Europ.J.Hort.Sci., **71** (1). S. 36–44, 2006, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Influence of Air Temperature on Productivity and Quality of some Medicinal Plants under Controlled Environment Conditions

A. E. Manukyan[1)] and W. H. Schnitzler[2)]
([1)]G.S. Davtyan Institute of Hydroponics Problems, National Academy of Sciences, Yerevan, Armenia and [2)]Chair of Vegetable Science, Quality of Vegetal Foodstuff, Center of Life Sciences Weihenstephan, Technische Universität München, Freising-Weihenstephan, Germany)

Summary

Cultivation of medicinal plants under controlled environment is a good opportunity to study the influence of main exogenic factors on herbal yield and quality. Such studies can give new data about the yield raise of species, as well as about improvement of content and composition of bioactive substances with practical applications. The main purpose of this study was to investigate the effect of different air temperatures (15, 20, 25 °C) under controlled environment conditions on herbal yield and content, composition and antioxidative capacity of secondary metabolites of lemon catmint (*Nepeta cataria* L. f. *citriodora*), lemon balm (*Melissa officinalis* L.) and sage (*Salvia officinalis* L.). It was found, that tested air temperatures had substantial influence on productivity of studied medicinal plants under controlled environment conditions. In case of lemon catmint 20 °C gave maximum raw medicinal material, for lemon balm 25 °C was more effective, while in sage, both 20 and 25 °C were optimal compared to the lowest temperature treatment (15 °C). In lemon catmint and sage, 15 and 25 °C temperatures provided high content of essential oil, while 20 and 25 °C treatments gave maximum recovery of essential oil. In case of lemon balm, 25 °C temperature gave high content and yield of essential oil. The composition of herbs' essential oil essentially depended on air temperature, as well as on harvest rounds. Air temperatures had less effect on content of flavonoids. However some differences were noted in connection with harvest rounds. Antioxidative capacity of flavonoid-rich extracts by ABTS-system showed differences according to air temperatures and first or second cut.

Zusammenfassung

Einfluss der Lufttemperatur unter kontrollierten Umgebungsbedingungen auf die Produktivität und Qualität einiger Arzneipflanzen. Die Kultivierung von Arzneipflanzen unter kontrollierten Anbaubedingungen im hydroponischen System bietet eine gute Möglichkeit, viele exogene Einflussfaktoren auf Kräuterertrag und Qualität zu ermitteln. Durch solche Experimente können neue Erkenntnisse über die Erhöhung des Ertrags der Pflanzenarten sowie über die Veränderung der Inhaltsstoffe und die Zusammensetzung von biologisch aktiven Substanzen für praktische Anwendungen gewonnen werden. Hauptsächlich sollte mit dieser Arbeit die Wirkung von verschiedenen Lufttemperaturen (15, 20, 25 °C) unter kontrollierten Umgebungsbedingungen auf Kräuterertrag, Inhalt und Zusammensetzung der ätherischen Öle, Flavonoidgehalt und antioxidative Kapazität von flavonoid-reichen Extrakten in Zitronen-Katzenminze (*Nepeta cataria* L. f. *citriodora*), Zitronenmelisse (*Melissa officinalis* L.) und Salbei (*Salvia officinalis* L.) untersucht werden. Lufttemperaturen haben einen wesentlichen Einfluss auf die Produktivität der untersuchten Arzneipflanzen. Die Zitronen-Katzenminze gab bei 20 °C maximalen Kräuterertrag, für die Zitronenmelisse waren 25 °C wirksamer, während bei Salbei sowohl 20 und 25 °C verglichen mit der niedrigsten Temperaturbehandlung (15 °C) optimal waren. Bei Zitronen-Katzenminze und Salbei führten Temperaturen von 15 und 25 °C zu einer Erhöhung des Gesamtgehalts an ätherischem Öl, während der Ertrag bei 20 und 25 °C sein Maximum hatte. Im Falle der Zitronenmelisse waren 25 °C als optimal anzusehen. Die Zusammensetzung der ätherischen Öle der untersuchten Kräuterarten hing im wesentlichen von der Lufttemperatur sowie vom Erntezeitpunkt ab. Auf den Gehalt an Flavonoiden hatte die Lufttemperatur keinen größeren Einfluss. Jedoch wurden Unterschiede im Zusammenhang mit dem Erntezeitpunkt bemerkt. Die antioxidative Kapazität von flavonoid-reichen Extrakten im ABTS-System zeigte Unterschiede hinsichtlich Lufttemperatur und Erntezeitpunkt.

Key words. antioxidative capacity – climatic chamber – essential oils – flavonoids – *Melissa officinalis* L. – *Nepeta cataria* L. f. *citriodora* – *Salvia officinalis* L.

Introduction

Recent years have witnessed tremendous interest in the cultivation of medicinal plants. The increasing popularity and success of herbal products in the markets, especially in the developed countries, has attracted attention of pharmaceutical companies of all stature. Besides herbal drugs, also natural health products, phytocosmetics and personal care products have created special niches by consumers. As a result, more and more companies are producing and marketing herbal products (ICS-UNIDO 2002).

Voluminous information is being generated on different aspects of medicinal plants and their products all over the world. This industry has rapidly grown over the last years, generating yearly sales of more than 10 billion US Dollar in North America alone (DORAIS et al. 2001). The worldwide sale during last decade has increased with an annual growth rate averaging between 5 and 15 % (ICS-UNIDO 2002). Conservative estimates of world turnover of medicinal plants and their products put them at around 40 billion US Dollar for the year 1996 (DORAIS et al. 2001), which is expected to reach 5 trillion US Dollar by 2050 (ICS-UNIDO 2002). The fact that herbal remedies continue to be in demand by the general public despite presence of large number of synthetic drugs in the market speaks of their inherent strength. Encouraging results in scientific investigations have added to enhance their acceptability among people, who hitherto were endorsing the use of conventional drugs. Health professionals and planners are showing keen interest in medicinal plants, heralding a new era for the development of herbal drugs (ICS-UNIDO 2002).

Currently, at least 25 % of medicines used in the world are derived from plants and many synthetic analogues build on prototype compounds are isolated from plants (KOSEV 2001). In 1998, research at Harvard University found that 40 % of Americans were using some form of complementary medicine on a regular basis (EISENBERG et al. 1998). European countries are among the top world importers with 120,000 metric tons annually (25 % of world trade) (CRAKER 2003). According to the World Health Organisation, 80 % of the population of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs (BANNERMAN 1993).

Traditionally, vegetables and ornamentals have been grown under soilless cultivation, but new medicinal and aromatic plants become economically important objects for hydroponics and controlled environment agriculture (BETTRAY and VÖMEL 1992; MIRZA et al. 1998; MAIRAPETYAN and TADEVOSYAN 1999; DORAIS et al. 2001; MAIRAPETYAN and MANUKYAN 2002; MANUKYAN 2003). This tendency must be supported by good research and experiments for soilless and controlled environment production of herbs, which will also ecologically benefit the wild herb flora.

One of the major factors of plants' growth and productivity is air temperature effecting yield and quality of herbs. The aim of this study is to investigate for some herbs the effect of different air temperatures on herbal yield, content of secondary metabolites (essential oils and flavonoids), their composition, and the antioxidative potential when grown hydroponically in climatic chambers. We have focused on above-mentioned bioactive compounds as important for the quality of herbs, because these plants are also well known as aromatic with valuable, high-demand essential oils, at the same time flavonoids are chosen as important group of health promoters. Such studies can give new data about the improvement of herbal yield and content, composition of bioactive substances with practical applications.

Materials and Methods

The experiments were conducted at the research station of the Chair of Vegetable Science - Quality of Vegetal Foodstuff of the Center of Life Sciences Weihenstephan, Technical University München. As medicinal plants lemon catmint (*Nepeta cataria* L. f. *citriodora*), lemon balm (*Melissa officinalis* L.), and sage (*Salvia officinalis* L.), all three belonging to the family *Lamiaceae*, were studied. Cultivation was started on August 19, 2003, by sowing homogenous seeds derived from hydroponically grown plants in Armenia (Armenian endemic species) in common peat in a greenhouse. Seeds were stratified by application of 2 g l^{-1} NO_3 solution (not for lemon balm) and kept at 10 °C for one week (all species). Pricking was done on September 10, 2003 (sage) and September 15, 2003 (lemon catmint and lemon balm) into cell trays with peat substrate.

Each climatic chamber had 2 ebb and flood tables with 2.7 m^2 surface area. Seedlings were planted in plastic pots (appr. 3 l) 18 plants per 1 m^2. For all species uncomposted wood fibre "Toresa special (Ts) " was used as substrate, which had pH 5.9 and contained 0.8 g l^{-1} water-soluble salts (233 mg l^{-1} N, 9 mg l^{-1} P_20_5 and 90 mg l^{-1} K_20). The nutrient solution was made up from 70 g "Flory" basis 1 (P-43; K-220; Mg-21; B-0,1; Cu-0,0015; Fe-1; Mn-0,2; Mo-0,003; Zn-0,0025 mg l^{-1}) in addition to 21.4 g ammonium nitrate and 48.2 g calcium nitrate per 100 l of water with pH of 5.5–7.1 and EC of 1.7–3.2 mS cm^{-1}. Once a week, pH and EC was measured and the necessary nutrients and water were added. Irrigation was automatically once a day for the duration of 10 minutes.

Plants were grown under three different air temperatures (Table 1) and harvested once at 8 weeks, and the regrowth for the second time cut at 16 weeks after transplanting. The criterion for lemon catmint harvest was the time of flower emergence (only for 15 °C treatment there was 2 weeks lateness from regular cuts); the other two species were harvested almost at the same time having minimum 50 (for lemon balm) and 10 g $plant^{-1}$ (for sage) fresh biomass. The fresh medicinal material was dried at 35 °C for 5–7 days.

Table 1. Set points for environmental conditions of plants in climatic chambers.

Day-night interval	Light intensity (lux)	Air temperature* (°C)			Air humidity** (%)
Day (15 hours)	8000	15	20	25	70
Night (9 hours)	–	15	20	25	70

* ±0.5 °C; ** ±2.5 %

For distillation, 200 g of fresh plant material was placed in a 2-liter round-bottom flask with approximately 1200 ml deionized water. The essential oil was extracted by steam distillation and pentane extraction for 1 h (SDE). The extracts were dried over sodium sulfate and concentrated under a stream of nitrogen. The amount of essential oil was determined gravimetrically. The composition of the essential oil was analysed, by GC and GC-MS. The oils were diluted in acetone (split 1:40) with separation of the compounds by GC (Fisons Instruments Mega 5360, Italy) on a Supelco-Wax capillary column (60 m, i.d. = 0.32 mm, 0.25 µm film thickness) with helium as carrier gas (0.8 ml min^{-1}) and a temperature program: 50 °C (3 min), 10 °C min^{-1}, 120 °C (2 min), 2 °C min^{-1}, 155 °C (0 min), 8 °C min^{-1}, 240 °C. Identification of essential oil main compounds was performed with a GC-MS system (HP 5890 Series II/HP 5971 A, HEWLETT PACKARD) on the same column and with the same temperature program by electron impact ionisation at 70 eV. Mass spectra were evaluated by comparison of retention times and mass spectra (WILEY 1990) and with an own terpenoid mass spectra database. Identification was confirmed by comparing retention times with available standard substances.

For determination of flavonoids and antioxidative capacity of flavonoid-rich extracts, plant material was freeze-dried and ground. Flavonoids were extracted from 0.4 g dry material with 80 % MeOH followed by liquid/liquid-extraction with ethylacetate. The amount of flavonoids was determined gravimetrically. The ABTS-system was used to evaluate the antioxidative capacity of flavonoid-rich extracts (AMAO 1999). The ability to reduce the peroxidase (myeloglobin/H_2O_2)-gener-

Table 2. Influence of air temperatures on productivity of herbs.

Species	Temperature (°C)	Harvests	Fresh medicinal material (g $plant^{-1}$)	Raw medicinal material	
				(g $plant^{-1}$)	(%)
		First	78.8	7.3	9.3
	15	Second	11.5	1.6	13.9
		Total	90.3 c	8.9 c	9.9 b
		First	95.0	11.8	12.4
Lemon catmint	20	Second	34.1	5.0	14.7
		Total	129.1 a	16.8 a	13.0 a
		First	87.5	11.6	13.3
	25	Second	20.4	3.1	15.2
		Total	107.9 b	14.7 b	13.6 a
LSD_{05}*	–	–	15.5	2.0	1.6
		First	17.7	2.4	13.6
	15	Second	15.1	2.0	13.2
		Total	32.8 b	4.4 c	13.4 a
		First	67.9	7.0	10.3
Lemon balm	20	Second	46.5	5.5	11.8
		Total	114.4 a	12.5 b	10.9 b
		First	78.3	9.0	11.5
	25	Second	46.6	6.5	13.9
		Total	124.9 a	15.5 a	12.4 ab
LSD_{05}*	–	–	15.0	1.9	1.5
		First	5.1	0.9	17.6
	15	Second	3.8	0.5	13.2
		Total	8.9 b	1.4 b	15.7 a
		First	38.1	5.6	14.7
Sage	20	Second	21.0	2.8	13.3
		Total	59.1 a	8.4 a	14.2 a
		First	35.0	5.3	15.1
	25	Second	16.8	2.2	13.1
		Total	51.8 a	7.5 a	14.5 a
LSD_{05}*	–	–	8.8	1.0	1.9

* Within the same species and total harvest

ated ABTS•+ [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation] has been used to rank the antioxidant activity of various agents including dietary flavonoids and chalcones (CHAN et al. 2003). In this system, myoglobin and H_2O_2 oxidise ABTS to the green ABTS•+ radical cation. 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation (ABTS•+) is a stable chromophore which absorbs strongly at 734 nm. This reaction can be followed photometrically. The lower rate of ABTS•+ formation indicates reducing or Fe-chelating properties of the extract. The analyses were performed with 3–4 replications. The statistical analyses were performed according to DOSPEKHOV (1985).

Results

Productivity of herbs was mainly affected by the air temperature in amplitudes of 15-20-25 °C under controlled environment conditions. Table 2 shows that lowest temperature (15 °C) was unfavourable for productivity of all species. From the tested temperatures, 20 °C was optimal for high productivity of lemon catmint (16.8 g plant^{-1} raw medicinal material) and further increasing the temperature negatively influenced yield of herb. In case of lemon balm, there was direct correlation between the temperature increase and productivity and highest temperature (25 °C) gave maximum raw medicinal material (15.5 g plant^{-1}). In sage, there was no statistical difference between the 20 and 25 °C treatments with regard to the raw medicinal material (8.4 and 7.5 g plant^{-1}, respectively). In case of lemon catmint, minimum percent of dry medicinal material was at 15 °C, in lemon balm at 20 °C, while in sage, there was no statistic difference between the temperature treatments.

Tested temperatures have a considerable influence on essential oil content of herbs under the controlled environment conditions. It can be seen from Fig. 1 that for the first as well as for the second harvest, the treatments with 15 and 25 °C provided maximum content of essential oil in lemon catmint (in average 0.106 and 0.115 %, respectively) and in sage (0.129 and 0.138 %, respectively). In case of lemon balm, high content of essential oil registered at 25 °C only (in average 0.091 %). At the same, air temperature was more essential for essential oil biosynthesis and accumulation in lemon balm. The difference between the high and low treatments was more than 2.5 times.

Calculations for essential oil total yield showed that the treatments with 20 and 25 °C provided maximum yield in case of lemon catmint (0.116 and 0.118 ml plant^{-1}, respectively) and sage (0.065 and 0.072 ml plant^{-1}, respectively), while in lemon balm maximum yield of essential oil was determined at 25 °C (0.107 ml plant^{-1}) (Fig. 2).

The substance composition of the essential oil in lemon catmint depended on air temperature, as well as on sequence of harvests under controlled environment conditions (Table 3). The main compounds of lemon catmint essential oil were geraniol (6.46–27.60 %), geranial (9.58–20.52 %), citronellol (10.96–18.94 %), neral (6.01–16.12 %), trans-3-hexen-1-ol (1.01–9.36 %) and nerol (1.85–8.21 %). Eighteen identified compounds represented appropriately 60, 59, 60 (first harvest) and 93, 95, 95 % (second harvest) of the total oil under 15, 20, 25 °C, respectively. Different air temperatures had a considerable influence on the contents of almost all identified compounds in lemon catmint essential oil, except for neral. The increase of air temperature from 15 to 25 °C decreased the contents of some main compounds, such as geraniol, geranial (only for the first harvest cut), and trans-3-hexen-1-ol, while in case of citronellol and nerol (only at the second harvest) it was on the contrary. Comparatively high contents of geraniol, geranial, citronellol, neral, and nerol were for the second harvest cut, while only the content of trans-3-hexen-1-ol was higher at the first harvest. It was also noted that the contents of neryl acetate and β-ionone were mainly differed depending on harvest time.

Lemon balm essential oil consisted mainly of geranial (8.90–44.45 %), neral (5.44–33.93 %) and geraniol (6.48–21.41 %) under controlled environment conditions (Table 4). Fifteen identified compounds represented about 51, 70, 88 % (first harvest) and 76, 94, 93 % (second harvest) of the total oil under 15, 20, 25 °C temperatures conditions, respectively. The contents of all identified compounds were affected by air temperature and differed depending on harvest time. It has been also noted that the increase of air temperature from 15 to 25 °C increased significantly the contents of geranial and neral in lemon balm essential oil. In case of geraniol, the increase of temperature from 15 to 20 °C increased its content, while further temperature increase up to 25 °C had negative influence on geraniol content in essential oil. The amount of citronellal and geranyl acetate greatly depended on environmental

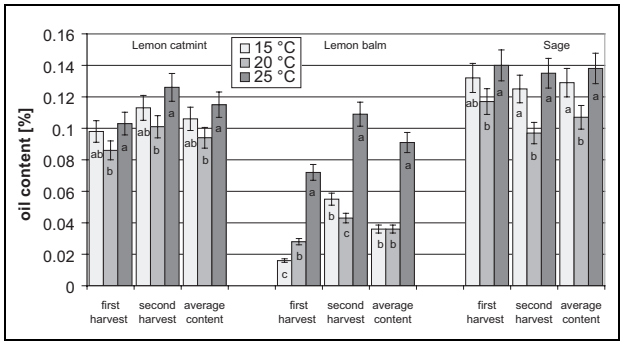


Fig. 1. Influence of air temperatures on essential oil content of herbs.

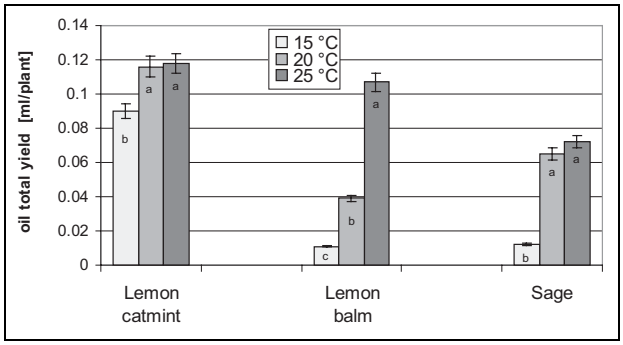


Fig. 2. Influence of air temperatures on essential oil total yield of herbs.

Table 3. Influence of air temperatures on lemon catmint essential oil composition.

Compound*	Content in essential oil (%)					
	first harvest cut			second harvest cut		
	15 °C	20 °C	25 °C	15 °C	20 °C	25 °C
cis-3-hexenal	0.85 a**	0.67 b	0.57 b	0.17 b	0.22 a	0.22 a
trans-ocimene	0.06 b	0.04 c	0.11 a	0.01 a	0.00 b	0.01 a
cis-3-hexenyl acetate	0.28 a	0.23 b	0.23 b	0.03 a	0.02 b	0.03 a
6-methyl-5-hepten-2-on	1.69 a	1.66 a	1.52 a	1.58 a	1.51 a	0.96 b
trans-3-hexen-1-ol	9.36 a	8.71 ab	7.59 b	2.19 a	2.04 a	1.01 b
cis-3-hexen-1-ol	0.52 b	0.62 a	0.36 c	0.34 a	0.38 a	0.20 b
trans-2-hexen-1-ol	0.05 b	0.08 a	0.08 a	0.01 b	0.02 a	0.01 b
citronellal	1.33 a	1.37 a	1.14 b	2.61 a	2.46 a	1.91 b
citronellyl formiat	1.65 b	1.74 b	2.22 a	1.16 b	1.38 a	1.52 a
citronellyl acetate	0.33 ab	0.29 b	0.36 a	0.08 c	0.10 b	0.12 a
neral	6.71 a	6.40 a	6.01 a	15.66 a	16.12 a	14.98 a
neryl acetate	1.53 a	1.17 b	1.04 b	0.00 a	0.00 a	0.00 a
geranial	11.42 a	10.27 ab	9.58 b	20.11 a	20.52 a	19.44 a
geranyl acetate	0.06 a	0.04 b	0.04 b	0.04 b	0.04 b	0.09 a
citronellol	10.96 c	13.50 b	17.58 a	15.23 b	15.93 b	18.94 a
nerol	1.87 a	1.85 a	2.08 a	7.08 b	7.33 ab	8.21 a
geraniol	8.18 a	7.24 ab	6.46 b	27.18 a	26.48 a	27.60 a
β-ionone	2.83 b	3.11 ab	3.52 a	0.01 b	0.02 a	0.01 b
not idetified	7.38 a	6.91 a	6.98 a	0.06 a	0.05 a	0.01 b

* Only identified and not identified main substances of about or more than 1 % are presented
** Statistics are brought within the same compound and harvest among the three temperatures

Table 4. Influence of air temperatures on lemon balm essential oil composition.

Compound*	Content in essential oil (%)					
	First harvest cut			Second harvest cut		
	15 °C	20 °C	25 °C	15 °C	20 °C	25 °C
1,8-cineole	0.28 b**	0.34 b	0.54 a	0.32 a	0.16 b	0.28 a
6-methyl-5-hepten-2-on	1.99 a	2.00 a	1.57 b	0.84 b	1.48 a	1.51 a
trans-3-hexen-1-ol	0.95 b	1.11 a	1.07 ab	2.65 a	1.04 b	1.18 b
trans-2-hexen-1-ol	0.94 a	0.53 b	0.28 c	1.05 a	0.28 b	0.18 b
1-octen-3-ol	0.75 a	0.36 b	0.06 c	0.44 a	0.01 b	0.01 b
citronellal	7.47 a	0.17 b	0.06 b	10.44 a	0.15 b	0.05 b
linalool	1.39 a	0.20 c	0.45 b	0.74 c	1.14 b	1.39 a
neral	5.44 c	19.50 b	31.21 a	16.63 c	28.74 b	33.93 a
geranyl formiat	0.96 b	2.00 a	0.32 c	0.21 a	0.23 a	0.23 a
geranial	8.90 c	28.39 b	41.34 a	22.15 c	37.47 b	44.45 a
geranyl butyrat	1.05 a	0.63 b	0.45 c	1.17 a	0.48 b	0.53 b
geranyl acetate	12.15 a	0.24 b	0.21 b	3.55 a	0.15 b	0.03 b
nerol	0.39 c	1.04 a	0.79 b	0.70 c	1.30 a	0.91 b
geraniol	6.48 c	11.91 a	9.66 b	15.35 b	21.41 a	8.72 c
β- ionone	2.29 a	1.22 b	0.02 c	0.04 a	0.00 b	0.00 b
not identified	1.33 a	1.01 b	0.41 c	0.02 a	0.01 b	0.01 b
not identified	7.20 a	5.89 b	1.61 c	0.05 a	0.02 c	0.04 b
not identified	0.61 a	0.40 b	0.07 c	9.45 a	1.82 b	1.67 b

* Only identified and not identified main substances of about or more than 1 % are presented
** Statistics are brought within the same compound and harvest among the three temperatures

Table 5. Influence of air temperatures on sage essential oil composition.

Compound*	Content in essential oil (%)					
	First harvest cut			Second harvest cut		
	15°C	20°C	25°C	15°C	20°C	25°C
α-pinene	1.76 b**	1.60 b	2.67 a	0.98 b	0.63 b	3.28 a
camphene	1.70 b	2.02 b	2.82 a	1.17 b	0.86 b	2.89 a
β-pinene	6.97 a	3.85 b	2.96 c	4.88 a	3.81 b	3.72 b
sabinene	0.47 a	0.37 b	0.24 c	0.39 a	0.41 a	0.23 b
β-myrcene	0.81 a	0.71 a	0.72 a	0.00 b	0.00 b	0.04 a
α-terpinene	0.16 a	0.11 b	0.13 b	0.10 b	0.11 b	0.13 a
limonene	0.52 c	0.71 b	1.11 a	0.39 b	0.00 c	1.20 a
1,8-cineole	8.16 a	7.56 ab	6.85 b	5.87 b	6.19 b	10.16 a
cis-ocimene	0.10 b	0.20 a	0.18 a	0.11 b	0.19 a	0.18 a
cis-3-hexenal	0.26 a	0.08 b	0.03 c	0.25 a	0.08 b	0.03 c
γ-terpinene	0.05 a	0.02 b	0.01 c	0.04 a	0.02 b	0.01 c
p-cymole	0.07 b	0.07 b	0.11 a	0.04 c	0.06 b	0.08 a
terpinolene	0.14 c	0.19 b	0.31 a	0.05 c	0.17 b	0.32 a
trans-3-hexen-1-ol	1.10 a	1.21 a	1.16 a	0.87 b	1.26 a	1.03 b
trans-2-hexen-1-ol	0.07 c	0.16 b	0.23 a	0.08 c	0.20 a	0.14 b
α-thujone	35.15 a	36.77 a	29.35 b	28.44 b	34.94 a	25.66 b
β-thujone	12.38 a	6.03 b	6.63 b	15.38 a	14.82 a	6.35 b
camphor	8.35 c	15.71 b	23.21 a	6.46 c	11.36 b	20.11 a
bornyl acetate	1.65 a	1.38 b	1.31 b	1.25 a	1.25 a	1.10 a
β-caryophyllene	5.18 a	4.80 a	4.16 b	3.66 a	3.67 a	3.20 a
α-humulene	1.46 b	1.78 a	2.00 a	1.68 a	1.52 ab	1.41 b
borneol	0.02 b	0.08 a	0.03 b	0.05 a	0.05 a	0.00 b
myrtenol	0.37 a	0.40 a	0.30 b	0.40 a	0.22 b	0.10 c
viridiflorol	5.57 a	5.05 a	3.00 b	4.47 a	3.74 b	2.59 c
not identified	0.26 c	0.58 b	0.74 a	0.30 b	0.34 b	0.66 a
manool	3.33 b	4.54 a	4.88 a	2.30 b	1.97 b	3.39 a

* Only identified and not identified main substances of about or more than 1 % are present
** Statistics are brought within the same compound and harvest among the three temperatures

temperature and treatment with low temperature (15 °C) gave maximum results in both harvests.

Among the 25 identified compounds in sage essential oil, α-thujone (25.66–36.77 %), camphor (6.46–23.21 %), β-thujone (6.03–15.38 %), 1,8-cineole (5.87–10.16 %), β-pinene (2.96–6.97 %), viridiflorol (2.59–5.57 %), β-caryophyllene (3.20–5.18 %) were the major constituents under controlled environment conditions (Table 5). Identified compounds represented about 96, 95, 94 % (first harvest) and 79, 88, 87 % (second harvest) of the total oil under 15, 20, 25 °C, respectively. Different air temperatures had a considerable influence on the content of all identified compounds in sage essential oil. The variations depended also on harvest time. The increase of temperature from 15 to 25 °C decreased the contents of some main compounds, such as α-thujone, β-thujone, β-pinene, viridiflorol, β-caryophyllene (only for the first harvest cut), while the content of camphor increased. Air temperature increase decreased the content of 1,8-cineole at the first harvest and increased it at the second harvest.

The influence of tested air temperatures on flavonoid content of studied herbs was less critical (Fig. 3) than in case of essential oils content (Fig. 1). Nevertheless, there were certain differences between the treatments in both harvests (exception of lemon catmint at first harvest), while in average there were significant differences only in case of lemon balm and 15 and 20 °C provided maximum content of flavonoids (3.4 %). In average, lemon balm and sage contained more flavonoids than lemon catmint (Fig. 3).

Antioxidative capacity of flavonoid-rich extracts from studied herbs was also compared by the ABTS-system. Lemon balm and sage had higher antioxidative capacity even at a dilution of 1:200 (Fig. 4, 5) compared to lemon catmint at only 1:20 (Fig. 6). As shown in Fig. 4, 5, 6 the extracts of the studied herbs grown under different air temperatures corresponded in their antioxidative capacity according to their amount of flavonoids. In case of lemon catmint, the extracts obtained from herbs grown under 20 °C (first harvest) and under 15 °C (second harvest) showed a comparatively stronger inhibition than the other treatments (Fig. 6), while in lemon balm, 15, 20 °C provided high antioxidative capacity for both harvest times (Fig. 4). In case of sage, if low temperature treatment

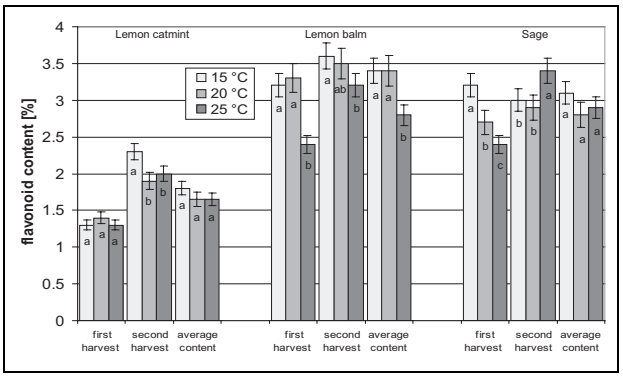


Fig. 3. Influence of air temperatures on flavonoid content of herbs.

(15 °C) provided high antioxidative capacity at the first harvest, then the high temperature treatment (25 °C) at the second harvest provided a stronger inhibition (Fig. 5). Additionally, the shapes of the curves differ considerably with a comparatively longer lag phase for 15 °C at second harvest for lemon catmint, for 15, 20 °C at second harvest for lemon balm and for 25 °C at second harvest for sage. In general, it can be stated that there was a stronger inhibition with the extracts from the second harvest cutting, especially in the case of lemon catmint, which probably stipulated a comparatively higher content of flavonoids (Fig. 3).

Discussion

Presented data from this research trial confirm that air temperature is an important external factor to influence productivity and quality of the herbs studied under controlled environment conditions. From the tested temperatures 20 °C can be recommended for high productivity of lemon catmint and sage, while in case of lemon balm 25 °C seems to be more effective. The reaction of species to changes in air temperature differed with differences between the minimum and maximum yield of lemon catmint, lemon balm and sage approximately 1.9, 3.5, 6.0 times, respectively.

The contents of essential oils in lemon catmint and lemon balm obtained under controlled conditions in the climatic chambers correspond with the literature data for outside cultivation of these herbs (ADZET et al. 1992; MAIRAPETYAN et al. 2001), while sage essential oil content was reduced (SEYBOLD 1998). It is known that sage oil content and composition highly depends on light intensity (LI et al. 1996). The observed decrease may have been stipulated by the influence of the comparatively low (8000 lux) light intensity under test situation in the chambers compared with the average natural light intensity during the natural vegetation period for optimisation of essential oil biosynthesis and accumulation in sage. Temperatures of 20 and 25 °C were more effective for high yield of essential oil in case of lemon catmint and sage, while for lemon balm 25 °C gave maximum results.

The analysis of the data on air temperatures showed some direct correlation between composition of essential oils and air temperature changes. In particular, the increase of air temperature from 15 up to 25 °C increased the contents of neral, geranial (in lemon balm) and α-pinene, terpinolene, camphor (in sage), while the contents of citronellal (in lemon catmint and lemon balm), geranyl acetate (in lemon balm) and γ-terpinene (in sage) decreased. Such information is useful for management of essential oil quality in respect to environmental control for hydroponically grown herbs un-

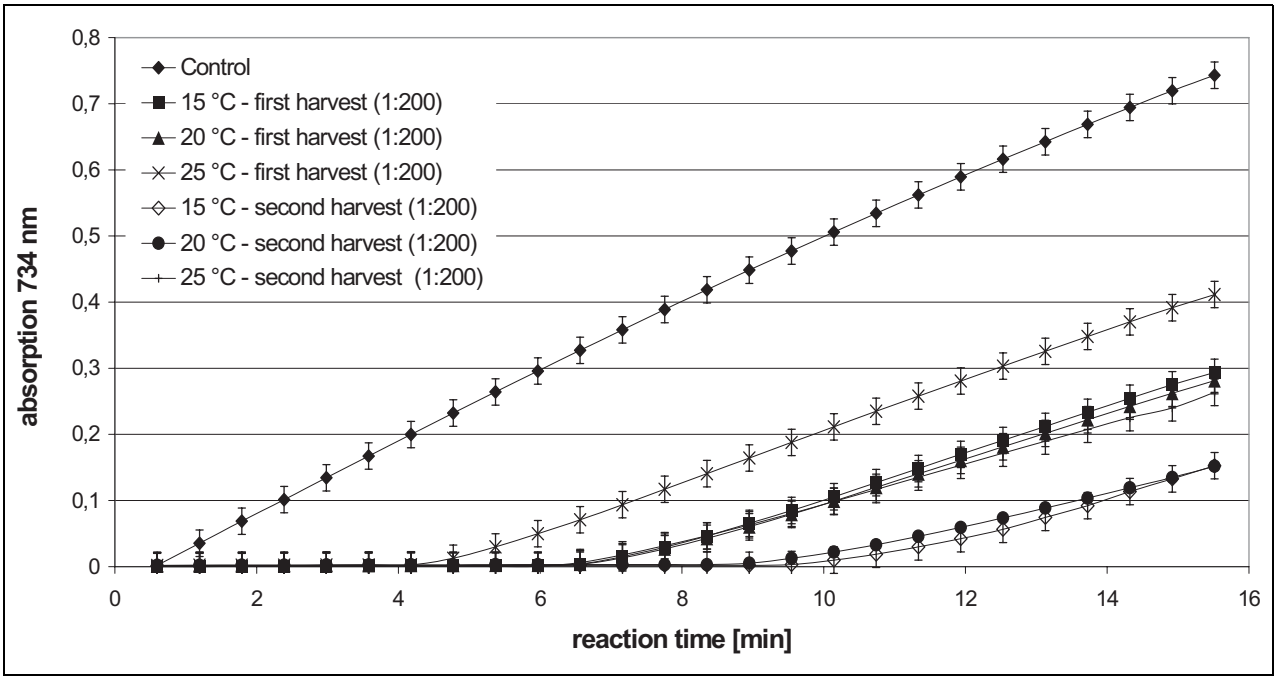


Fig. 4. Antioxidative capacity by ABTS model system with flavonoid-rich extract from lemon balm grown under three air temperature conditions.

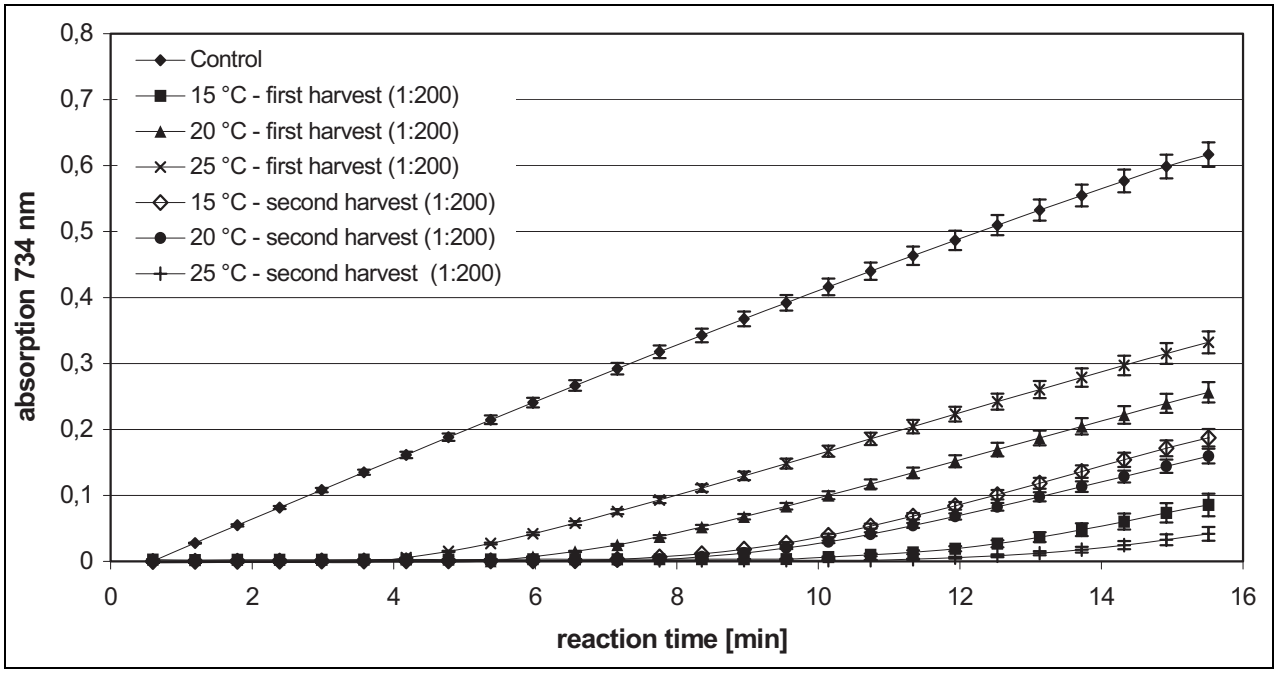


Fig. 5. Antioxidative capacity by ABTS model system with flavonoid-rich extract from sage grown under three air temperature conditions.

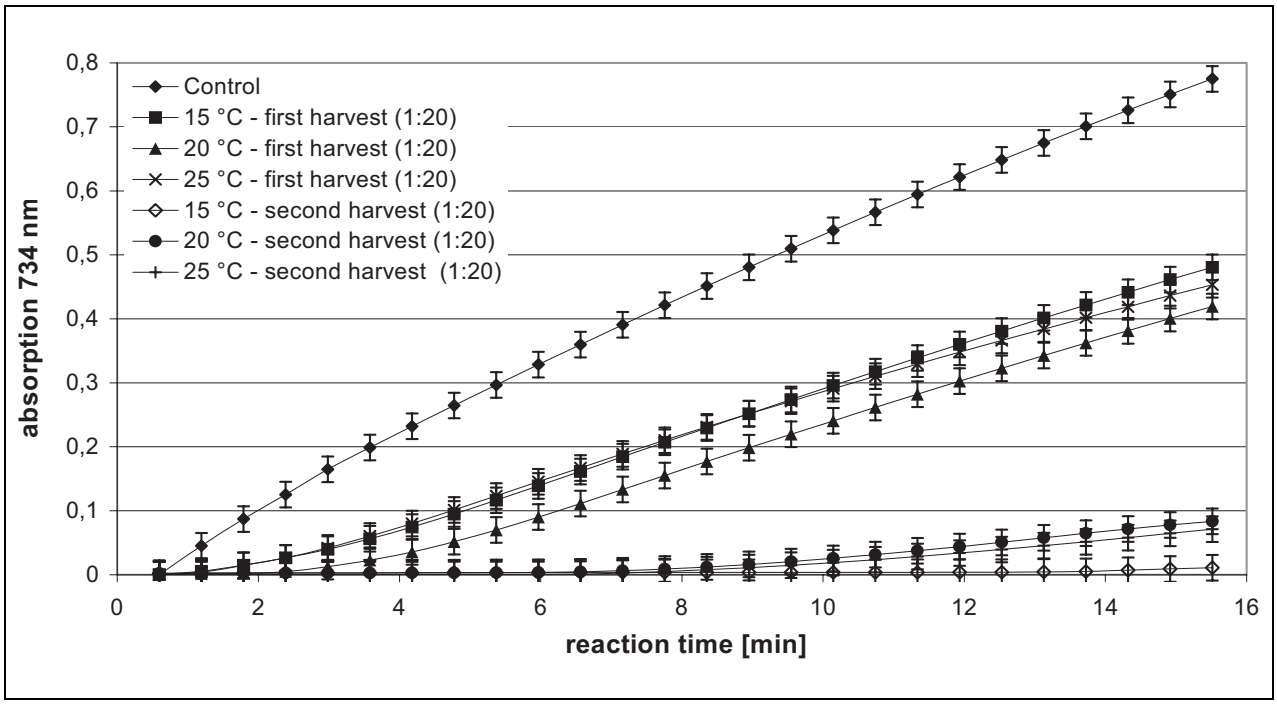


Fig. 6. Antioxidative capacity by ABTS model system with flavonoid-rich extract from lemon catmint grown under three air temperature conditions.

der protected cultivation. At the same time, in case of some volatile compounds of oils, stronger changes have been noted depending on harvest time (plant age). The variation of lemon catmint essential oil composition depending on natural climatic changes during one day was already described for open-air hydroponics conditions (MAIRAPETYAN et al. 2001).

The tested air temperatures had less effect on the content of flavonoids of the three studied herbs, however, antioxidative capacity of flavonoid-rich extracts by the ABTS-system showed significant differences. The differences between some treatments were not consistent in the content of flavonoids and the antioxidative capacity by the ABTS-system was not proportional. This can be

explained by the fact that some main flavonoids in the extracts of these herbs, affected by the temperatures may have specific inhibition potentials.

Several authors have published data on the influence of temperature on productivity and quality of plants including herbs under controlled environment conditions. In particular, MÁTHÉ et al. (1992) reported about the yield variations of essential oil in *Salvia officinalis* L. depended on climatic conditions. BETTRAY and VÖMEL (1992) tested the influences of several air temperatures on yield and active compounds of four different Camomile [*Chamomilla recutita* (L.) Rausch.] genotypes in a phytotron experiment. It was found, besides the genotypic differences, there were positive effects on flower drug yield, flowering speed, and essential oil content as determined by increasing temperature under controlled conditions. Herb quantity and single flowerhead weight reacted negatively to higher temperature. A comparison of the response to differences between day and night temperatures and a short (about 2 hours) temperature drop on plant height (internode length) and other plant characteristics of *Melissa officinalis* L. has been investigated by MOE et al. (1995). ROEBER and HAAS (1997) studied the influence of temperature and water quality on plant quality and growth of *Hydrangea* x *hybrida* and found that the plants cultivated "cool and dry" were more compact, healthy, and possessed higher quality grade.

Conclusions

In conclusion, tested air temperatures had a considerable influence on lemon catmint, lemon balm and sage productivity and quality under controlled environment conditions. Raw medicinal material, essential oil content, yield and the amount of some volatile compounds depended on air temperatures. The flavonoid content of the three studied herbs also changed, but to a lesser extent, while the flavonoid-rich extracts significantly differed in their antioxidative capacity.

Acknowledgements

This work was partly supported by the International Centre for Scientific Culture (ICSC) - World Laboratory (Lausanne, Switzerland).

References

ADZET, T., R. PONZ, E. WOLF and E. SCHULTE 1992: Genetic variability of the essential oil content of *Melissa officinalis*. Planta Med **58**, 558–561.

AMAO, M. 1999: Methods of measure the antioxidant activity in plant material. A comparative discussion. Free Radic Res **31**, 89–96.

BANNERMAN, R. 1993: Traditional Medicine and Healthcare Coverage. WHO, Geneva.

BETTRAY, G. and A. VÖMEL 1992: Influence of temperature on yield and active principles of *Chamomilla recutita (*L.*)* Rausch. under controlled conditions. Acta Horticulturae **306**, 83–87.

CHAN, T., G. GALATI, A. PANNALA, C. RICE-EVANS and P. O'BRIEN 2003: Simultaneous detection of the antioxidant and pro-oxidant activity of dietary polyphenolics in a peroxidase system. Free Radic Res **37**, 787–794.

CRAKER, L. 2003: Production and demand – a view to the future of MAP. Acta Horticulturae **597**, 15–21.

DORAIS, M., A.P. PAPADOPOULOS, X. LUO, S. LEONHART, A. GOSSELIN and K. PEDNEAULT 2001: Soilless greenhouse production of medicinal plants in northeastern Canada. Acta Horticulturae **554**, 297–303.

DOSPEKHOV, B. 1985: Field experiment methodology. Agropromizdat, Moscow (in Russian).

EISENBERG, D., R. DAVIS, S. ETTNER, S. APPEL, S. WILKEY and M. VAN ROMPAY 1998: Trends in alternative medicine use in the United States, 1990–1997: Results of a Follow-up National Survey. J. Am. Med. Assoc. **280**, 1569–1575.

ICS-NIDO 2002: Workshop on "Regulations for production and marketing of medicinal plant products" 18–20 November 2002, Trieste, Italy (http://www.ics.trieste.it/documents/environment/medicinalplants/activities/ws-Nov2002/ AideMemoire.pdf).

KOSEV, P. 2001: Full reference book of medicinal plants. Expo-Press, Moscow, 3–7 (in Russian).

LI, Y., L. CRACKER and T. POTTER 1996: Effect of light level on essential oil production of sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*). Acta Horticulturae **426**, 419–426.

MAIRAPETYAN, S. and A. TADEVOSYAN 1999: Plant mineral nutrition optimization under hydroponic conditions. Gitutyun, Yerevan, Armenia (in Russian).

MAIRAPETYAN, S., A. MANUKYAN, G. MAMIKONYAN and Yu. DANGYAN 2001: The quantitative and qualitative changes of catmint essential oil during one day under hydroponics conditions. Armenian Chemical Journal **54**, 119–127 (in Russian).

MAIRAPETYAN, S. and A. MANUKYAN 2002: Catmint culture. Practical Hydroponics & Greenhouses **62**, 63–65.

MANUKYAN, A. 2003: Hydroponic production of medicinal herbs – Greater celandine. Practical Hydroponics & Greenhouses **70**, 30–31.

MÁTHÉ, Jr. I., L. OLÁH, A. MÁTHÉ, V. MIKLÓSSY, J. BERNÁTH and G. BLÜDEN 1992: Changes in the essential oil production of *Salvia officinalis* under climatic conditions of the temperature belt. Planta Med **58**, A680.

MIRZA, M., M. YOUNUS, Y. HOYANOB and R. CURRIE 1998: Greenhouse production of Echinacea and other medicinal plants. Paper presented at Opportunities and Profits II: Special Crops into the 21st Century. Edmonton, AB, Canada.

MOE, R., K. WILLUMSEN, I. IHLEBEKK, A. STUPA, N. GLOMSRUD and L. MORTENSEN 1995: DIF and temperature drop responses in SDP and LDP, a comparison. Acta Horticulturae **378**, 27–33.

ROEBER, R. and H. HAAS 1997: Plant quality and growth of *Hydrangea x hybrida* as influenced by temperature and water quantity. Acta Horticulturae **450**, 425–431.

SEYBOLD, A. 1998: Die Gattung Salvia – Geschichte, Biologie, Anzucht und Verwendung. Drogenreport **11**(19), 35–36.

WILEY, J. 1990: Wiley 138K mass spectral database. John Wiley and Sons, INC, New York.

Received September 24, 2004 / Accepted August 01, 2005

Addresses of authors: Dr. A. E. Manukyan (corresponding author), G.S. Davtyan Institute of Hydroponics Problems, National Academy of Sciences, Noragyugh 108, 375082 Yerevan, Armenia, and Prof. Dr. W. H. Schnitzler, Chair of Vegetable Science, Quality of Vegetal Foodstuff, Center of Life Sciences Weihenstephan, Technische Universität München, Dürnast 2, 85350 Freising, Germany, e-mail: artmanukyan@yahoo.com.