
		medium	3.82 cd			4.74 a			4.66 cd			4.34 cd								
		late	3.10 e			4.89 a			4.18 d			5.12 ab								
	'Lemona'	early	5.39 a			4.37 a			5.69 abc			5.36 a								
		medium	3.85 cd			4.53 a			4.86 bcd			4.77 abc								
		late	4.04 cd			4.42 a			5.02 bcd			4.50 bcd								
	'NLC'	early	4.94 ab			4.40 a			6.33 a			5.01 abc								
		medium	3.88 cd			4.67 a			5.95 ab			3.96 d								
		late	3.50 de			4.52 a			5.00 bcd			4.37 bcd								
Natural light	'Aufr. Typ'	early	4.45 abcdef	0.27	< 0,001	4.40 a	0.22	0.506	5.21 abc	0.35	0.715	5.67 a	0.24	0.288	n.d.			n.d.		
		medium	4.15 bdefg			4.67 a			4.21 c			4.29 bcd								
		late	2.72 h			4.76 a			4.13 c			5.12 abc								
	'Lemona'	early	5.33 ac			4.17 a			5.79 abc			5.37 ab								
		medium	4.29 abcdefg			4.29 a			4.43 bc			4.97 abcd								
		late	4.29 abcdefg			4.09 a			4.93 abc			4.42 bcd								
	'NLC'	early	5.02 abcd			4.09 a			6.37 a			4.82 abcd								
		medium	3.86 efg			4.53 a			6.06 ab			4.07 cd								
		late	4.12 bdefg			4.51 a			4.98 abc			4.38 bcd								
Shading	'Aufr. Typ'	early	4.52 abcdef			4.26 a			5.41 abc			5.05 abcd								
		medium	3.49 fgh			4.81 a			5.11 abc			4.39 bcd								
		late	3.49 fgh			5.02 a			4.23 c			5.12 abc								
	'Lemona'	early	5.46 ab			4.57 a			5.58 abc			5.35 ab								
		medium	3.42 fgh			4.76 a			5.29 abc			4.57 abcd								
		late	3.80 defgh			4.74 a			5.12 abc			4.58 abcd								
	'NLC'	early	4.86 abcde			4.70 a			6.28 a			5.19 abc								
		medium	3.91 cdefgh			4.80 a			5.83 abc			3.85 d								
		late	2.89 gh			4.53 a			5.01 abc			4.36 bcd								

Tab. A 15: Plant height [cm], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Plant height [cm]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			27.4 b	0.8	< 0,001	94.7 a	0.9	0.057	31.1 b	0.9	< 0,001	61.6 a	1.5	0.125	25.6 b	1.5	< 0,001
Shading						40.6 a			91.8 a			45.4 a			65.4 a			35.9 a		
	'Aufr. Typ'		73.7 b	1.3	< 0,001	36.9 a	0.8	< 0,001	93.7 a	0.7	0.199	40.9 a	0.9	< 0,001	63.3 a	1.4	0.865	32.3 a	1.5	0.170
	'Lemona'		81.0 a			31.2 b			92.8 a			35.6 b			63.7 a			29.3 a		
		early	67.4 c	1.5	< 0,001	36.6 a	1.0	< 0,001	76.4 c	0.8	< 0,001	45.6 a	1.1	< 0,001	n.d.			n.d.		
		medium	76.2 b			34.7 a			100.1 b			41.3 b								
		late	88.5 a			30.7 b			103.3 a			27.9 c								
Natural light	'Aufr. Typ'		n.d.			30.5 c	1.1	0.719	94.5 a	1.0	0.055	33.6 c	1.3	0.697	62.1 a	1.9	0.525	27.6 bc	2.1	0.663
	'Lemona'					24.4 d			95.0 a			28.7 d			61.2 a			23.6 c		
Shading	'Aufr. Typ'					43.3 a			93.0 a			48.3 a			64.6 a			37.0 a		
	'Lemona'					37.9 b			90.6 a			42.5 b			66.1 a			34.9 ab		
Natural light		early	n.d.			29.7 b	1.3	0.248	76.2 c	1.1	< 0,001	42.9 b	1.5	< 0,001	n.d.			n.d.		
		medium				29.4 b			99.9 b			29.9 c								
		late				23.1 c			108.1 a			20.6 d								
Shading		early				43.5 a			76.6 c			48.2 ab								
		medium				40.1 a			100.4 b			52.6 a								
		late				38.3 a			98.4 b			35.2 c								
	'Aufr. Typ'	early	65.4 d	2.0	0.237	39.7 a	1.3	0.087	76.0 c	1.0	< 0,001	47.5 a	1.5	0.335	n.d.			n.d.		
		medium	72.6 cd			36.0 ab			99.2 b			45.2 a								
		late	83.2 b			35.0 ab			106.0 a			30.0 c								
	'Lemona'	early	69.4 d			33.5 b			76.8 c			43.7 ab								
		medium	79.7 bc			33.5 b			101.1 b			37.3 b								
		late	93.9 a			26.4 c			100.5 b			25.8 c								
Natural light	'Aufr. Typ'	early	n.d.			33.3 bc	1.9	0.031	76.5 e	1.4	0.006	47.3 b	2.1	0.016	n.d.			n.d.		
		medium				32.6 bc			98.5 cd			32.7 cd								
		late				25.6 cd			108.5 a			20.7 ef								
	'Lemona'	early				26.2 cd			75.9 e			38.6 bc								
		medium				26.3 cd			101.4 c			27.2 def								
		late				20.6 d			107.7 ab			20.4 f								
Shading	'Aufr. Typ'	early				46.1 a			75.6 e			47.7 ab								
		medium				39.4 ab			99.9 c			57.8 a								
		late				44.3 a			103.6 abc			39.3 bc								
	'Lemona'	early				40.9 ab			77.7 e			48.8 ab								
		medium				40.8 ab			100.9 bc			47.5 ab								
		late				32.2 bc			93.3 d			31.1 cde								

Tab. A 16: Leaf Area Index (LAI), Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Leaf Area Index			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			n.d.			10.8 b	0.3	0.021	6.1 b	0.3	< 0,001	13.1 a	0.4	0.510	6.4 b	0.3	< 0,001
Shading									11.8 a			7.8 a			13.4 a			8.9 a		
	'Aufr. Typ'								11.6 a	0.3	0.186	7.8 a	0.3	< 0,001	13.0 a	0.4	0.312	8.5 a	0.3	0.001
	'Lemona'								11.0 a			6.1 b			13.5 a			6.8 b		
		early							13.5 a	0.4	< 0,001	8.1 a	0.3	< 0,001	n.d.			n.d.		
		medium							10.9 b			6.2 b								
		late							9.4 c			6.6 b								
Natural light	'Aufr. Typ'								11.2 a	0.4	0.463	7.3 a	0.4	0.069	12.6 a	0.5	0.465	7.2 bc	0.4	0.826
	'Lemona'								10.4 a			4.9 b			13.5 a			5.6 c		
Shading	'Aufr. Typ'								11.9 a			8.3 a			13.4 a			9.8 a		
	'Lemona'								11.7 a			7.3 a			13.5 a			8.0 b		
Natural light		early							13.8 a	0.5	0.047	8.8 a	0.4	< 0,001	n.d.			n.d.		
		medium							10.3 bc			4.8 b								
		late							8.3 c			4.8 b								
Shading		early							13.3 a			7.4 a								
		medium							11.6 ab			7.5 a								
		late							10.5 bc			8.5 a								
	'Aufr. Typ'	early							14.1 a	0.5	0.300	9.0 a	0.4	0.313	n.d.			n.d.		
		medium							11.4 bc			7.3 ab								
		late							9.2 c			7.1 b								
	'Lemona'	early							12.9 ab			7.2 ab								
		medium							10.5 c			5.0 c								
		late							9.6 c			6.2 bc								
Natural light	'Aufr. Typ'	early							14.6 a	0.7	0.980	10.0 a	0.6	0.378	n.d.			n.d.		
		medium							10.8 bcd			6.7 bcd								
		late							8.3 d			5.4 cde								
	'Lemona'	early							13.0 abc			7.6 abc								
		medium							9.8 cd			3.0 e								
		late							8.3 d			4.2 de								
Shading	'Aufr. Typ'	early							13.7 ab			8.1 abc								
		medium							11.9 abc			8.0 abc								
		late							10.1 cd			8.9 ab								
	'Lemona'	early							12.9 abc			6.7 abcd								
		medium							11.3 abcd			7.0 abcd								
		late							10.8 bcd			8.1 abc								

Tab. A 17: SPAD values, Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

SPAD			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			36.9 a	0.6	0.024	30.4 b	0.4	< 0,001	29.8 b	0.7	0.003	32.6 b	0.3	0.039	24.8 a	1.0	0.392
Shading						34.2 b			34.5 a			34.7 a			33.6 a			26.2 a		
	'Aufr. Typ'		33.8 a	0.3	0.338	34.0 b	0.5	< 0,001	32.9 a	0.4	0.057	31.3 b	0.6	0.002	33.2 a	0.3	0.539	23.3 b	0.8	< 0,001
	'Lemona'		33.4 a			37.1 a			32.0 a			33.3 a			32.9 a			27.7 a		
		early	33.7 a	0.3	0.001	35.7 ab	0.6	0.015	28.7 c	0.5	< 0,001	25.9 b	0.6	< 0,001	n.d.			n.d.		
		medium	32.5 b			34.5 b			35.9 a			35.2 a								
		late	34.6 a			36.5 a			32.8 b			35.7 a								
Natural light	'Aufr. Typ'		n.d.			35.0 bc	0.7	0.255	30.7 b	0.5	0.395	29.0 c	0.8	0.623	32.8 a	0.4	0.618	22.7 c	1.1	0.808
	'Lemona'					38.7 a			30.1 b			30.7 bc			32.4 a			27.0 ab		
Shading	'Aufr. Typ'					33.0 c			35.2 a			33.6 ab			33.6 a			23.9 bc		
	'Lemona'					35.4 b			33.8 a			35.8 a			33.5 a			28.4 a		
Natural light		early	n.d.			37.7 a	0.8	0.121	27.4 e	0.7	0.014	24.6 c	0.9	0.050	n.d.			n.d.		
		medium				35.2 ab			32.8 bc			32.2 b								
		late				37.6 a			31.0 cd			32.7 b								
Shading		early				33.6 b			29.9 de			27.3 c								
		medium				33.8 b			39.0 a			38.2 a								
		late				35.3 ab			34.6 b			38.7 a								
	'Aufr. Typ'	early	34.0 ab	0.5	0.535	34.6 bc	0.7	0.109	29.4 c	0.6	0.043	25.4 c	0.8	0.019	n.d.			n.d.		
		medium	32.9 ab			33.3 c			37.0 a			35.0 ab								
		late	34.5 a			34.2 bc			32.4 b			33.5 b								
	'Lemona'	early	33.4 ab			36.7 ab			27.9 c			26.5 c								
		medium	32.1 b			35.7 bc			34.8 ab			35.4 ab								
		late	34.7 a			38.8 a			33.2 b			37.9 a								
Natural light	'Aufr. Typ'	early	n.d.			36.5 abc	1.0	0.967	28.3 fg	0.9	0.484	24.0 f	1.1	0.097	n.d.			n.d.		
		medium				33.7 c			33.3 bcde			33.0 bc								
		late				35.0 bc			30.4 defg			29.9 cde								
	'Lemona'	early				39.0 ab			26.6 g			25.2 ef								
		medium				36.8 abc			32.3 cdef			31.4 bcd								
		late				40.3 a			31.5 cdef			35.5 ab								
Shading	'Aufr. Typ'	early				32.8 c			30.5 defg			26.7 def								
		medium				33.0 c			40.7 a			36.9 ab								
		late				33.3 c			34.4 bcd			37.0 ab								
	'Lemona'	early				34.4 bc			29.2 efg			27.8 cdef								
		medium				34.6 bc			37.2 ab			39.4 a								
		late				37.3 abc			34.9 bc			40.3 a								

Tab. A 18: Shoots per plant, Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Shoots/plant			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			99.1 a	3.7	0.031	75.8 a	1.7	< 0,001	93.7 a	2.1	< 0,001	88.6 a	3.7	0.083	107.3 a	7.9	0.009
Shading						87.3 b			53.7 b			62.6 b			77.8 a			64.4 b		
	'Aufr. Typ'		87.4 a	6.0	0.546	98.1 a	3.7	0.068	64.5 a	1.7	0.820	80.6 a	2.1	0.123	75.5 b	3.1	0.004	87.0 a	7.5	0.824
	'Lemona'		91.6 a			88.2 a			65.0 a			75.7 a			90.9 a			84.7 a		
		early	83.1 a	6.8	0.288	93.5 a	4.6	0.892	75.8 a	2.1	< 0,001	73.2 b	2.7	0.003	n.d.			n.d.		
		medium	96.6 a			91.5 a			61.2 b			75.2 b								
		late	88.8 a			94.5 a			57.3 b			86.1 a								
Natural light	'Aufr. Typ'		n.d.			101.9 a	5.3	0.438	74.7 a	2.4	0.471	99.0 a	3.0	0.068	79.2 b	4.4	0.354	108.5 a	10.6	0.995
	'Lemona'					96.2 ab			77.0 a			88.4 a			98.0 a			106.2 ab		
Shading	'Aufr. Typ'					94.3 ab			54.3 b			62.1 b			71.9 b			65.6 ab		
	'Lemona'					80.3 b			53.1 b			63.0 b			83.7 ab			63.2 b		
Natural light		early	n.d.			96.3 a	6.4	0.698	89.7 a	2.9	0.229	96.4 a	3.7	< 0,001	n.d.			n.d.		
		medium				99.2 a			70.1 b			91.5 ab								
		late				101.8 a			67.8 b			93.2 ab								
Shading		early				90.8 a			61.9 bc			50.0 c								
		medium				83.8 a			52.3 cd			58.9 c								
		late				87.2 a			46.9 d			78.9 b								
	'Aufr. Typ'	early	78.9 a	9.0	0.897	99.4 a	6.4	0.270	77.5 a	2.9	0.089	71.9 b	3.7	0.254	n.d.			n.d.		
		medium	95.3 a			90.7 a			57.1 b			79.2 ab								
		late	88.1 a			104.2 a			58.9 b			90.6 a								
	'Lemona'	early	87.4 a			87.6 a			74.1 a			74.4 ab								
		medium	97.9 a			92.3 a			65.3 ab			71.2 b								
		late	89.4 a			84.8 a			55.7 b			81.5 ab								
Natural light	'Aufr. Typ'	early	n.d.			101.0 a	9.1	0.921	92.4 a	4.1	0.440	95.5 a	5.2	0.394	n.d.			n.d.		
		medium				94.8 a			63.2 cde			98.1 a								
		late				110.0 a			68.4 bcd			103.3 a								
	'Lemona'	early				91.5 a			86.9 ab			97.2 a								
		medium				103.5 a			76.9 abc			85.0 ab								
		late				93.5 a			67.1 bcd			83.2 abc								
Shading	'Aufr. Typ'	early				97.9 a			62.5 cde			48.4 e								
		medium				86.6 a			50.9 de			60.2 bcde								
		late				98.4 a			49.4 de			77.8 abcd								
	'Lemona'	early				83.7 a			61.2 cde			51.7 de								
		medium				81.0 a			53.7 de			57.5 cde								
		late				76.1 a			44.3 e			79.9 abc								

Tab. A 19: Biomass yield (FM) [dt FM/ha], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Biomass yield [dt FM/ha]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			130.4 a	5.2	0.038	374.9 a	6.7	< 0,001	95.6 a	5.3	0.065	256.4 a	7.0	0.295	71.6 a	4.7	0.151
Shading						111.1 b			301.3 b			112.6 a			245.2 a			81.7 a		
	'Aufr. Typ'		279.5 b	11.5	0.004	126.9 a	4.4	0.019	344.1 a	5.6	0.053	115.2 a	4.5	< 0,001	232.9 b	6.0	0.002	83.0 a	4.7	0.080
	'Lemona'		310.0 a			114.6 b			332.0 a			93.0 b			268.7 a			70.4 a		
		early	285.4 a	12.3	0.353	117.8 a	5.1	0.679	350.0 a	6.4	0.021	122.3 a	5.2	< 0,001	n.d.			n.d.		
		medium	298.4 a			121.4 a			335.6 ab			106.3 b								
		late	300.5 a			123.0 a			328.6 b			83.7 c								
Natural light	'Aufr. Typ'		n.d.			138.7 a	6.2	0.397	382.7 a	8.0	0.566	108.8 a	6.3	0.412	241.0 ab	8.4	0.493	74.2 a	6.6	0.279
	'Lemona'					122.2 ab			367.1 a			82.4 b			271.9 a			69.1 a		
Shading	'Aufr. Typ'					115.1 ab			305.6 b			121.6 a			224.8 b			91.8 a		
	'Lemona'					107.1 b			297.0 b			103.5 ab			265.5 a			71.7 a		
Natural light		early	n.d.			122.2 ab	7.2	0.096	417.7 a	9.0	< 0,001	135.7 a	7.2	< 0,001	n.d.			n.d.		
		medium				138.8 a			358.7 b			82.7 bc								
		late				130.3 ab			348.2 bc			68.4 c								
Shading		early				113.4 ab			282.4 d			108.8 ab								
		medium				104.0 b			312.4 cd			129.9 a								
		late				115.8 ab			309.1 cd			99.0 bc								
	'Aufr. Typ'	early	261.5 a	14.6	0.391	128.7 a	6.6	0.282	359.0 a	8.2	0.536	133.5 a	6.6	0.526	n.d.			n.d.		
		medium	285.5 a			122.5 a			336.9 ab			120.8 a								
		late	291.5 a			129.4 a			336.6 ab			91.4 bc								
	'Lemona'	early	309.3 a			106.9 a			341.1 ab			111.1 ab								
		medium	311.2 a			120.3 a			334.3 ab			91.8 bc								
		late	309.6 a			116.7 a			320.7 b			76.1 c								
Natural light	'Aufr. Typ'	early	n.d.			139.4 a	9.4	0.362	438.9 a	11.6	0.041	154.3 a	9.3	0.260	n.d.			n.d.		
		medium				142.5 a			360.2 bc			98.8 bcdef								
		late				134.2 a			349.0 bcd			73.3 def								
	'Lemona'	early				105.1 a			396.5 ab			117.1 abc								
		medium				135.1 a			357.2 bc			66.6 ef								
		late				126.5 a			347.5 bcd			63.6 f								
Shading	'Aufr. Typ'	early				118.1 a			279.1 e			112.6 abcde								
		medium				102.5 a			313.5 cde			142.7 ab								
		late				124.6 a			324.2 cde			109.5 abcdef								
	'Lemona'	early				108.7 a			285.7 e			105.0 abcdef								
		medium				105.5 a			311.3 cde			117.0 abcd								
		late				106.9 a			293.9 de			88.5 cdef								

Tab. A 20: Biomass yield (DM) [dt DM/ha], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Biomass yield [dt DM/ha]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			31.8 a	1.3	0.003	77.5 a	1.3	< 0,001	24.2 a	1.3	0.359	55.0 a	1.8	0.003	18.3 a	1.0	0.554
Shading						22.3 b			52.7 b			22.3 a			42.9 b			17.4 a		
	'Aufr. Typ'		55.5 a	1.5	0.209	28.8 a	1.2	0.024	67.7 a	1.3	0.009	25.8 a	1.2	0.001	46.0 b	1.5	0.007	18.6 a	1.0	0.301
	'Lemona'		58.2 a			25.3 b			62.5 b			20.7 b			51.9 a			17.1 a		
		early	45.4 c	1.8	< 0,001	25.1 a	1.4	0.169	49.3 c	1.6	< 0,001	24.6 a	1.4	0.059	n.d.			n.d.		
		medium	54.3 b			28.1 a			66.8 b			24.3 a								
		late	70.9 a			28.0 a			79.2 a			20.7 a								
Natural light	'Aufr. Typ'		n.d.			34.3 a	1.7	0.273	80.2 a	1.9	0.838	27.4 a	1.7	0.351	53.0 ab	2.1	0.227	18.6 a	1.4	0.531
	'Lemona'					29.2 ab			74.7 a			21.0 b			57.0 a			18.0 a		
Shading	'Aufr. Typ'					23.3 bc			55.1 b			24.1 ab			38.9 c			18.7 a		
	'Lemona'					21.4 c			50.3 b			20.4 b			46.9 b			16.2 a		
Natural light		early	n.d.			28.5 ab	2.0	0.283	63.6 cd	2.3	0.347	29.6 a	1.9	0.001	n.d.			n.d.		
		medium				34.4 a			78.1 b			22.5 ab								
		late				32.5 a			90.7 a			20.3 b								
Shading		early				21.6 b			34.9 e			19.6 b								
		medium				21.8 b			55.5 d			26.2 ab								
		late				23.6 b			67.7 c			21.1 ab								
	'Aufr. Typ'	early	43.1 c	2.6	0.508	27.9 a	1.9	0.585	51.3 d	2.3	0.893	27.1 a	1.8	0.855	n.d.			n.d.		
		medium	54.7 b			29.2 a			69.5 bc			27.3 a								
		late	68.6 a			29.3 a			82.2 a			22.8 ab								
	'Lemona'	early	47.7 bc			22.3 a			47.3 d			22.1 ab								
		medium	53.9 bc			27.0 a			64.1 c			21.3 ab								
		late	73.1 a			26.7 a			76.1 ab			18.7 b								
Natural light	'Aufr. Typ'	early	n.d.			32.9 ab	2.7	0.623	66.9 cdef	3.2	0.668	34.1 a	2.6	0.415	n.d.			n.d.		
		medium				36.5 a			80.6 abc			26.1 ab								
		late				33.6 ab			93.1 a			21.9 ab								
	'Lemona'	early				24.1 ab			60.3 def			25.2 ab								
		medium				32.3 ab			75.5 bcd			18.9 b								
		late				31.3 ab			88.3 ab			18.8 b								
Shading	'Aufr. Typ'	early				22.9 b			35.6 g			20.2 b								
		medium				22.0 b			58.3 ef			28.6 ab								
		late				25.0 ab			71.4 cde			23.6 ab								
	'Lemona'	early				20.4 b			34.3 g			18.9 b								
		medium				21.7 b			52.7 f			23.7 ab								
		late				22.1 b			64.0 def			18.6 b								

Tab. A 21: Leaf fresh matter yield [dt FM/ha], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Leaf yield [dt FM/ha]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			86.8 a	4.7	0.029	144.4 a	3.1	< 0,001	51.4 a	2.2	0.139	129.4 a	4.7	0.025	50.4 a	3.0	0.841
Shading						67.6 b			109.6 b			56.7 a			109.5 b			49.5 a		
	'Aufr. Typ'		134.6 b	4.2	0.020	80.6 a	3.9	0.090	135.7 a	3.1	< 0,001	59.7 a	2.0	< 0,001	116.6 a	3.9	0.215	53.0 a	3.0	0.178
	'Lemona'		143.7 a			73.8 a			118.4 b			48.3 b			122.3 a			47.0 a		
		early	122.3 b	4.5	< 0,001	79.3 a	4.3	0.635	139.0 a	3.8	0.001	66.8 a	2.4	< 0,001	n.d.			n.d.		
		medium	143.8 a			77.6 a			124.7 b			50.5 b								
		late	151.5 a			74.8 a			117.4 b			44.8 b								
Natural light	'Aufr. Typ'		n.d.			92.5 a	5.5	0.242	153.2 a	4.4	0.952	59.4 a	2.8	0.064	125.8 ab	5.6	0.740	51.6 a	4.2	0.407
	'Lemona'					81.0 ab			135.6 b			43.4 b			133.0 a			49.2 a		
Shading	'Aufr. Typ'					68.7 b			118.2 c			60.1 a			107.3 b			54.3 a		
	'Lemona'					66.6 b			101.1 d			53.3 ab			111.7 ab			44.8 a		
Natural light		early	n.d.			86.6 ab	6.1	0.086	165.7 a	5.4	0.018	75.1 a	3.2	< 0,001	n.d.			n.d.		
		medium				93.4 a			138.8 b			39.7 c								
		late				80.3 ab			128.8 bc			39.4 c								
Shading		early				72.0 ab			112.3 c			58.6 b								
		medium				61.7 b			110.7 c			61.3 ab								
		late				69.2 ab			106.0 c			50.3 bc								
	'Aufr. Typ'	early	113.9 c	5.4	0.270	87.2 a	5.5	0.272	147.8 a	5.4	0.943	72.2 a	3.1	0.065	n.d.			n.d.		
		medium	142.6 ab			79.1 a			134.2 ab			59.9 a								
		late	147.5 ab			75.5 a			125.0 abc			47.2 b								
	'Lemona'	early	130.6 bc			71.4 a			130.1 abc			61.4 a								
		medium	145.0 ab			76.0 a			115.3 bc			41.1 b								
		late	155.5 a			74.0 a			109.8 c			42.5 b								
Natural light	'Aufr. Typ'	early	n.d.			99.8 a	7.8	0.540	181.4 a	7.7	0.112	84.1 a	4.3	0.410	n.d.			n.d.		
		medium				94.8 ab			144.4 abc			52.3 bcd								
		late				82.9 ab			133.8 bcd			41.7 cde								
	'Lemona'	early				73.5 ab			149.9 ab			66.1 ab								
		medium				91.9 ab			133.2 bcde			27.0 e								
		late				77.6 ab			123.8 bcde			37.0 de								
Shading	'Aufr. Typ'	early				74.7 ab			114.2 bcde			60.4 bc								
		medium				63.4 ab			124.0 bcde			67.4 ab								
		late				68.2 ab			116.3 bcde			52.6 bcd								
	'Lemona'	early				69.2 ab			110.3 cde			56.7 bcd								
		medium				60.1 b			97.4 de			55.3 bcd								
		late				70.3 ab			95.7 e			48.0 bcde								

Tab. A 22: DM leaf yield [dt DM/ha], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different (p < 0.05). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Leaf yield [dt DM/ha]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			20.6 a	1.2	0.007	30.9 a	0.7	< 0,001	15.2 a	0.5	0.163	30.1 a	1.0	0.002	14.1 a	0.7	0.006
Shading						13.4 b			20.4 b			14.0 a			22.0 b			10.7 b		
	'Aufr. Typ'		27.0 b	0.7	0.001	17.6 a	1.0	0.214	27.1 a	0.7	0.005	16.0 a	0.5	< 0,001	25.3 a	0.8	0.086	13.0 a	0.7	0.247
	'Lemona'		31.0 a			16.4 a			24.2 b			13.3 b			26.8 a			11.8 a		
		early	25.0 b	0.9	< 0,001	17.0 a	1.1	0.472	24.1 a	0.9	0.102	16.8 a	0.6	< 0,001	n.d.			n.d.		
		medium	24.2 b			17.8 a			26.4 a			14.1 b								
		late	37.7 a			16.3 a			26.4 a			13.0 b								
Natural light	'Aufr. Typ'		n.d.			21.6 a	1.4	0.360	32.5 a	1.0	0.886	17.1 a	0.7	0.090	29.6 a	1.2	0.554	14.9 a	1.0	0.822
	'Lemona'					19.5 ab			29.4 a			13.3 b			30.7 a			13.4 ab		
Shading	'Aufr. Typ'					13.6 bc			21.8 b			14.8 ab			21.0 b			11.2 ab		
	'Lemona'					13.3 c			19.0 b			13.3 b			23.0 b			10.2 b		
Natural light		early	n.d.			20.1 ab	1.6	0.135	30.8 a	1.2	0.066	19.8 a	0.8	< 0,001	n.d.			n.d.		
		medium				22.7 a			31.8 a			12.9 b								
		late				18.9 ab			30.2 a			13.0 b								
Shading		early				13.9 b			17.4 c			13.8 b								
		medium				12.8 b			21.0 bc			15.3 b								
		late				13.7 b			22.6 b			12.9 b								
	'Aufr. Typ'	early	22.6 c	1.2	0.140	18.8 a	1.4	0.172	25.2 a	1.2	0.863	17.9 a	0.8	0.044	n.d.			n.d.		
		medium	23.7 c			18.2 a			28.1 a			16.6 ab								
		late	34.7 b			15.8 a			28.0 a			13.4 bcd								
	'Lemona'	early	27.5 c			15.1 a			23.0 a			15.8 abc								
		medium	24.7 c			17.3 a			24.8 a			11.6 d								
		late	40.7 a			16.7 a			24.8 a			12.5 cd								
Natural light	'Aufr. Typ'	early	n.d.			23.1 a	2.0	0.585	33.0 a	1.7	0.348	21.9 a	1.2	0.309	n.d.			n.d.		
		medium				23.1 a			32.9 a			16.3 ab								
		late				18.7 abc			31.5 ab			13.3 bc								
	'Lemona'	early				17.0 abc			28.6 abc			17.7 ab								
		medium				22.3 ab			30.7 ab			9.5 c								
		late				19.0 abc			28.8 abc			12.7 bc								
Shading	'Aufr. Typ'	early				14.6 abc			17.4 d			13.9 bc								
		medium				13.3 abc			23.3 bcd			16.9 ab								
		late				12.9 bc			24.6 abcd			13.5 bc								
	'Lemona'	early				13.2 abc			17.4 d			13.8 bc								
		medium				12.3 c			18.8 d			13.7 bc								
		late				14.4 abc			20.7 cd			12.4 bc								

Tab. A 23: DM content of the leaves [%], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Leaf DM content [%]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			23.7 a	0.4	< 0,001	21.6 a	0.5	0.005	31.2 a	0.6	< 0,001	23.3 a	0.3	< 0,001	27.9 a	0.5	< 0,001
Shading						19.8 b			18.6 b			24.7 b			20.1 b			21.6 b		
	'Aufr. Typ'		20.0 b	0.6	0.007	21.5 a	0.4	0.335	20.0 a	0.4	0.208	27.2 b	0.5	0.006	21.5 a	0.3	0.491	24.7 a	0.4	0.603
	'Lemona'		21.4 a			22.0 a			20.3 a			28.7 a			21.9 a			24.9 a		
		early	20.4 b	0.7	< 0,001	21.1 a	0.4	0.079	17.2 c	0.4	< 0,001	25.0 b	0.6	< 0,001	n.d.			n.d.		
		medium	16.9 c			22.5 a			21.0 b			29.4 a								
		late	24.9 a			21.6 a			22.3 a			29.5 a								
Natural light	'Aufr. Typ'		n.d.			23.3 a	0.5	0.549	21.5 a	0.5	0.725	29.8 b	0.7	0.026	23.5 a	0.4	0.147	28.7 a	0.6	0.008
	'Lemona'					24.1 a			21.8 a			32.6 a			23.2 a			27.2 a		
Shading	'Aufr. Typ'					19.7 b			18.4 b			24.6 c			19.6 b			20.6 b		
	'Lemona'					19.9 b			18.8 b			24.9 c			20.5 b			22.7 b		
Natural light		early	n.d.			23.1 ab	0.6	0.991	18.7 bc	0.6	0.026	26.4 b	0.8	< 0,001	n.d.			n.d.		
		medium				24.5 a			22.9 a			33.9 a								
		late				23.5 a			23.3 a			33.3 a								
Shading		early				19.1 c			15.6 d			23.5 b								
		medium				20.6 bc			19.1 c			25.0 b								
		late				19.6 c			21.2 ab			25.7 b								
	'Aufr. Typ'	early	19.9 c	0.8	0.159	21.2 a	0.6	0.210	16.9 c	0.4	0.439	24.5 c	0.7	0.506	n.d.			n.d.		
		medium	16.7 d			22.6 a			20.8 b			28.2 ab								
		late	23.6 b			20.7 a			22.3 a			28.8 a								
	'Lemona'	early	21.0 c			21.0 a			17.5 c			25.5 bc								
		medium	17.1 d			22.5 a			21.2 ab			30.6 a								
		late	26.2 a			22.4 a			22.2 a			30.1 a								
Natural light	'Aufr. Typ'	early	n.d.			23.1 abc	0.9	0.972	18.3 cdef	0.6	0.548	26.0 c	1.0	0.094	n.d.			n.d.		
		medium				24.3 ab			22.7 ab			31.3 b								
		late				22.5 abc			23.6 a			32.1 ab								
	'Lemona'	early				23.1 abc			19.2 cde			26.8 c								
		medium				24.6 a			23.1 a			36.5 a								
		late				24.5 ab			23.1 a			34.5 ab								
Shading	'Aufr. Typ'	early				19.3 c			15.4 f			22.9 c								
		medium				20.8 abc			18.8 d			25.1 c								
		late				18.9 c			21.1 abc			25.6 c								
	'Lemona'	early				19.0 c			15.8 ef			24.1 c								
		medium				20.3 bc			19.3 bcd			24.8 c								
		late				20.4 abc			21.4 abc			25.7 c								

Tab. A 24: Leaf:stem ratio, Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Leaf:stem ratio			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			1.9 a	0.1	0.040	0.7 a	0.0	0.821	1.2 a	0.1	0.291	1.1 a	0.0	0.253	2.5 a	0.1	< 0,001
Shading						1.5 b			0.7 a			1.1 a			1.1 a			1.8 b		
	'Aufr. Typ'		1.1 a	0.0	0.382	1.6 a	0.1	0.174	0.7 a	0.0	0.876	1.1 a	0.1	0.076	1.2 a	0.0	0.030	2.0 b	0.1	0.015
	'Lemona'		1.2 a			1.8 a			0.7 a			1.2 a			1.0 b			2.4 a		
		early	1.2 a	0.0	0.266	2.1 a	0.1	< 0,001	0.9 a	0.0	< 0,001	1.3 a	0.1	< 0,001	n.d.			n.d.		
		medium	1.2 a			1.6 b			0.7 b			0.9 b								
		late	1.1 a			1.4 b			0.5 c			1.3 a								
Natural light	'Aufr. Typ'		n.d.			1.8 ab	0.1	0.673	0.7 a	0.0	0.397	1.2 a	0.1	0.323	1.2 a	0.1	0.585	2.4 ab	0.1	0.435
	'Lemona'					2.0 a			0.7 a			1.3 a			1.1 a			2.6 a		
Shading	'Aufr. Typ'					1.4 b			0.7 a			1.0 a			1.1 a			1.6 c		
	'Lemona'					1.6 ab			0.7 a			1.2 a			1.0 a			2.1 bc		
Natural light		early	n.d.			2.4 a	0.2	0.122	0.9 a	0.0	0.561	1.3 a	0.1	0.052	n.d.			n.d.		
		medium				1.8 ab			0.7 b			1.0 b								
		late				1.4 b			0.5 c			1.4 a								
Shading		early				1.8 ab			1.0 a			1.4 a								
		medium				1.4 b			0.6 b			0.9 b								
		late				1.4 b			0.5 c			1.1 ab								
	'Aufr. Typ'	early	1.1 a	0.1	0.471	2.0 a	0.2	0.325	0.9 a	0.0	0.380	1.3 ab	0.1	0.039	n.d.			n.d.		
		medium	1.2 a			1.6 ab			0.7 b			1.0 bc								
		late	1.0 a			1.2 b			0.5 c			1.1 abc								
	'Lemona'	early	1.2 a			2.2 a			1.0 a			1.4 a								
		medium	1.2 a			1.6 ab			0.6 b			0.9 c								
		late	1.1 a			1.6 ab			0.5 c			1.4 a								
Natural light	'Aufr. Typ'	early	n.d.			2.3 ab	0.2	0.159	0.9 a	0.0	0.675	1.3 abc	0.1	0.419	n.d.			n.d.		
		medium				1.8 abc			0.7 b			1.1 abc								
		late				1.4 bc			0.5 bc			1.3 abc								
	'Lemona'	early				2.5 a			0.9 a			1.4 abc								
		medium				1.9 abc			0.7 b			0.8 bc								
		late				1.5 abc			0.5 bc			1.6 a								
Shading	'Aufr. Typ'	early				1.7 abc			0.9 a			1.3 abc								
		medium				1.5 abc			0.7 b			0.8 c								
		late				1.0 c			0.5 bc			0.9 bc								
	'Lemona'	early				1.9 abc			1.0 a			1.4 ab								
		medium				1.2 c			0.6 bc			0.9 bc								
		late				1.7 abc			0.5 c			1.3 abc								

Tab. A 25: Essential oil content [%], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different (p < 0.05). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Essential oil [%]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			0.4 a	0.0	0.171	0.1 b	0.0	< 0,001	0.4 a	0.0	< 0,001	0.1 a	0.0	0.456	0.7 a	0.0	0.163
Shading						0.4 a			0.2 a			0.3 b			0.1 a			0.6 a		
	'Aufr. Typ'		0.2 b	0.0	< 0,001	0.3 b	0.0	< 0,001	0.1 b	0.0	< 0,001	0.3 b	0.0	< 0,001	0.1 b	0.0	0.005	0.6 b	0.0	0.023
	'Lemona'		0.3 a			0.4 a			0.2 a			0.4 a			0.2 a			0.7 a		
		early	0.2 b	0.0	0.020	0.4 a	0.0	0.001	0.1 c	0.0	< 0,001	0.5 a	0.0	< 0,001	n.d.			n.d.		
		medium	0.2 ab			0.4 ab			0.2 b			0.3 b								
		late	0.3 a			0.4 b			0.2 a			0.3 b								
Natural light	'Aufr. Typ'		n.d.			0.3 c	0.0	0.218	0.1 d	0.0	0.012	0.3 a	0.0	0.205	0.1 b	0.0	0.031	0.7 a	0.0	0.865
	'Lemona'					0.4 ab			0.2 b			0.4 a			0.2 a			0.8 a		
Shading	'Aufr. Typ'					0.4 bc			0.1 c			0.3 b			0.1 b			0.6 a		
	'Lemona'					0.5 a			0.2 a			0.3 a			0.1 ab			0.7 a		
Natural light		early	n.d.			0.4 ab	0.0	0.170	0.1 d	0.0	< 0,001	0.5 a	0.0	0.526	n.d.			n.d.		
		medium				0.4 ab			0.1 c			0.3 b								
		late				0.3 b			0.2 b			0.3 b								
Shading		early				0.5 a			0.1 d			0.4 a								
		medium				0.4 ab			0.2 b			0.3 bc								
		late				0.4 ab			0.3 a			0.2 c								
	'Aufr. Typ'	early	0.1 c	0.0	0.865	0.4 bcd	0.0	0.955	0.0 e	0.0	< 0,001	0.4 b	0.0	0.112	n.d.			n.d.		
		medium	0.2 bc			0.3 cd			0.1 d			0.3 c								
		late	0.2 bc			0.3 d			0.2 c			0.2 c								
	'Lemona'	early	0.3 ab			0.5 a			0.1 d			0.5 a								
		medium	0.3 ab			0.4 ab			0.2 b			0.3 c								
		late	0.4 a			0.4 abc			0.3 a			0.3 c								
Natural light	'Aufr. Typ'	early	n.d.			0.4 abc	0.0	0.242	0.0 e	0.0	0.077	0.4 ab	0.0	0.421	n.d.			n.d.		
		medium				0.3 bc			0.1 d			0.3 cd								
		late				0.3 c			0.1 d			0.3 cd								
	'Lemona'	early				0.4 abc			0.1 de			0.6 a								
		medium				0.5 ab			0.2 c			0.3 cd								
		late				0.4 bc			0.2 bc			0.3 cd								
Shading	'Aufr. Typ'	early				0.4 abc			0.0 e			0.4 bc								
		medium				0.3 bc			0.1 d			0.2 d								
		late				0.3 bc			0.2 bc			0.2 d								
	'Lemona'	early				0.5 a			0.1 d			0.5 ab								
		medium				0.4 abc			0.3 b			0.3 cd								
		late				0.4 ab			0.4 a			0.2 d								

Tab. A 26: Total phenolic content [mg GAE/g DM], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Total phenolic content [mg GAE/g DM]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			95.3 a	2.5	0.003	111.9 a	2.1	< 0,001	115.0 a	2.9	< 0,001	177.8 a	3.4	< 0,001	163.9 a	3.5	< 0,001
Shading						78.6 b			83.8 b			88.8 b			154.2 b			123.7 b		
	'Aufr. Typ'		139.7 a	4.5	0.781	83.9 a	2.3	0.055	95.8 a	2.1	0.180	98.9 a	2.7	0.099	162.2 a	3.4	0.141	144.7 a	3.5	0.725
	'Lemona'		141.3 a			89.9 a			99.9 a			104.9 a			169.8 a			142.9 a		
		early	131.6 a	5.3	0.120	89.6 a	2.8	0.398	73.3 b	2.6	< 0,001	122.9 a	3.3	< 0,001	n.d.			n.d.		
		medium	145.9 a			86.6 a			107.7 a			102.9 b								
		late	144.0 a			84.6 a			112.6 a			80.0 c								
Natural light	'Aufr. Typ'		n.d.			95.9 a	3.3	0.022	110.4 a	3.0	0.686	117.8 a	3.8	0.002	168.1 ab	4.8	0.030	170.3 a	4.9	0.043
	'Lemona'					94.7 a			113.3 a			112.2 ab			187.4 a			157.4 a		
Shading	'Aufr. Typ'					71.9 b			81.1 b			80.0 c			156.3 b			119.0 b		
	'Lemona'					85.2 a			86.5 b			97.5 b			152.1 b			128.4 b		
Natural light		early	n.d.			98.0 a	3.9	0.623	92.5 b	3.7	0.049	138.7 a	4.5	0.535	n.d.			n.d.		
		medium				96.8 a			117.5 a			114.0 b								
		late				91.1 ab			125.7 a			92.5 c								
Shading		early				81.2 ab			54.1 c			107.0 bc								
		medium				76.5 b			97.8 b			91.8 c								
		late				78.0 b			99.5 b			67.5 d								
	'Aufr. Typ'	early	126.5 a	7.3	0.377	90.4 a	3.8	0.227	71.1 b	3.7	0.749	111.6 b	4.3	0.008	n.d.			n.d.		
		medium	143.8 a			81.4 a			104.2 a			102.5 b								
		late	148.7 a			80.0 a			112.0 a			82.7 c								
	'Lemona'	early	136.7 a			88.9 a			75.4 b			134.1 a								
		medium	148.0 a			91.9 a			111.1 a			103.2 b								
		late	139.2 a			89.1 a			113.2 a			77.3 c								
Natural light	'Aufr. Typ'	early	n.d.			101.4 a	5.4	0.900	91.1 d	5.3	0.785	129.2 ab	6.1	0.008	n.d.			n.d.		
		medium				95.6 ab			115.9 abcd			127.6 ab								
		late				90.8 abc			124.4 ab			96.8 cde								
	'Lemona'	early				94.7 ab			93.9 cd			148.1 a								
		medium				98.0 a			119.1 abc			100.3 bcd								
		late				91.4 abc			127.1 a			88.2 cde								
Shading	'Aufr. Typ'	early				79.3 abc			51.2 e			94.1 cde								
		medium				67.2 c			92.5 d			77.5 de								
		late				69.3 bc			99.6 bcd			68.6 e								
	'Lemona'	early				83.1 abc			57.0 e			120.0 abc								
		medium				85.7 abc			103.2 abcd			106.2 bcd								
		late				86.8 abc			99.4 bcd			66.4 e								

Tab. A 27: Antioxidant capacity [µmol TE/g DM], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Antioxidant capacity [µmol TE/g DM]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	**Cultivar**	**Development**	**LS mean**	**SE**	**p**	**LS mean**	**SE**	**p**	**LS mean**	**SE**	**p**	**LS mean**	**SE**	**p**	**LS mean**	**SE**	**p**	**LS mean**	**SE**	**p**
Natural light			n.d.			1810.0 a	41.8	< 0,001	1884.2 a	34.1	< 0,001	1814.2 a	43.8	< 0,001	2837.3 a	29.4	< 0,001	2726.0 a	57.2	< 0,001
Shading						1414.6 b			1472.8 b			1314.5 b			2487.6 b			2086.6 b		
	'Aufr. Typ'		2030.1 a	44.3	0.059	1547.5 b	41.8	0.035	1616.5 b	30.6	0.003	1538.2 a	43.8	0.411	2630.1 a	29.4	0.145	2399.6 a	51.9	0.845
	'Lemona'		2143.9 a			1677.1 a			1740.5 a			1590.5 a			2694.8 a			2412.9 a		
		early	1948.9 b	52.4	0.011	1656.2 a	51.2	0.520	1361.8 b	36.0	< 0,001	1828.2 a	55.9	< 0,001	n.d.			n.d.		
		medium	2164.8 a			1572.9 a			1798.8 a			1643.6 a								
		late	2147.2 a			1607.9 a			1874.8 a			1221.3 b								
Natural light	**'Aufr. Typ'**		n.d.			1787.5 a	59.2	0.162	1814.2 a	43.3	0.675	1881.5 a	62.0	0.005	2781.1 a	41.5	0.274	2787.0 a	73.4	0.082
	'Lemona'					1832.6 a			1954.2 a			1747.0 a			2893.5 a			2664.9 a		
Shading	**'Aufr. Typ'**					1307.5 b			1418.8 b			1194.9 b			2479.0 b			2012.2 b		
	'Lemona'					1521.7 b			1526.8 b			1434.0 b			2496.1 b			2160.9 b		
Natural light		**early**	n.d.			1803.5 a	72.5	0.450	1564.9 b	50.9	0.155	2117.9 a	75.9	0.668	n.d.			n.d.		
		medium				1811.2 a			1959.9 a			1866.9 ab								
		late				1815.4 a			2127.8 a			1457.9 c								
Shading		**early**				1508.8 ab			1158.7 c			1538.4 bc								
		medium				1334.6 b			1637.8 b			1420.2 c								
		late				1400.3 b			1621.9 b			984.8 d								
	'Aufr. Typ'	**early**	1843.3 b	71.2	0.259	1611.7 a	72.5	0.691	1263.9 b	48.6	0.376	1727.8 a	75.9	0.268	n.d.			n.d.		
		medium	2091.3 ab			1472.0 a			1743.9 a			1649.4 a								
		late	2155.5 ab			1558.7 a			1841.5 a			1237.4 b								
	'Lemona'	**early**	2054.4 ab			1700.6 a			1459.6 b			1928.5 a								
		medium	2238.4 a			1673.8 a			1853.8 a			1637.7 a								
		late	2138.8 ab			1657.1 a			1908.2 a			1205.3 b								
Natural light	**'Aufr. Typ'**	**early**	n.d.			1770.1 ab	102.5	0.738	1475.5 de	68.7	0.443	2056.8 ab	107.3	0.059	n.d.			n.d.		
		medium				1759.6 ab			1915.0 abc			2075.5 ab								
		late				1832.7 a			2052.0 a			1512.2 cd								
	'Lemona'	**early**				1836.9 a			1654.3 cd			2179.0 a								
		medium				1862.8 a			2004.7 ab			1658.4 abc								
		late				1798.2 a			2203.6 a			1403.5 cde								
Shading	**'Aufr. Typ'**	**early**				1453.4 abc			1052.4 f			1398.8 cde								
		medium				1184.4 c			1572.9 de			1223.3 cde								
		late				1284.6 bc			1631.1 cd			962.6 e								
	'Lemona'	**early**				1564.3 abc			1265.0 ef			1678.0 abc								
		medium				1484.7 abc			1702.8 bcd			1617.1 bc								
		late				1515.9 abc			1612.7 cd			1007.0 de								

Tab. A 28: Rosmarinic acid content [%], Rauischholzhausen 2013–2015. Results of the three-factorial analysis. Values with different letters within a factor or factor combination are significantly different ($p < 0.05$). LS mean = least squares mean; SE = standard error; p = p-value; n.d. = not determined.

Rosmarinic acid [%]			2013						2014						2015					
			Cut 1			Cut 2			Cut 1			Cut 2			Cut 1			Cut 2		
Light intensity	Cultivar	Development stage	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p	LS mean	SE	p
Natural light			n.d.			3.8 a	0.1	< 0,001	3.8 a	0.1	< 0,001	4.2 a	0.1	< 0,001	5.8 a	0.1	< 0,001	5.3 a	0.2	< 0,001
Shading						2.2 b			2.2 b			2.7 b			4.4 b			3.2 b		
	'Aufr. Typ'		4.1 a	0.1	0.194	3.0 a	0.1	0.719	3.0 a	0.1	0.828	3.5 a	0.1	0.384	4.9 a	0.1	0.106	4.2 a	0.1	0.860
	'Lemona'		4.3 a			2.9 a			3.0 a			3.4 a			5.2 a			4.3 a		
		early	3.7 b	0.2	0.005	2.9 a	0.1	0.356	2.1 b	0.1	< 0,001	3.7 a	0.2	< 0,001	n.d.			n.d.		
		medium	4.6 a			2.9 a			3.5 a			3.8 a								
		late	4.3 ab			3.1 a			3.3 a			2.9 b								
Natural light	'Aufr. Typ'		n.d.			4.0 a	0.1	0.003	3.8 a	0.1	0.483	4.4 a	0.2	0.149	5.6 a	0.1	0.377	5.5 a	0.2	0.010
	'Lemona'					3.6 a			3.7 a			4.0 a			6.0 a			5.1 a		
Shading	'Aufr. Typ'					2.0 b			2.1 b			2.7 b			4.3 b			2.9 b		
	'Lemona'					2.3 b			2.2 b			2.8 b			4.4 b			3.4 b		
Natural light		early	n.d.			3.6 a	0.1	0.581	2.9 b	0.2	0.382	4.8 a	0.2	0.026	n.d.			n.d.		
		medium				3.7 a			4.2 a			4.2 ab								
		late				3.9 a			4.2 a			3.5 bc								
Shading		early				2.2 b			1.3 c			2.7 cd								
		medium				2.1 b			2.8 b			3.3 bc								
		late				2.2 b			2.4 b			2.3 d								
	'Aufr. Typ'	early	3.6 b	0.2	0.950	3.2 ab	0.1	0.008	2.1 b	0.2	0.306	3.5 ab	0.2	0.025	n.d.			n.d.		
		medium	4.5 ab			2.9 ab			3.4 a			4.0 a								
		late	4.2 ab			2.9 ab			3.4 a			3.2 ab								
	'Lemona'	early	3.9 ab			2.7 b			2.1 b			4.0 a								
		medium	4.7 a			2.9 ab			3.6 a			3.5 ab								
		late	4.4 ab			3.3 a			3.2 a			2.6 b								
Natural light	'Aufr. Typ'	early	n.d.			3.9 a	0.2	0.341	2.9 c	0.2	0.054	4.4 abc	0.3	0.043	n.d.			n.d.		
		medium				4.0 a			4.3 a			4.9 ab								
		late				4.0 a			4.2 ab			3.9 abcd								
	'Lemona'	early				3.3 ab			3.0 c			5.2 a								
		medium				3.5 ab			4.1 ab			3.5 bcde								
		late				3.9 a			4.2 ab			3.2 cde								
Shading	'Aufr. Typ'	early				2.4 cd			1.4 de			2.5 de								
		medium				1.9 cd			2.5 c			3.1 cde								
		late				1.8 d			2.6 c			2.6 de								
	'Lemona'	early				2.0 cd			1.3 e			2.9 cde								
		medium				2.2 cd			3.2 bc			3.6 bcd								
		late				2.7 bc			2.2 cd			2.0 e								

Declaration / Erklärung

I declare: this dissertation submitted is a work of my own, written without any illegitimate help by any third party and only with materials indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At any time during the investigations carried out by me and described in the dissertation, I followed the principles of good scientific practice as defined in the “Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice”.

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

Place, Date / Ort, Datum

Marco Russo