SZENT ISTVÁN UNIVERSITY

AFFECTING FACTORS OF PRODUCTION AND ACTIVE AGENTS OF COMMON YARROW (*ACHILLEA COLLINA* BECKER)

PhD THESIS

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1. BACKGROUND AND OBJECTIVES

Achillea species are well known medicinal plants all over the world, their drug –*Millefolii herba*- is constantly demanded material in the medicinal, cosmetic and food industry. Due to their medicinal effect, they are applied both in traditional medicine and modern phytotherapy against inflammation and spasmodic conditions of the digestive system, hepato-biliary disorders, as an appetizer and externally for skin inflammations and wounds. However the field of application of yarrow species presumably will broaden, since new application possibilities were justified in the last decades, such as antioxidant, hepatoprotective, antidiabetic, immuno-suppresive and anxiolytic effects. (Karlová, 2006; Németh et Bernáth, 2008).

In Central-Europe *Achillea collina* Becker is the most common and often applied amongst the species of the genus. According to the literature data and practical experiences this species is able to produce quality drug corresponding the pharmacopoeia standards and national regulations.

At the same time drug quality is often inadequate and do not meet the standards. According to a study revealed in Austria in 2008, only the half of the investigated samples -*Millefolii herba*, and *Millefolii flos*- of European origin reached the standards of the current European Pharmacopoeia (Benedek et al., 2008). Based on the results it was obvious that the way of drug production –collection or cultivation- defines the drug quality very much: problems with quality occurred more often in case of collected samples (Benedek et al., 2008, Raal et al., 2011; Ŝpinarová et Petřiková, 2003).

The solution for these quality problems would be the cultivation of common yarrow (Benedek et al., 2008; Ŝpinarová et Petřiková, 2003), nevertheless cultivation is struggling with many practical problems because of the heterogeneity of the cultivated stocks, the difficulties of mechanic weed control and also has economical uncertainties. However the cultivation technology of the plant is known, according to recent scientific results some parts of it considered to be out-of-date, and at the same time some technological developments necessary for large scale production are still missing. For successful cultivation of common yarrow cultivars producing high biomass and active agent content under Hungarian climatic conditions would be needed, furthermore the more complex exploration of affecting factors of yarrow's active ingredients.

Therefore during my doctoral work our objectives were:

- to explore the intraspecific variability of *A. collina* in respect of its morphological, productional and chemical traits,
- to broaden our knowledge on chemosyndrome of common yarrow with the composition of roots, which seems to have high scientific relevance recently,
- to learn more about the effect of ontogenesis on accumulation of active substances,
- to define the effect of environmental factors (temperature, lighting) on the morphology, production and chemism of this plant,
- to establish the technological development of cultivation with the examination of the competitive ability and herbicide tolerance of yarrow.
- furthermore, with our results we wanted to contribute
    - to the breeding and utilization of varieties,
    - to the active agent content optimized harvest date
    - and to the introduction of an up-to-date, integrated chemical weed control technology in yarrow cultivation.

2. MATERIALS AND METHODS

2.1. The experimental sites

Our field experiments were installed at two experimental sites, at the research station of Department of Medicinal and Aromatic Plants in Soroksár and at the fields of farmer Imre Kovács, near Kál, from 2012 to 2014. Both experimental sites can be characterized with sandy soil with minor differences in their other soil parameters. During the experimental years the mean temperature was about the season's average values at both experimental sites, while significant differences in the amount of precipitation occurred during the growing season. 2013 proved to be a quite dry year with only 103 mm precipitation during the examined period, while in 2014 the total precipitation was higher than usual at both experimental sites (340 mm at Soroksár and 257 mm at Kál).

2.2. Plant material and treatments

2.2.1. The intraspecific variability of common yarrow

The plant material of the trial was 11 *A. collina* Becker taxa, with Hungarian ('Azulenka') and European breeded cultivars ('Alba', 'Proa', 'Spak'), cultivated stocks (Földes, Gyula, Kál) and

wild growing originated genotypes from the genebank of the department (Gb9, Gb10, Gb22, Gb47) amongst them. The plant propagation was carried out in 2012 by sowing, the seedling were pricked in 1-2 leaf stage development. The seedlings were kept in green house without heating until planting them to open-field in May 2012 at Soroksár to 50 x 25 cm spacing. The trial was set on small plots (1x4 m) in 3 repetitions with 32 plants in each plot. The roots of the three-year-old plants were harvested in 2014 for the *Achillea* root analysis, the sampling was carried out in July after the flowering of the stands from 24 plants in taxa.

2.2.2. The effect of ontogenetic factors on active agent of common yarrow

The plant material of the experiments were one-year-old *A. collina* 'Proa' plantations. The propagation of the plants was carried out in Kál, the sowing was carried out in early spring to peat-soil mixture, seedlings were planted to field at the end of April-beginning of May. The seeds were sowed to cell trays, the seedlings were grown without pricking, but cut repeatedly and kept in heated polytunnel. The seedlings were planted to field in 5-7 leaf stage development to 50 x 25 cm spacing at Soroksár and Kál. The experiment was set on smaller plots (30 m^2) at Soroksár, while in large-scale plot size (1-2 ha) at Kál. The plant density was 8 plant/m^2.

As treatments the plants were harvested during flowering in 5 development stages according to the general BBBCH scale (http://www.bba.de/veroeff/bbch/bbcheng.pdf). Bulk samples were taken from 50 plants in each development stage, cutting the flowering shoots with 30 cm long stems.

2.2.3. The effect of climatic conditions on the morphology, production and active agent content of common yarrow

The plant material of the experiment was 10 one-year-old *A. collina* plant divided into two parts and planted to pots (16.0 x 15,5 cm).

As treatments to weather programs were installed in climatic chambers (Fitotron SGC120, Weiss Gallenkamp Ltd.) in 2012: one with a sub-Mediterranean-like „warm" weather (15 weeks 15,0°C/8,0°C -> 27°C/14,0°C; 14260 lux, 413 μmol/m^2s) and a cooler „cold" weather treatment (25 weeks 12,5°C/7,5°C -> 27°C/14,0°C; 5700 lux, 162 μmol/m^2s).

2.2.4. The control of interspecific competitions in common yarrow cultivation

The plant material of the experiments were one-year-old *A. collina* 'Proa' plantations in every experimental year. The propagation of the plant material carried out in the same way as it was described in chapter 2.2.2. The experiments were installed in Soroksár and Kál in every year from 2012 until 2014.

As treatments pre-planting incorporated (PPI) and post-emergent (POST) herbicide treatments were set with untreated control, in 12 combinations in 2012 and 2013, while in 11

combinations in 2014. The plot size was different in the experimental years: 10 m^2 in 2012, 20m^2 at Soroksár, 2100m^2 at Kál in 2013, while 20m^2 in 2014.

The plant material of the experiments received regular nutrient and water supply, and plant protection during the experimental years.

2.3. Measurements

2.3.1. The phenological, morphological and productional features of common yarrow

The plants development and flowering time was observed from the beginning of the experiments. The morphological features were measured in full flowering: we made a brief morphological description about the plants including their habitus, leaf colour and other specific features. For the determination of plant height the highest flowering shoots were measured on each plant in 6 repetitions. In addition we measured the length of the flowering horizon (difference of the height of the flowering shoots of one plants) in 6 repetitions in our experiments on intraspecific variability of yarrow. In our experiments examining the effect of climatic conditions, leaf length, leaf width, number of inflorescences and number of branches were also measured in 10 repetitions.

For the examination of productional features sampling of the plants was made in full flowering from 5 or 10 plants in our experiments on intraspecific variability and on effect of climatic conditions. We harvested the plants by hand, cut them with 30 cm long stems, then dried the plant material naturally and measured the drug mass in the dried samples. The measurement unit was given in g/plant.

For the determination of ratio of different plant parts we separated 20 g of the samples to inflorescences, leaves and stems, measured their mass, then calculated the values into percentages. In our experiments on the effect of ontogenesis we measured the ratio of plant parts in all the 5 development stages.

2.3.2. The interspecific competition and herbicide tolerance of common yarrow

For the determination of interspecific competition, we observed and described the weed cover on the plots by bonitation and gave the values in percentages. The spectra of the occurring weed species and their cover was also observed, we evaluated the results by a special scale system.

For the determination of yarrow's herbicide tolerance, the cover, growth, development and visible damages were observed by bonitation. The plant height was also measured in full flowering in 10 repetitions.

2.3.3. Active agent content and composition

2.3.3.1. Determination of essential oil content in the flowering shoots

Essential oil content of the flowering shoots was determined by steam distillation by the method recommended in the VII. Hungarian Pharmacopoeia (*Achilleae herba*, Pharmacopoeia Hungarica, 1986) in 3 repetitions. Essential oil content was expressed in g/100 g d.w.

2.3.3.2. Determination of proazulene content

Proazulene content of the essential oils was measured by the method recommended in VIII. Hungarian Pharmacopoeia (*Millefolii herba*, Pharmacopoeia Hungarica, 2003) in 3 repetitions. Proazulene was expressed in chamazulene %.

2.3.3.3. Determination of total flavonoid content

The total flavonoid content was performed by the method listed in the Ph. Hg. VIII. (*Crataegi folium cum flore*, Pharmacopoeia Hungarica, 2003) and given in hyperoside %. The measurements were performed in 3 repetitions in the samples from experiments on intraspecific variability and effect of ontogenetic factors.

2.3.3.4. Determination of total-phenolic content

The total polyphenol content was determined 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 x °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 total phenolic content of the samples was expressed as mg GAE/g d.w. The measurements were carried out in 3 replications in the samples of our experiments on yarrow's intraspecific variability, effect of ontogenesis and climatic conditions.

2.3.3.5. Determination of essential oil content in the roots

For the determination of essential oil content in the roots, 50 g grinded root was hydrodistilled in a Clevenger-type apparatus for 4 hours. The essential oil content was expressed in ml/ 100 g dry matter.

2.3.3.6. Preparation of HS extracts

For the preparation of HS extracts 500 mg chopped dried root was soaked with distilled water (2 mL) in a 20 mL headspace vial. The samples were heated at 80 °C for 20 minutes with the next mixing program: shaking for 5 seconds, pause for 2 seconds and 500 μL of vapour in split ratio 10:1 was injected directly via a transfer line to GC.

2.3.3.7. Preparation of dichloromethane extracts

For preparation of dichloromethane extracts 2 g chopped root was soaked in 10 mL of dichloromethane. The samples were put into ultrasonic bath for 20 minutes, then placed in dark at room temperature for 3 days. From sample solutions 2 mL was filtered through a 0.45 μm membrane PTFE filter (Rotilabo-Spritzenfilter 13 mm) and transferred to vial for GC-MS and GC-FID analysis.

2.3.3.8. Determination of the composition of root oil, HS and dichloromethane extracts

The identification of the root oil, HS and dichloromethane extracts composition was carried out by GC-MS-method based on mass spectra, librarian references (Wiley 6, NIST02- and AMDIS-32 software) and the calculation of their linear retention indexes (Van den Dool and Kratz, 1963).

2.4. Statistical analysis

The results were statistically evaluated by Microsoft Office 2010 and IBM SPSS Statistics 22 softwares. For the evaluation of common yarrow's intraspecific variability, the effects of ontogenetic factors and interspecific plant competition one- and two-way ANOVA were carried out, while for evaluation of the effects of climatic conditions we used paired t-test. Data normality and homogeneity of deviation was examined during test condition. Levene-test was used to examine the homogeneity of deviation while Kolmogorov-Smirnov-test was used to examine the normality. For the pairwise comparisons of treatments Tukey HSD post hoc test was ran in case of homogeneity of deviation, while Games-Howell test was ran in case of inhomogeneity of deviation. For data evaluation 95% confidence level ($p \leq 0.05$) was chosen. For the characterisation of the correlation of the different data series Pearson-correlation coefficient value was calculated.

3. RESULTS

3.1. Intraspecific variability of common yarrow

3.1.1. Phenological and morphological variability of common yarrow

We observed differences in the flowering time of the taxa: most of them proved to be early flowering type, while the flowering of 'Azulenka' and Gb22 started in two week delay compared to the other genotypes in all the three years.

The taxa showed significant variability regarding their macromorphological features. Leaf colour was uniform in almost every taxa (except Gb22), while regarding plant habitus more than the half of them was heterogeneous. Taxa with special morphological features were found (varieties 'Azulenka', 'Proa' and wild growing Gb22).

The plant height was 43.1±5.3 cm in 2012 and 51.0±6.6 cm in 2013 on average. We didn't find significant differences between the taxa, while the difference between the years proved to be statistically significant. The length of flowering horizon varied between 12.8 cm and 21.0 cm in 2012 and between 15.8 cm and 24.0 cm in 2013. We measured the lowest values in Gb22 (12.8±5.3 cm and 15.8±6.4 cm). Significant differences were observed both between taxa and years.

3.1.2. Productional variability of common yarrow

We measured 19.9±4.7 g/plant drug mass on average in the first year of the experiment without any statistical difference between the taxa. In the second year of cultivation the drug mass increased (33.8±10.4 g/plant) with the highest value (55.9±7.2 g/plant) measured in 'Azulenka'. By this time the difference between taxa was relevant, similarly to the difference between the years.

3.1.3. Chemical variability of overground parts of common yarrow

The examined *A. collina* taxa presented considerable qualitative variability regarding their active agent content. Highest essential oil content was detected in Gb47 (0.348 g/100 g on average) in all the three years, while the lowest values were measured in Gb9 (0.199±0.017 g/100 g, and 0.147±0.015 g/100 g) in 2012-2013, and in Kál (0.172±0.010 g/100g) in 2014**.** Significant differences were proved both between taxa and years.

Highest proazulene content was detected in Gb22 (0.173±0.010%) in 2012, in Gb47 and Gb22 (0.115±0.007% and 0.113±0.115%) in 2013, and in Gb22 (0.140±0.008%) in 2014. Lowest proazulene content was measured in Gb10 (0.064±0.003%) in 2012, while in 2013 and 2014 in Kál (0.043±0.007%, and 0.064±0.005%). We detected significant differences both between taxa and years.

Total flavonoid content of the taxa varied between 1.373±0.051% ('Proa') and 1.972±0.025% (Gb22) in 2012. In 2013 we detected highest values in Gb47 (2.379±0.137%), lowest in Gyula (0.540±0.210%). Difference between taxa and years was statistically significant.

We measured highest total phenolic content in the aqueous extracts of'Proa' (220.042±2.612 mg GAE/g d.w.) in 2012, in Gb47 (179.882±1.168 mg GAE/g d.w.) in 2013, while lowest phenolic content was found in Gb22 (160.918±2.710 mg GAE/g d.w.) and Gyula (133.872±4.414 mg GAE/g d.w.). The total phenolic content of the alcoholic extracts varied between 81.233±1.090 mg GAE/g d.w. (Gb22) and 122.143±1.792 mg GAE/g d.w. ('Proa') in 2012, while in 2013 highest content was detected in Gb22 (82.707±4.841 mg GAE/g d.w.), lowest in 'Spak' (30.193±2.388 mg GAE/g d.w.). The difference between taxa and years was proved to be significant in case of both extraction methods.

3.1.4. Chemical variability of the underground parts of common yarrow

We detected low oil content in the roots of the examined *A. collina* taxa, on average 0.036 ml/100 g. Maximum (0.052 ml/100 g) oil content was measured in 'Proa', Földes and Gb47, while the accumulation level was only 0.021 ml/100 g in case of 'Azulenka', 'Spak' and Gb10.

The main compound of the root oils was 7-heptadecanone-en (28.9-4.0%) in every taxa. Terpene compounds amounted 51.2% on average of the oils. The ratio of monoterpene fraction varied between 0.2% to 13.8%, while the sesquiterpene fraction proved to be predominant in every taxon (32.6-49.9%). Alismol (3.1-18.9%), β-sesquiphellandrene (0.5-9.8%), neryl isovalerate (0.2-10.5%), γ-humulene (1.1-6.8%), cis-cadine-4-en-7-ol (0.3-7.4%) and β-eudesmol (0.2-7.3%) were present in the oil of all the examined taxa. At the same time 4-hydroxi-4-methyl-2-pentanone, δ-elemene, modeph-2-ene, guaiol and bulnesol occured only in some specific taxa's oil. Alkamide constituents were also present in half of the taxa, however only in small (0.3-2.5%) concentrations.

Main constituents of HS extracts in every taxa were albene (20.8-52.1%) and β-pinene (8.3-47.1%), while hexanal (1.0-8.4%), α-pinene (1.4-8.1%) and neryl isovalerate (0.4-2.7%) were also universally present in the extracts. On contrary camphene (0.2-15.4%), sabinene (0.2-18.1%), α-isocomene (0.2-7.2%), trans-caryophyllene (0.2-10.2%), γ-humulene (0.2-7.6%) and β-sesquiphellandrene (0.2-7.6%) could be detected in specific taxa, but in considerable amounts. In 'Azulenka', 'Alba', Földes, Gyula and Gb10 taxa the predominance of monoterpene compounds (53.5-59.6%) was characteristic, while in the HS extracts of 'Proa', 'Spak', Gb9, Gb22, Gb47 sesquiterpenes were predominant (51.5-79.8%).

The main constituents of dichloromethane extracts in each taxa were 7-heptadecanone-en (5.3-17.8%), linoleic acid (0.3-12.0%), an alkamide (1.4-14.4%), three sterols (RI 3280: 5.3-7.7%, RI 3338: 4.2-11.8%, RI 3360: 3.7-13.0%) and a triterpene compound (RI 3456: 2.4-8.5%), together amounted 34.4-61.7% of the extracts. The ratio of sterols varied from 19.1% to 34.7% in the extracts, while other triterpenes (4.7-14.1%) and alkamides (2.3-16.6%) also reached considerable ratios. Monoterpenes (0.4-1.4%) and sesquiterpenes (4.1-9.6%) amounted only smaller concentrations.

3.1.5. Correlation among examined parameters of common yarrow

Among morphological parameters we found strong positive correlation between plant height and length of flowering horizon (r=0,712). Moderately strong positive (r=0,436) correlation was detected between plant height and drug mass, while only weak positive correlation (r=0,325) between the ratio of stems and the length of flowering horizon.

We detected weak positive correlation between the ratio of inflorescences and essential oil content (r=0,302), the ratio of inflorescences and total phenolic content of aqueous extracts (r=0,261) and strong correlation between ratio of inflorescences and proazulene content (r=0,329). The ratio of stems showed weak negative correlation with essential oil (r= -0,319) and proazulene content (r= -0,244).

We detected moderately strong positive correlation between essential oil and proazulene content (r=0,566), while strong positive correlation between the total phenolic content of aqueous and alcoholic extracts (r=0,757). Significant correlations were found between essential oil and total phenolic content of alcoholic extracts (r=0,305), and between proazulene and total phenolic content (aqueous extracts: r=0,286, alcoholic extracts r=0,285).

Positive correlation between the essential oil of above and underground parts was observed (r=0,370).

3.2. Effect of ontogenetic factors on the active materials of common yarrow

Accumulation level of essential oil in *A. collina* followed an optimum curve, increased from the beginning of generative phase, then decreased. In Soroksár the maximum (0.295±0.048 g/100 g) was reached in white bud phase in every year, but didn't decreased significantly in the following early and full flowering phases, only in overblown phase. In Kál we determined the peak (0.336 g/100 g) in early flowering every year and the essential oil content considerably decreased in overblown phase. The difference between growing sites and years proved to be significant, while between development stages only in samples originating from Kál.

Proazulene content, similarly to essential oil content, increased from the beginning of generative phase, and then gradually decreased. We detected maximal proazulene content in white bud stage in Soroksár (0.183±0.017% in 2012, 0.123±0.005% in 2013 and 0.134±0.018% in 2014). In Kál the maximum was measured in different development stages: in 2012 at early flowering (0.203±0.024%), in 2013 at full flowering (0.141±0.014%), while in 2014 in white bud stage (0.173±0.015%). Significant differences were proved between growing sites, years and development stages.

The accumulation of total flavonoid showed variable dynamic during generative phase in the examined years. In 2012 slight decrease from green bud stage and a second peak in overblown phase in total flavonoid content was observed, while in 2013 and in 2014 total dynamic of flavonoid content was optimum curve-like: it increased from the beginning of generative phase and decreased only from the second half of flowering. Maximum values were measured at green bud stage

(0.900±0.135%) in 2012, and at early flowering (2.837±0.070% and 2.018±0.085%) in 2013 and in 2014. The differences between years and development stages proved to be significant.

In total phenolic content of aqueous extracts we detected two maximums: the first one at the beginning of generative phase, the second one at the end of flowering. The first peak was reached in green bud stage in every year with 207.182±2.379 mg GAE/g d.w. total phenolic content on average. The second peak was detected in overblown phase in 2012 (214.169±1.002 mg GAE/g d.w.) and in 2014 (170.857±7.063 mg GAE/g d.w.), and in full flowering in 2013 (258.455±2.402 mg GAE/g d.w.). Significant differences were observed between years and development stages.

3.3. Effect of climatic conditions

3.3.1. Effect of climatic conditions on the phenological and morphological features of common yarrow

The investigated temperature and light settings resulted in significant changes in the development of *A. collina*. In "cold" treatment the flowering of the plant delayed by 9 weeks to the plant of "warm" treatment. The effect of the treatments manifested itself on the macromorphological features as well: in „cold" treatment plants with elongated stems and large, pendant leaves grew. Plant height increased by 50% in „cold" treatment compared to the average height in „warm" treatment (54.4±7.9 cm). Length of the leaves, both from the leaf rosettes (22.9±6.3 cm) and from the stems (8.5±1.6 cm), and leaf width (2.0±0.3 cm) reached higher values on „cold" treated plants than in „warm" treatment (16.9±6.8 cm, 7.1±1.9 cm, 1.7±0.6 cm). The generative organs were less affected by the treatments: the number of inflorescences (12.8±4.8 on average) and the number of the branches (9.6±3.14) were stable.

3.3.2. Effect of climatic conditions on the productional features of common yarrow

In „warm" treatment plants produced 81.1±23.6 g fresh mass and 16.0±4.0 g drug mass on average, while under „cold" conditions fresh plant mass increased by 40% and drug mass by 63% (117.2±2.0 g/plant, and 26.2±4.8 g/plant). The changes manifested itself in the ratio of plant organs as well: in „warm" the mass of inflorescences and leaves together amounted 9.9±2.2 g/plant, while in „cold" treatment the values were higher by 17% (11.6±0.4 g/plant).

3.3.3. Effect of climatic conditions on the active materials of common yarrow

Only slight difference in essential oil content of *A. collina* was found between the treatments (0.160 g/100±0.023 g in „warm" and 0.159 ml/100±0.032 g in „cold" treatment). We detected higher proazulene content in samples from „cold" treatment (0.106±0.010%) than from „warm" conditions (0.071±0.062%), although the values statistically did not differ from each other. Similarly, we found that total phenolic content of the samples were stable both in aqueous („warm":

91.62±9.65 mg GAE/g d.w.; „cold”: 96.52±10.93 mg GAE/g d.w.) and in alcoholic extracts („warm”: 45.75±6.50 mg GAE/g d.w.; „cold”:45.05±6.94 mg GAE/g d.w.).

3.4. Control of interspecific plant competition

3.4.1. Weed cover and spectra

Weed cover ranged from 45% to 100% in 2012, we observed the lowest weed cover in Soroksár at the control plot. POST Pledge and POST Galigan treatments at both experimental sites, while POST Benefex and POST Pendigan treatments had no effect on weeds. Weed growth and development were moderately cut back by POST Leopard treatment in Soroksár, POST Starane, POST Pendigan and POST Dual Gold treatments in Kál, where weed cover did not exceed 60-70%. In 2013-ban weed cover varied between 10% and 100% in the treatments. The lowest covers were detected in PPI Benefex+POST Pendigan, PPI Benefex+POST Pendigan+Galigan treatments in Soroksár, while POST Agil and PPI Agil treatments showed no efficacy. In Kál the most advantageous results were found in POST Pendigan+Galigan and PPI Pendigan treatments, where the weed cover varied between 10% and 50% on the plots. In 2014 we observed the lowest weed cover in the control plot (15%) in Soroksár, while the highest weed cover (95%) in PPI Afalon+POST Leopard combination and in control plot in Kál. Prosperous results with 27.5-40% weed cover were detected in PPI combinations of Racer.

According to the results of our weed survey, the presence of some of important weed species which cause serious problems throughout the country were crucial in Soroksár and Kál as well. Hence the presence of *Abutilon theophrasti, Ambrosia artemisifolia, Amaranthus blitoides, A. retroflexus, Capsella bursa-pastoris, Cynodon dactylon, Echinochloa crus-galli, Portulaca oleracea, Setaria viridis* on the growing sites were moderately strong-strong during the examined years.

3.4.2. Cover and plant height of common yarrow

In 2012 only POST Agil and POST Pendigan treatments did not decreased the cover of *A. collina.* We observed considerable loss in plant cover (40-70%) in POST Starane, POST Pulsar at both experimental sites and in POST Devrinol in Soroksár. In 2013 among the installed 12 treatments only PPI Benefex+POST Pendigan+Galigan (70%), POST Afalon+ Pendigan (45%), POST Pendigan+Galigan (80-90%) and PPI Afalon+Pendigan (50%) caused lower yarrow cover in the experimental sites. In 2014 the installed treatments did not affect negatively the growth of yarrow, its cover remained 100% in the treated plots both in Soroksár and Kál.

In 2013 plant height of common yarrow varied from 33.7 cm to 52.1 cm in Soroksár. Among treatments POST Afalon+Pendigan (33.7±7.0 cm) and POST Pendigan+Galigan (36.1±6.7 cm in Soroksár and 42.0±7.2 cm in Kál) cut back the plant height significantly. We measured the highest plants (67.6±9.0 cm) in Kál at the control plots. The effect of treatments on plant height did not manifested itself in 2014, we didn't find significant differences between the treatments: the average values were 34.8±5.0 cm in Soroksár and 49.6±7.2 cm in Kál. The difference between the results of the two experimental sites proved to be significant in both years and treatments.

3.4.3. Active materials of common yarrow

In 2012 essential oil content of common yarrow varied between 0.188 g/100 g and 0.447 g/100 g in the treatments. We determined high (>0.35 g/100 g) essential oil content in POST Pulsar, POST Fusilade, POST Agil and a POST Dual Gold treatments in Soroksár, while highest values, exceeding 0.3 g/100 g oil content, were found in POST Fusilade, POST Agil, POST Devrinol, POST Pendigan treatments and in control plots in Kál. We observed significantly lower essential oil content in POST U-46 (0.207±0.005 g/100 g), POST Starane (0.192±0.004 g/100 g) treatments in Soroksár, and POST Benefex (0.210±0.005 g/100 g) treatment in Kál. Significant differences between treatments were proved in both experimental sites. The essential oil content of the samples varied between 0.187 g/100 g and 0.400 g/100 g in 2013. We measured maximum in PPI Pendigan+Galigan treatment (0,374±0,023 g/100 g) in Soroksár, while in Kál POST Pendigan+Galigan combination resulted in the highest essential oil content (0,320±0,055 g/100 g). Among the applied herbicide combinations only PPI Benefex+POST Pendigan+Galigan decreased oil content significantly (0.196±0.008 g/100 g). Statistically significant difference between the treatments were found only in Soroksár in 2013. In 2014 essential oil content of the plants varied between 0.116 g/100 g and 0.568 g/100 g. In Soroksár we detected the highest values, (more than 0.400 g/100), in PPI Racer and PPI Afalon combinations, while essential oil content significantly decreased in PPI Afalon+POST Stomp Super (0.245±0.067 g/100 g) and PPI Stomp Super+POST Afalon (0.202±0.075 g/100 g) treatments. High (more than 0.3 g/100 g) essential oil content was detected in PPI Afalon+POST Stomp Super, PPI Afalon+POST Leopard, PPI Stomp Super+POST Stomp Super combinations in Kál. PPI Racer+POST Stomp Super (0.210±0.041 g/100 g), and PPI Racer+POST Leopard (0.198±0.006 g/100 g) treatments affected essential oil content negatively. The effect of growing site on essential oil content of common yarrow was approved statistically in 2012 and 2014.

The proazulene content of the samples varied from 0.060% to 0.211% in 2012. We detected highest values in POST Fusilade (0.210±0.001%) in Soroksár and in POST U-46 (0.165±0.007%) in Kál, while we found lowest proazulene content in POST U-46 (0.061±0.004%) in Soroksár and

in POST Benefex (0.104±0.001%) combination in Kál. Significant differences were observed between the treatments in both experimental sites. In 2013 we detected highest proazulene content in PPI Pendigan treatment (0.198±0.007%) in Soroksár and in POST Pendigan+Galigan (0.141±0.024%) combination is Kál, while lowest proazulene content was measured in PPI Benefex+POST Pendigan+Galigan (0.094±0.004%) in Soroksár. Significant differences were found only in Soroksár between the treatments proazulene content. The average values of proazulene content of yarrow reached 0.138±0.043% in Kál, while in Kál the results varied from 0.089±0.013% to 0.159±0.008%. We observed significant differences between the treatments only in Kál: highest values were detected in PPI Afalon+POST Stomp Super and PPI Afalon+POST Leopard combinations (0.159±0.008% and 0.153±0006 %), while PPI Racer+POST Stump Super treatment resulted lower (0.089±0.013%) proazulene content. Significant differences in proazulene content between the growing sites were found in all the three years of our experiments.

3.5. New scientific results

During my doctoral work the following new scientific and practically relevant results were achieved:

1. The examined varieties, cultivated stocks and wild growing populations do not vary from each other regarding the average values and amplitude of the examined morphological, productional and chemical parameters, on the other hand their homogeneity varies depending the exact feature.

2. We established that flowering time, leaf colour, habitus, length of flowering horizon, drug mass, ratio of plant organs, essential oil, proazulene and total flavonoid content are genetically determined, genotype dependent characteristics, while the total phenolic content of the taxa does not have well recognizable taxa specific pattern.

3. We proved that all of the examined *A. collina* taxa accumulate small amounts of volatile containing oil in their root, whose accumulation level is taxa specific feature.

4. The presence of 7-heptadecanone, 7-hexadecanone, alismol, β-sesquiphellandrene, γ-humulene, cis-cadine-4-en-7-ol, β-eudesmol, neryl isovalerate in the root oil, and α-,β-pinene, albene, hexanal, neryl isovalerate in the HS extract is universal characteristics of *A. collina*.

5. Regarding their root oil composition, in the selected varieties dominance of guaian skeleton type, while in wild originating populations bigger ratios of eudesmanes, bisabolanes and humulanes are typical.

6. We established that accumulation of volatile and phenolic agents of common yarrow follows characteristic dynamic during generative phase: essential oil, proazulene and total flavonoid content shapes optimum curve-like, while total phenolic content has two maximum peeks, one at beginning of generative phase and another after flowering.

7. We established that climatic conditions affect the accumulation of active agents differently: in case of essential oil, proazulene and total phenolic content they determine the accumulation level, while in case of total flavonoid content not only the level, but the accumulation dynamics also.

8. According to our results optimal drug quality can be achieved by harvesting *A. collina* in early flowering and in the first part of flowering, in 61-65 phase according to BBCH scale.

9. We demonstrated, that under the simulated cold and cloudy climatic conditions, the leaf size of *A. collina* was bigger, the plant height increased with 50%, while the fresh and drug mass with 40-60%.

10. We proved, that the constant, 25 week long 2,5-5,0/0,5-2,5 °C temperature and 8560 lux illumination difference affected essential oil and proazulene content only slightly.

11. According to our results the following herbicide active ingredients, doses and applications can be recommended for the chemical weed control of common yarrow. For PPI treatment: benefin (8 l/ha) 2-3 weeks before planting, incorporated into the upper 5-7 cm layer of the soil; fluorchloridon (2 l/ha) and pendimethalin (4 l/ha) in combination, immediately before planting, incorporated into the upper 2-3 cm layer of the soil; pendimethalin (6 l/ha) immediately before planting, incorporated to the upper 2-3 cm layer of the soil. For POST treatment: pendimethalin (6 l/ha) at 2-3 leaf phase of weeds; quizalofop-p-ethyl (3 l/ha) at 2-3 leaf phase of the weeds.

4. CONCLUSIONS AND PROPOSALS

The results of our comparative investigation deriving from the same growing site prove the intraspecific variability of *A. collina*. The examined yarrow taxa presented considerable variability regarding their development and macropmorphological characteristics. In this regard even the selected varieties proved to be instable, which draws attention to the fact that in breeding the homogeneity of appearance is not achieved until now. Based on our results, among the examined features leaf colour and habitus are those qualitative features, which can be connected to exact genotypes.

We established that plant height (48.6-55.2 cm) of common yarrow is attained full growth in two-year-old populations and can be considered as a characteristic feature of this species. Similarly length of flowering horizon is reached its full growth in the second year of cultivation, which is specific for *A. collina*. On the contrary, the length of flowering horizon (15.8-24.0 cm) presented significant genotype dependence during our investigations.

From the significant differences in the taxa productional features and from their changes depending on plant age, we can conclude, that the values of drug mass and its time of manifestation are taxa specific characteristics. Regarding the drug mass of the investigated groups, the cultivars and cultivated stocks proved to be much more homogeneous than the taxa of wild origin.

The active agent content of the examined taxa presented considerable quantitative variability. Regarding the level of essential oil accumulation we found that the taxa's potential is quite variable (0.147-0.401 g/100 g). Not all of the examined genotypes were able to produce quality drug concerning pharmacopoeia standards in Soroksár, even the selected varieties ('Alba', 'Proa', 'Spak') didn't reach the standards. This phenomenon emphasizes the importance of breeding programs under exact climatic conditions. At the same time, some genotypes from wild growing origins, like Gb22, Gb47 and the own selected 'Azulenka' produced remarkable amounts of essential oil in every experimental year. Regarding the essential oil content of the groups, the homogeneity of wild growing taxa exceeded cultivars and cultivated stocks. During our investigations the effect of environmental conditions on chemosyndrome also manifested itself in the variable level of essential oil accumulation. In 2013 because of the dry and hot weather conditions the volatile content of the taxa decreased, then in 2014 under weather conditions free from externities the essential oil content increased again.

The proazulene content of the examined taxa presented significant variability (0.043-0.173%) and taxa specific characteristic. The level reached the pharmacopoeia standards (0.02%) in every taxa. In proazulene content the selected cultivars had similar potential and their homogeneity

proved to be good, on the contrary to wild growing genotypes whose homogeneity was adequate, but their proazulene content proved to be variable. From this regard the potential of cultivated stocks is low due to their heterogeneity and low proazulene concentration. We established that proazulene content was variable during the three years of examination, which can be explained by the climatic conditions, which affirms the results of Tyihák et al. (1963), who claimed that ecological factors determine the quantity of the compounds, not the presence.

The accumulation of total flavonoids (0.540-2.379%) presented taxa effect, although the differences between the examined genotypes did not appear as markedly as in case of essential oil and proazulene content. Effect of year on flavonoid content showed up, higher values were measured in 2013 thanks to the hot and dry weather during growing season.

Total phenolic content presented high intraspecific variability both in aqueous (133.872-220.042 mg GAE/g d.w.) and in alcoholic extracts 30.193-122.813 mg GAE/g d.w.) during our experiments, however the values did not proved the effect of genotype. The effect of environmental factors and year effect revealed itself significantly, although the difference between years and genotypes proved to be variable and was the opposite with total flavonoid content: we detected higher values under less hot and dry growing season of 2012.

The established correlations between the investigated chemical features (between essential oil and proazulene, total phenolics of aqueous and alcoholic extract, essential oil and total phenolic, proazulene and total phenolic content) can be interpreted indirectly. The correlations derive from the applied extraction methods and from the similar sensitivity to environmental factors of active agents, therefor they add new, practical information about drug quality.

Intraspecific variability of *A. collina* can be concluded from the different amount of volatile oil accumulated in the roots (0.021-0.052 ml/100 g) and from its correlation with essential oil of flowering shoots. We established that the composition of distilled root oil, HS and dichloromethane extracts is different from each other and from the flowering shoots and presents significant variability as well. Beyond universal constituents, which seems to be specific to the species (7-heptacanone-en, linoleic acid, albene, pinenes, hexanal alismol, neryl isovalerate, γ-humulene, cis-cadine-4-en-7-ol, β-eudesmol), we detected taxa specific constituents in the roots of some genotypes (4-hydroxi-4-methyl-2-pentanone, δ-elemene, modeph-2-ene, guaiol, bulnezol, camphene, sabinene, α-isocomene, *transz*-caryophyllene, γ-humulene, β-sesquiphelleandrene). According to our results and literature data, presumably some constituents in the roots, like alkilamides, have taxonomical relevance, while others, like mono- and sesquiterpenes, have a role in ecological adaption. Anyhow the exploration of their role and biosynthesis in the roots needs further investigations.

Our results demonstrate that during ontogenesis significant changes takes place regarding the differentiation of the organs and accumulation of active agents in *A. collina*. The active agent content of the plant parts is different (Németh et al., 2007.; Gudaityte et Venskutonsis, 2007), hence the ratio of different plant parts on the shoots is important. The ratio of inflorescences and leaves in the drug constantly increased during ontogenesis from green bud stage (BBCH 55). However in our experiments accumulation of active agent content did not follow clearly the differentiation and growth of inflorescences. We established that essential oil content maximum cannot be connected to one well-defined development stage, the peak is reached in early flowering and before, in BBCH 59-61 stages. Proazulene content is followed the accumulation dynamics of essential oil, similarly its maximum is cannot be defined with one exact development stage, it is reached in BBCH 55-65 phases. Accumulation of total flavonoids presented significant differences in the detected values and in dynamics as well. Based on this, it seems that flavonoid accumulation of common yarrow is quite sensitive to external, environmental factors. Total phenolic content reached the first peak in green bud stage, BBCH 55, the second one in BBCH 65-69 stages. On the whole, considering the accumulation dynamics of active agents, the influence of environmental factors and year effect, under Hungarian climatic conditions optimal harvest date for one-year-old *A. collina* is during the first half of flowering, in BBCH 61-65 development stages.

The installed temperature and illumination caused considerable changes in the phenological, morphological and productional features of *A. collina* in our experiment. In „cold" treatment low and slowly increasing temperature delayed the flowering of yarrow, while low light intensity influenced plant habitus, bigger leaves, and longer stems developed (Salisbury et Ross, 1992). These changes manifested itself in the production of yarrow as well. Based on our results, accumulation level of essential oil and proazulene did not changed by the consequence of the installed climatic conditions, which is in accordance with the results of Giorgi et al. (2010) and Hofmann et Fritz (1993), who did not detect the effect of changing environmental conditions. Also, from the stable level of total phenolic content we can conclude that, our experimental parameters were still in the tolerance range of the plant. However, because of the specific experimental parameters of the trial, it is important to note, that in vitro experimental parameters differ from natural weather phenomena: they are free from environmental externities, changes in temperature and water supply are constant, so presumably plants can adapt to this environment more easily. In this regard the results of our experiment can be rather interpreted for different climatic conditions (growing sites), than for different weather conditions of continental climate.

During our experiment on reducing interspecific plant competition we observed dissimilar efficacy of the treatments. Based on our results pendimethalin (Pendigan, Stomp Super) was more effective by PPI application against weed seedlings. POST application of pendimethalin and

oxifluorfen (Pendigan and Galigan) in combination found to be prosperous: lower dose of pendimethalin was compensated by oxifluorfen, which covered soil surface as a film layer after application. PPI application of Benefin successfully reduced first generation of weeds, but it needs a POST complement treatment later in May. Fluorchloridon (Racer) presented strong herbicide activity applied both separately and in combination, however monocotyledon herbicides complemented its effects well during our experiments. We observed the effect of different growing sites on the efficacy of the treatments, like in case of PPI linuron (Afalon) combination in 2014, while in some other treatments (2012. Pendigan, Dual, 2014. PPI Afalon-POST Stomp Super, PPI Stomp Super -POST Afalon, PPI Stomp Super-POST Boxer) the difference might be caused by the dissimilar weed spectra and development.

We found considerable differences in the effect of herbicide treatments on *A. collina* in every year of the experiment and both growing sites. The tested herbicides proved to be selective on yarrow in greater or lesser extent. In case of PPI applied active ingredients this meant position selectivity: in case of incorporated active agents, like linuron (Afalon), the herbicide and plant roots are situated in different soil layer. Applied as soil herbicides, benefin and pendimethalin is effective on germinating seeds, they interfere with cell division by inhibiting the rearrangement of tubulins (Hunyadi, 2000), fluorchloridon has an effect on germinating seeds as well. Quizalofop-p-ethyl and propaquizafop (Leopard and Agil) are monocotyledon herbicides, selective for every dicotyledonous plant regardless of their development. Besides, on the hairy leaves of common yarrow herbicides absorption is worse (Hunyadi, 2000). The difference between experimental sites can be explained by the characteristics of growing sites, dissimilar development stage of yarrow during herbicide application (2012. Devrinol and Fusilade combinations) and by damages caused by weather conditions –high amount of precipitation, as we experienced at Soroksár in 2014.

The effect of treatments on plant height was significant in 2013 at both experimental sites, and the differences between combinations could be traced back to the adverse effect of some active ingredients. In 2014 treatments didn't show any effect on plant height, the applied combination didn't cut back the plant growth at all neither at Soroksár, nor at Kál.

The chemical weed control treatments effected significantly the active agent content of yarrow, differences in essential oil and proazulene content were observed, but not in every experimental year and site. Lower active agent content derived from the damage of common yarrow, however other factors -development stage of plants, effect of growing site, effect of herbicide active ingredients on biosynthesis of plant metabolites- has an influence on it as well, whose have to be clarified in the future.

Based on our results, cultivation technology of yarrow and effects of herbicide active agents, early or late PPI application of herbicides can be advised in yarrow cultivation, since logistic

reasons and their less influence on growth and production of the plant. These active ingredients can protect the plantation against first generation of weeds, but their effect should be completed later with POST treatment or with mechanical weeding.

The results of our experiments on the production and active agents of common yarrow have several practically important aspects as well.

- With the cultivation of taxa with different flowering time, harvest can be prolonged, ensuring proper harvest timing without quality loss of plant material.
- Cultivars/taxa with characteristic and stable morphological features can be advantageous in breeding because of the easier selection of the disparate plants.
- For optimal drug quality, cultivars with good chemical features and short, homogeneous flowering horizon should be chosen by the farmers, which can produce drug with high active agent content and favourable plant part ratios. Among the examined taxa perspective genotypes were found with optimal drug quality and outstanding production. Under Hungarian climatic conditions 'Azulenka' variety and Gb22 wild originating genotype can be advised for cultivation. Taxa Gb47 with its high essential oil content can serve as breeding material in the future.
- The characteristics of growth and development of *A. collina,* according to which, some of the features (macromophological features, active agent content) are manifested in first year, while others (plant height, length of flowering horizon, production) only in second year of cultivation, can serve as practical reference point for variety and DUS testing.
- By revealing the accumulation dynamics of active agents of common yarrow, harvest of plant material in early flowering better drug quality can be achieved and the active agent optimized harvest date can be defined.
- Our results about the competitive ability and herbicide tolerance of *A. collina* have great importance in introducing the chemical weed control of yarrow into cultivation and with this, reducing the manual work needs.

Based on these, we can establish that our results contribute to the creditable and more economic cultivation of *A. collina* in the future.

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