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Effect of Weather Conditions on the Morphology, Production and Chemical Composition of Two Cultivated Medicinal and Aromatic Species

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Summary

Two medicinal plant species (*Achillea collina* Becker and *Hyssopus officinalis* L.) normally occurring and frequently cultivated in our Central European region, were grown in controlled environment simulating "cold" and "hot" weather conditions. A "cold" environment (slowly increasing temperatures from 12.5/7.5 °C to 27.0/14.0 °C and 5700 lux illumination) prolonged the appearance of the first inflorescences by 9 weeks in both species. In yarrow, "cold" treatment resulted in elevated plant height (approximately by 50 %) and by up to 35 % larger leaves. In addition, plants produced more biomass (0.117 kg plant^{-1}) compared to the "hot" treatment. The accumulation of essential oil, the proazulene ratio and the total phenol content of the herb have only been moderately effected by the different climatic conditions. In case of hyssop, however, the generative features like number and length of inflorescences were significantly increased by 180 and 108 %, respectively, in the "hot" chamber. Biomass and accumulation of essential oil were also favoured by "hot" treatment. The content of 1.22 ml 100 g oil^{-1} exceeded the mean value of plants grown in the "cold" chambers by almost 150%. The composition of the oil showed characteristic quantitative changes due to weather conditions. It is concluded that, while in cultivation of yarrow the effect of the year may considerably affect yield, in stands of hyssop it is more important for the quality.

Key words. biomass – climate – essential oil – hyssop – proazulene – total phenolic content – weather – yarrow

Introduction

The goal of the experiment was to detect the influence of temperature and light on behaviour of two aromatic species – yarrow and hyssop – originating from different natural habitats. Although both are now widely cultivated in Central European region, their different responses on external factors might significantly influence the success of the production.

Yarrow species are perennial, herbaceous, widespread and popular medicinal plants all over the world. In Central-Europe, the most common species is *Achillea collina* Becker. The most important active agent of the flowering shoots is the essential oil with proazulenes as main compound, but the drug contains other important ingredients too, like flavonoids, non-volatile sesquiterpenes, etc. Numerous therapeutic effects of yarrow have been proven, among others spasmolytic, analgesic, anti-inflammatory and digestive activities (Németh and Bernáth 2008).

Hyssop (*Hyssopus officinalis* L.) is a xerophyte, perennial aromatic shrub originating from the Mediterranean region. The dried flowering shoots and their essential oil is widely applied for culinary purposes and in the phytotherapy due to the antispasmodic, anti-inflammatory, antiviral, antibacterial and antioxidant activities (Sváb 1992). Decoction of the drug is used as an antitussive agent, against inflammation of the gastrointestinal tract, angina and skin diseases.

Common yarrow grows as a weed in several ruderal plant associations in Central and Eastern Europe, while the natural habitat of hyssop lies in the hot, dry and calcareous hillsides around the Mediterranean Sea. To assure stable yields and quality, however, both species have been introduced into agricultural cultivation and are now cultivated in several countries of the EU. Although environmental conditions may considerably influence the production, there is relatively few information about the effect of weather conditions on their development, yield and, especially, on their contents of bioactive compounds in these important species.

The impact of environmental conditions on yarrow has been investigated in different habitats. Already in the 60'ies it was reported that soil characteristics, hydration and climate affected growth and morphological charac-

teristics of yarrow, while they had less influence on proazulene content (Máthé et al. 1963). Field experiments of Cernaj et al. (1991) revealed that edafic and climatic conditions might affect the production of essential oil. Increasing altitude caused significant changes in height, number of inflorescences and total phenolic content of *A. millefolium,* but the content of essential oil was not affected (Giorgi et al. 2005, 2010). In our investigations in 45 Hungarian populations, no correlation between the habitat and the chamazulene content of the essential oil could be established (Németh et al. 2007). According to Michler et al. (1992) proazulene is detectable in yarrow only in meadow populations on soils rich in magnesium, manganese and phosphate. The authors however, consider that ecological circumstances determine only indirectly the presence of azulenes by favouring distribution of tetraploid taxa. Investigations under controlled conditions are sporadic. Our results with *A. crithmifolia* showed, that not only morphology, but to some extent the composition of the oil might be affected by climatic factors (Németh et al. 1993).

In case of hyssop, scientific experiments mainly focused on the variability of agronomic and morphological features (Galambosi et al. 1993; Zavislak 2011). Although considerable chemical variability of the composition of essential oil has been described in wild growing populations, only theoretical assumptions could be made about the possible influence of climate and habitat (Piccaglia et al. 1999). Based on long-term (1993–2000) investigations a significant negative correlation between the amount of precipitation during the development of flowers and their essential oil content was proven (Németh et al. 2001).

Based on literature references, the effect of environment on bioactive substances seems to be contradictious. Obviously, such kind of results is to be expected, when investigating natural populations, which, in fact, should not have been compared with each other. The studies are in numerous cases hardly reproducible and it is difficult to separate the ecological effects from the genetic variability of populations. Only chemosyndroms of well-defined genotypes detected under controlled conditions may provide reliable information in this respect. The lack of appropriate data initiated our recent study.

Material and Methods

Treatments

To investigate the effects of different environmental conditions, two weather programs as treatments were installed in phytotron chambers (Fitotron SGC120 growth chambers, Weiss Gallenkamp Ltd., Loughborough, Leicestershire, United Kingdom) in 2012. One program simulated a Submediterranean-like hot weather ("H") and another one a relatively cool vegetation period ("C"), however both normally occurring under our Central European natural climate. The details of the programming are summarized in Table 1.

Plant material and care of plants

One-year old individuals of *Achillea collina* Becker and *Hyssopus officinalis* L. originating from open field collection were chosen for the study. Ten individuals from both species were divided in two equal parts as sister-plants and these 10–10 sister-plants placed in two climatic chambers.

The plants were grown in pots (16.0 cm in diameter and 15.5 cm in height) filled with a standard soil mixture (Compo Sana Potting Soil). During the first nine weeks irrigation was carried out twice a week, then from week 10 on every two days, assuring 65–70 % RWC in the soil. The plants were fertilized fortnightly with Wuxal Super complex fertilizer by adding 0.2 l of 0.25 % solvent into each pot. During the experiment, the fungicid "Thiovit Jet" was applied twice against powdery mildew of hyssop. To protect the plants from mites and aphids, "Axoris Compo" quicksticks were put into each pot.

Measurements

Phenological and morphological features. The development of the plants was recorded every two weeks. Detailed morphological descriptions of the plants were carried out during full blooming. The plant height was determined on the tallest flowering stem of each plant. The length of the leaves at the central part of the shoots was recorded and in the case of yarrow also for the leaf rosettes. Besides, for each plant individual the number of inflorescences/plant, the number of branches/stem (3 replications plant^{-1}) and in case of hyssop the length of the inflorescences (3 measurements plant^{-1}) was determined.

Production. The biomass and drug production were determined for each plant species separately (10 replications) by harvesting the shoots in the state of full flowering. The shoots of yarrow were cut with 30–35 cm long stems, while hyssop plants were cut over the wooden parts and then measured by analytical scale. The plant material was dried at room temperature (22–24 °C) and measured again to determine the dry mass. To evaluate the ratio of the valuable plant organs in the dried plant material, separation of leaves and flowers from the stem was carried out by hand and the fraction containing the leaves and flowers was measured.

Essential oil content. The essential oil content was determined in the shoots collected in full flowering. The dried plant material of yarrow was distilled according to the European Pharmacopoeia VII. (2012) (*Millefolii herba*) in 5 replications per treatment. The distillation was carried out from 20 g plant material for 2 hours in a Clevenger-type apparatus, at a rate 2–3 ml min^{-1}. After the distilla-

Table 1. The climatic program of the controlled environment chambers (harvest in "hot" environment at the end of 15th week; harvest in "cold" environment at the end of 25th week).

Week	Hot weather regime		Cold weather regime	
	Temperature (°C) (14 h day/10 h night)	Relative humidity (%)	Temperature (°C) (14 h day/10 h night)	Relative humidity (%)
1.	15.0/ 8.0	70	12.5/ 7.5	70
2.	17.0/ 9.0	70	12.5/ 7.5	70
3.	18.0/ 9.5	50	12.5/ 7.5	50
4.	19.0/10.0	50	17.0/ 9.0	50
5.	20.0/10.5	50	17.0/ 9.0	50
6.	21.0/11.0	50	18.0/ 9.5	50
7.	22.0/11.5	50	18.0/ 9.5	50
8.	23.0/12.0	65	19.0/10.0	65
9.	24.0/12.5	65	19.0/10.0	65
10.	25.0/13.0	65	20.0/10.5	65
11.	26.0/13.5	65	20.0/10.5	65
12.	27.0/14.0	65	21.0/11.0	65
13.	27.0/14.0	65	21.0/11.0	65
14.	27.0/14.0	65	22.0/11.5	65
15.	27.0/14.0	65	22.0/11.5	65
16.	–	65	23.0/12.0	65
17.	–	65	23.0/12.0	65
18.	–	65	24.0/12.5	65
19.	–	65	24.0/12.5	65
20.	–	65	25.0/13.0	65
21.	–	65	25.0/13.0	65
22.	–	65	26.0/13.5	65
23.	–	65	26.0/13.5	65
24.	–	65	27.0/14.0	65
25.	–	65	27.0/14.0	65
Luminous emmitance	14260 lux		5700 lux	
Photosynthetically active radiation	413 µmol m^{-2} s^{-1}		162 µmol m^{-2} s^{-1}	

tion, the blue essential oil was rinsed with xylene from the graduated tube of the apparatus. Dried samples of hyssop were also hydrodistilled in a Clevenger apparatus according to Hungarian Pharmacopoeia VIII. (2004) (in lack of official standard for hyssop, the one for *Thymi herba* was used), from 20 g plant material for 2 hours, at a rate 2–3 ml min^{-1} in replications per reatment.

The essential oil content was calculated to the dry matter, so the water content of the drugs was also determined as the loss on drying of 4 g drug at 105 °C for 3 hours.

Proazulene content. The proazulene content of yarrow was determined in the essential oil-xylene mixture according to the method described in European Pharmacopoeia VII. (2012) (*Millefolii herba*) in 5 replications per treatment. The mixture was diluted to 50 ml with xylene, than the absorbance of the solution was measured at 608 nm to calculate the percentage content of proazulenes with xylene as compensation liquid.

Total phenol content. The total phenol content (TPC) was determined in the yarrow samples by the modified method of Singleton and Rossi (1965), both in aqueous and alcoholic extracts. For the aqueous extract 1 g drug was infused with 100 °C distilled water (100 ml) for 24 h. For the alcoholic extracts, the 1 g drug was extracted by ethanol (20 %) for 72 h. Then both types of extracts were filtered and stored in the freezer at 4 °C until measured. Sample solution of 0.5 ml was introduced into a test tube

and then 2.5 ml Folin–Ciocalteau's reagent (10 v/v%) was added. After incubation of 1 min, 2 ml of sodium carbonate (0.7 M) were added. The absorbance was measured at 760 nm after incubation in hot water (50 °C) for 5 min. Gallic acid (0.3 M) was used as chemical standard for calibration. The TPC of the sample was expressed as mg of gallic acid equivalent per g dry weight (DW). The measurements were carried out in 3 replications in the 5 yarrow samples.

Composition of essential oil. It was studied in samples of hyssop by GC-MS method. An Agilent Technologies 6890 N instrument was used, equipped with a HP-5MS capillary column (30 m × 0.25 mm, 0.25 film thickness). It was programmed as follows: 60 °C for 0.5 min, followed by a temperature increase of 3 °C min^{-1} until 240 °C, keeping the final temperature for 5 min. Injector and detector temperatures were kept at 230 °C. Helium was used as carrier gas with a constant flow rate of 1 ml min^{-1}, a split ratio of 30:1, and an injection volume of 0.2 ml (10 % n-hexane). The MS analyses were carried out using the same equipment with an Agilent Technologies MS 5975 detector. The ionization energy was 70 eV and the mass spectra were recorded in full scan mode, which revealed the total ion current (TIC) chromatograms. A mixture of aliphatic hydrocarbons (C9-C23) in n-hexane was injected under the above mentioned temperature program to calculate the linear retention indices. The mass spectra and linear retention indices (LRI) were compared with those of commercial (NIST, Whiley) library mass spectra built up from data obtained from pure compounds (Sigma/Aldrich). The proportion of the individual compounds was expressed as total area percentages. The GC-MS analysis was carried out in 5 replications per treatment.

Statistical analyses. The results were analysed with IBM SPSS Statistics 20 program. The paired samples T-test was used for the comparison of the treatments. The normality of the data series were proved with the K-S test ($P > 0.05$).

Results

Phenological and morphological features

The first flower buds appeared after 7 weeks on the plants in the so-called "hot" weather ("H") chamber, while buds were detected after 11 weeks in the "cold" chamber. Both species were in full blooming from the 10th week of the experiment (Fig. 1 and 2). The so-called "cold" weather treatment delayed the blooming by 9 weeks. The flowering of yarrow lasted 6–7 weeks in both "hot" and "cold" treat-



Fig. 1. Yarrow plants of the same genotype showing different morphological features after 10 weeks of treatment. On the left side: "hot" treatment, on the right side: "cold" treatment.



Fig. 2. Hyssop plants of same genotype showing different morphological features and habitus after 10 weeks of treatment. On the left side: "cold" treatment, on the right side: "hot" treatment.

ments, while the blooming period of hyssop was by 4 weeks longer under the cooler circumstances (data not shown).

Several morphological differences were detectable due to the different environments. The plants under "hot" conditions developed normally, while the "cold" treatment changed their habitus (Fig. 1 and 2). In yarrow, "cold" treatment resulted in approximately 50 % higher plants and up to 35 % larger leaves, while the difference was not significant in case of hyssop (Table 2 and 3). The generative features, however, changed significantly only in hyssop. The number of inflorescences/plant increased approximately by three fold and the number of braches/stem from 35 to 41 pieces in the "cold" treatment. However, a large proportion of these branches developed abnormally: single branches appeared in spite of their regular coupled development. Inflorescences of hyssop were significantly longer under "hot" conditions. They more than doubled their length compared to the "cold" treatment (Table 3).

Productional features

The biomass production of the two species showed different tendencies. Yarrow plants produced significantly higher fresh (FM) and dried masses (DM) in the "cold" environment (0.117 kg plant^{-1} FM and 0.026 kg plant^{-1} DM, Table 2). In contrast, hyssop responded to the "hot" weather by a higher production (0.048 kg plant^{-1} FM and 0.012 kg plant^{-1} DM), although the difference was not statistically significant (Table 3). The mass of valuable plant organs represented by the fraction of flowers and

Table 2. The effect of climatic conditions on yarrow (mean ± SD).

Parameter	Hot weather regime	Cold weather regime	SL
1. Plant height (cm)	54.42 ± 7.88	81.95 ± 10.37	***
2. Leaf length (on leaf rosettes)	16.90 ± 6.77	22.90 ± 6.34	+/*
(on stems)	7.10 ± 1.91	8.50 ± 1.59	
3. Number of inflorescences plant^{-1}	13.00 ± 5.07	12.50 ± 4.50	ns
4. Number of branches stem^{-1}	10.30 ± 4.76	8.9 ± 1.52	ns
5. Fresh mass (g plant^{-1})	81.10 ± 23.57	117.20 ± 20.00	**
6. Dried mass (g/plant)	16.00 ± 3.96	26.20 ± 4.84	***
7. Mass of the fraction of flowers and leaves (g plant^{-1})	9.90 ± 2.25	11.58 ± 0.44	*
8. Essential oil content (ml k g DW^{-1})	1.60 ± 0.23	1.59 ± 0.32	ns
9. Proazulene content (% DW)	0.07 ± 0.01	0.11 ± 0.06	ns
10. TPP content (mg GAE g DW^{-1})			
in water extract	91.62 ± 9.65	96.52 ± 10.93	ns
in alcoholic extract	45.75 ± 6.50	45.05 ± 5.94	Ns

SD: standard deviation; DW: Dry weight
SL: Level of Significance; +: P < 0.1; *: P < 0.05; **: P < 0.01; ***: P < 0.001; ns: not significant.

Table 3. The effect of climatic conditions on hyssop (mean ± SD).

Parameter	Hot weather regime	Cold weather regime	SL
1. Plant height (cm)	50.60 ± 7.31	55.90 ± 8.29	ns
2. Leaf length (cm)	2.70 ± 0.49	2.50 ± 0.49	+
3. Number of inflorescences plant^{-1}	24.70 ± 7.85	8.80 ± 3.78	**
4. Number of branches stem^{-1}	35.20 ± 9.98	40.90 ± 10.70	+
5. Length of inflorescences (cm)	14.00 ± 3.75	6.70 ± 3.13	***
6. Fresh mass (g plant^{-1})	47.60 ± 10.87	39.65 ± 19.18	ns
7. Dried mass (g plant^{-1})	11.87 ± 2.53	11.22 ± 5.83	ns
8. Mass of the fraction of flowers and leaves (g plant^{-1})	7.05 ± 0.88	6.67 ± 3.27	ns
9. Essential oil content (ml kg DW^{-1})	12.20 ± 1.90	4.90 ± 1.80	**

SD: standard deviation; DW: Dry weight
SL: Level of Significance; +: P < 0.1; *: P < 0.05; **: P < 0.01; ***: P < 0.001; ns: not significant.

leaves followed the same tendency as the plant mass in case of both species.

Essential oil content

The influence of temperature and light on the accumulation of essential oil showed considerable differences between the two species (Table 2 and 3). Hyssop plants in the "hot" environment produced an almost 150 % higher essential oil content (1.22 ml 100 g^{-1}) than the ones growing in the "cold" environment (0.49 ml 100 g^{-1}). In contrast, the accumulation of essential oil in yarrow was not affected by the examined weather factors (0.160 ml 100 g^{-1} in the "hot" and 0.159 ml 100 g^{-1} in "cold" environment).

Proazulene content of the drug

The content of proazulene was only moderately affected by the treatments. The ratio of proazulenes in the oil reached a high level either under "cold" (0.106 %) and in "hot" (0.071 %) environment. Both concentrations fulfil the criteria of the requirements set up in Ph.Eur. VII., which gives a minimum value of 0,02 % of the dried shoots.

Total phenolic content

The TPC was determined in yarrow samples (Table 2). It was established that the applied regimes of temperature and light did not significantly affect the TPC neither of the aqueous or of the alcoholic extracts.

Composition of the essential oil

From the oil of the hyssop samples from the "hot" environment 32 components were identified, while only 24 occurred in the samples from "cold" environment (Table 4). Especially the number of monoterpenes increased in the "hot" treatment from 13 to 20, parallel with their area ratio from 85.3 to 93.7 % when compared with the "cold" treatment. Beside, some components showed characteristic quantitative changes. The proportion of the main component isopinocamphone increased from 44.4 to 52.6 %, while the proportion of other characteristic compounds – among others trans-pinocamphone and pinocarvone – decreased.

Discussion

The different temperature and light regimes in the controlled environment chambers resulted in significant changes in morphological, productive and chemical characteristics of both yarrow and hyssop. However, the reactions of these species differed partly from each other. While in yarrow the environmental differences influenced mainly the vegetative traits, in hyssop their effect proved to be significant on the generative features.

The development of yarrow was slowed down by 9 weeks in the "cold" treatment. As consequence of lower light intensity larger leaves, longer stems were developed (Salisbury and Ross 1992) and higher biomass and dried plant mass was achieved. This difference reflects itself also in the mass of the fraction of flowers and leaves. Yarrow acclimatizes itself very easily to different environmental circumstances but as a result, morphological traits, habit of the plant might change drastically. Dabrowska (1977) described characteristic phenotypical changes both of *A. collina* and of *A. millefolium* as result of different water supply. Altitude as an ecological factor influences leaf dissection of *Achillea millefolium* (Gurevitch 1988). It was proved that compact leaves of high altitude plants could warm up above air tempera-

Table 4. Main compositional changes of hyssop oil as effect of the treatments. The results of the GC analysis are expressed as a percentage of the total peak area of the chromatogram.

Compound	RT	LRI	Hot weather regime (mean)	Cold weather regime (mean)
β-Pinene	6.64	980.856	2.1	5.2
β-Phellandrene	8.21	1029.064	0.7	1.2
Trans-pinocamphone	13.47	1162.896	20.9	17.5
Pinocarvone	13.61	1166.401	12.8	8.8
Isopinocamphone	13.77	1170.268	44.4	52.6
Myrtenol	14.78	1194.126	1.8	3.3
Germacrene D	26.18	1481.527	4.5	1.2
Elemol	28.86	1552.888	1.7	0.7
Total monoterpenes			93.7	85.3
Total sesquiterpenes			6.3	13.9

ture while the temperature of dissected leaves of lower altitude plants remained constant.

The accumulation of essential oil was not significantly influenced by the installed environmental conditions. This can be explained by the fact, that in spite of the increase in the fraction of flowers and leaves, the number of inflorescences have not changed, which is the most relevant organ in point of essential oil content (Hofmann and Fritz 1993). However, the essential oil content of the shoots depends on several other factors too, like the number of essential oil ducts and possible shifts in the numerous enzymatic reactions thorough the biosynthetic processes of essential oil compounds. Our results agree with the findings of Giorgi et al. (2010) who did not register significant changes in this feature as consequence of the environment but contradict to the results of Shalaby and Verzár-Petri (1975) who stated that high temperatures and intensive illumination enhance the accumulation of essential oil in yarrow. The special role of phenophase in the reaction of the plants was proven by Ruminska (1968): shadowing in earlier phenophases results in reduced flower mass and oil accumulation while in later developmental stage it might have positive effect. In our experiment, no temporal changes in illumination were applied.

Similarly, to the essential oil content, we could not prove a significant change in the proazulene content of the drug, either. It is in agreement with the results of Hofmann and Fritz (1993) who established that azulene content of the yarrow variety 'Proa' was not influenced by the temperature, length and intensity of illumination. In non-azulenogenic species however, compositional changes were described: in climatic chambers was found an increase of 10–20 % of the proportion of camphor and borneol in *A. crithmifolia* oil if the temperature was elevated by 3–5 °C (Németh et al. 1993). Similarly, Giorgi et al. (2005) describing the effects of altitude on the composition of *Achillea millefolium* oil detected the disappearance of some compounds, like α- and β-thujone. However, the majority of authors agree that ecological factors influence the accumulation of oil much more than its composition (Németh 2005).

In case of the accumulation of phenolics in yarrow our results represent significant additional information, as former studies did not focused on the effects of environment from this respect. It is known that phenolic compounds may play a role in stress defence (Heimler et al. 2009). Thus, its stable level might support the conclusion that our experimental parameters were still in the tolerance range of *A. collina*.

On the other side, our results showed that for hyssop, – although cultivated for several decades already in Central Europe, as a species of Southern European regions – this environment was much less tolerable. The "cold" treatment significantly prolonged the time until flowering and also negatively affected the number and length of flowers. Although the number of branches increased compared to the warmer environment, but the majority of them did not bear flowers, their generative development was disturbed.

Besides, in the "cold" chamber – in contrary to yarrow –, the essential oil content of the shoots was significantly reduced, too. Under open field conditions, we formerly proved that, the accumulation level of the oil can change by up to 50 % in different year, its variance is high (Németh et al. 2001). These findings have now been ascertained under controlled conditions.

Compositional changes in the essential oil may also occur in consequence of variable weather conditions. Novak et al. (2010) proved that the ratio of the main monoterpene components, thymol and carvacrol in oregano, depends on a large extent on the temperature. The question if the differential reaction of these compounds to temperature is regulated at the level of gene expression or rather simply an effect of temperature on the kinetic properties of the enzymes has not been clarified.

It can be concluded, that essential oil bearing plants of different origin may show different reactions on weather factors, which significantly influences yield and quality in open field cultivation. Although, few results on the effect of irrigation have been published (Radácsi et al. 2010), the majority of data about the influence of the environment originates from open field observations and there are hardly any exact data about the combined effect of temperature and light under controlled conditions (Bernáth et al. 1991). Based on our results it is supposed that in yarrow plantations the weather conditions or season trends may considerably affect plant growth and yield while in hyssop cultivation temperature and light conditions seem to be more important in respect of the quality (essential oil content and composition) of the drug. In our experiment, relatively balanced environments with moderate changes during plant development were installed. Nevertheless, in natural environmental conditions the ecological tolerance of the species might manifest differently because of the extremer alterations in weather conditions. To make conclusion about the effects of these sudden changes in temperature and light as possible stress factors further studies are necessary.

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