The European Journal of Plant Science and Biotechnology ©2007 Global Science Books

Conservation and Evaluation of Selected Medicinal and Aromatic Plants from Lithuania

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ABSTRACT

This review focuses on research of medicinal and aromatic plants in Lithuania and describes how the conservation of genetic resources of these plants is considered. Biological peculiarities of species and their sources of raw material determine the mode of conservation including *in situ* and *ex situ* methods. The data on evaluation of essential oils of native species (*Achillea millefolium*, *Acorus calamus*, *Origanum vulgare*, *Helichrysum arenarium*, *Hypericum perforatum*, *Thymus* ssp., *Tanacetum vulgare*) of commercial value in herbal medicine are presented. The morphological, chemical and ecological markers support the selection and taxonomical identification of intra-specific diversity and are essential for germplasm conservation and utilization. The exploitation of the chemical diversity of evaluated species may be a potential source of genetic variation to allow selecting the valuable material for breeding.

Keywords: essential oils, genetic recourses, intra-specific variability

Abbreviations: MAPs, medicinal and aromatic plants

CONTENTS

INTRODUCTION

Medicinal and aromatic plants (MAPs) represent a relevant part of the world's natural biodiversity. An estimated 40,000 to 50,000 plant species are used in folk and modern medicine throughout the world. A list compiled by the WHO contained over 21,000 names of plants that were reported to be used as medicinals (Heywood 1999). In Europe, at least 2000 MAP species are used on a commercial basis, of which 1200-1300 are native to Europe (Lange 1998). The new 5^{th} edition of the European Pharmacopoeia (2005) contains around 200 monographs on plant drugs and this trend is likely to continue. Out of the known 1500 species of vascular Lithuanian flora, about 460 species have been considered as medicinal plants used in folk or traditional medicines. Of these about 280 species are native origins (Radušiene and Janulis 2004). Increasing demand for herbal preparations is placing extensive harvesting of raw material from wild what is considered to be a major threat to MAPs in Europe. According to Large (1998, 2002) in eastern and south-eastern European countries, wild-collected botanicals still play a pivotal role. The pressure on natural resources due to numerous factors raises the importance of germplasm conservation. European efforts regarding conservation and sustainable use of MAP species are coordinated through the European Cooperative Program for Crop Genetic Resources (ECP/GR) and it's Working Group on MAPs.

Conservation activity of plant genetic recourses in Lithuania started in 1995, when the country joined the European PGR conservation network, participating in ECP/GR. The "*Catalogue of Lithuanian plant genetic resources*" was published in 1997. Current conservation activity is directed by the national PGR programme "Genefund" and the Plant Gene Bank founded by the Ministry of the Environment in 2004.

This review presents considerations arising from integrated research and conservation of MAPs performed in Lithuania based on published and new data. Integrating this data allows for the evaluation and conservation of MAPs with a view to providing better material for herbal medicine.

Received: 23 February, 2007. Accepted: 10 April, 2007.

Invited Review

RESOURCES, THREATS AND USE OF MAPs

In Lithuania as in other eastern bloc countries (Lange 2002) the changes in political and economic conditions after the fall of the Soviet Union have changed the trade of herbal pharmaceutics (Radušienė 2004). On the other hand, privatisation and changes of land use affected the distribution of wild populations endangering these recourses. The large areas of previously cultivated wasteland are currently used for gathering raw materials, the MAPs. Numerous populations of *Achillea millefolium* and *Hypericum perforatum* in waste lands have therefore become vulnerable because of unregulated exploitation. The habitat changes impacts on size and vitality of the populations of common species. Populations of *Acorus calamus*, *Menyanthes trifoliata*, *Drosera anglica* or *Valeriana officinalis* previously harvested from wetlands have become reduced because of draining of surrounding areas. On the other hand, the increasing demand in international herbal trade caused the decline of populations of the most used native species in herbal preparations: *Arctostaphyllos uva-ursi*, *Menyanthes trifoliata*, *Thymus serpyllum*, *Tussilago farfara*, *Polygonum aviculare*, *Urtica dioica*, *Frangula alnus*, and *Helichrysum arenarium* (Radušienė 2004). Species that had become threatened were placed under legal protection which involved controlled gathering or strict protection in the Red Data Book (Kundrotas 2003). The Ministry of the Environment governs the wild-harvesting of MAPs.

The flora has changed continuously by expansion of some aggressive invasive species. At present in Lithuania 470 species are registered as alien which spread in natural communities and are causing serious problems to the indigenous species (Gudžinskas 1998). *Heracleum sosnowskyi* and *Lupinus polyphyllus* can serve as evident examples of aggressive invasive species. It is difficult to estimate what impact invasive species will have on indigenous flora in general and on MAP species in particular, but it is important to monitor their spread.

CONSERVATION OF MAPs

The conservation of MAPs can be accomplished by *in situ* and *ex situ* measures. Combination of both methods originated a complementary conservation strategy, which is very important to promote sustainable use of recourses. Conservation of MAP germplasm is developed within the conservation model described by Maxted *et al*. (1997). The conservation initiative involves identification of priority species for conservation action, which appear to be endangered and/or highly used in pharmaceutics. The biological peculiarities of species and their sources of raw material determined the mode of conservation (Radušienė 2004). Although, at present, wild populations of some species are not endangered in their natural habitats, the survey of its distribution and sampling of their diversity have been initiated to ensure the conservation and further utilization of their germplasm.

In situ conservation

Conservation *in situ* is a measure for the rational exploitation and protection of MAPs in their natural habitats and to maintain their genetic diversity. This mode of conservation is aided by the conservation of the full range of MAPs and other plant germplasm in their natural environment. *In situ* conservation activities are mainly connected to protect ecosystems including red listed species and/or forest species and rarely targeted to specific plants, like MAPs (Heywood and Dulloo 2005). Protected areas of different categories encompass about 11% of all Lithuania territory, but there are no reserves specifically designated as reserves for the conservation of MAP germplasm. Legislation on MAP exploitation and conservation is directly connected with environmental protection in general. An inventory and monitoring of threatened species in protected areas is prioritized

Fig. 1 Typical population of *Allium ursinum* in lowland forest habitat. Photo: B. Karpavičiene.

Fig. 2 Forest ecotype of *Arnica montana* in Lithuania. Photo: J. Radušiene.

because of minimal and inexpensive management. In the selection process of *in situ* conservation areas for target MAP species the following criteria have been considered: ecological heterogeneity of the site; phenotypic diversity and concentration of the target taxa; relative degree of genetic erosion, location of the site with regard to protected areas (Labokas 1999).

The inventory of populations, monitoring of their vitality, renovation and coenodiversity changes have been carried out with wild *Allium* species and *Arnica montana*. All native species of *Allium* are subject to germplasm conservation in Europe. There are 7 spontaneous species of *Allium* in Lithuania: *A. oleraceum*, *A. senescens* ssp. *montanum*, *A. schoenoprasum*, *A. angulosum*, *A. scorodoprasum*, *A. ursinum*, and *A. vineale*. The last four species are listed in the Red Data Book of Lithuania (Kundrotas 2003). The inventory of *Allium* species distribution was based on scientific observations and questionnaires of foresters. The largest amount of data was collected on *A. ursinum* and *A. oleraceum* (Karpavičienė 2004, 2005). There are about 100 known habitats of *A. ursinum* which are concentrated in lowland forests in the central and western parts of Lithuania (**Fig. 1**). Other species are rare or very rare.

Arnica montana (Mountain Tobacco) is regarded as a critically endangered species in Belgium, Bosnia, Croatia, and Luxemburg; endangered in Belarus and the Netherlands; vulnerable in Estonia, Germany, Hungary, Latvia, Lithuania, Portugal, and Romania; and near threatened in Denmark and Norway (Lange 1998). The inventory and more detailed analysis of the status of *Arnica* populations have just begun in Lithuania. Two different habitat types; forest and meadow were distinguished within the range of *A.*

montana (Radušienė and Labokas 2005). The forest habitats of arnica are different from those described in other European areas where the species is distributed in mountain and submountain areas and in heath lands and grasslands of lowland areas (Luijten *et al*. 1996) (**Fig**. **2**). Specific habitats typical for Lithuania may be a result of the species performance on the north-eastern border of its distribution range. The whole population of *A*. *montana* seems to be in relatively good condition.

Ex situ conservation

Ex situ conservation is defined as germplasm conservation outside the natural habitat by cultivating and maintaining tissue culture, plants or their propagules in field collections and gene banks with potential their use as gene donors for new crops (Heywood 2003; Hajjar and Hodgkin 2005). *Ex situ* conservation of MAPs is focused on species which were prioritized according importance of plant production and both of biological and chemical diversity. Sustainable use of MAPs can be achieved by further introduction of "wild" plants into cultivation. Wild species are considered to be a source for the utilization in the improvement of cultivated medicinal plants and pharmaceuticals. The target species of detail evaluation were selected concerning the pharmacological significance in local and international scales. *Ex situ* is the most applicable method for MAP maintenance and evaluation of their intra-specific diversity. Field collections are used to represent the broad range of genetic diversity within species. Collections of MAP germplasm in Lithuania have been established at the Institute of Botany and Kaunas Botanical Garden and have focused on the conservation of both endangered and common wild species. Each collected accession was propagated in a field nursery and subjected to evaluation of its morphology, phenology and chemistry. An evaluation of the bio-active chemical compounds is of major importance for selection of MAP germplasm.

INVENTORY OF MORPHOLOGICAL AND CHEMICAL DIVERSITY

Variability of MAPs is generally a very important phenomenon which is used in selection and cultivation of valuable material. We have used several morphological, chemical and ecological tools for plant material quality identification. On the other hand, they could also serve as taxonomically useful markers. The evaluation and exploitation of the chemical diversity of plant species are rapidly increasing because of a growing demand for high quality herbal drugs and the search of new and highly active components.

MAP species differ substantially from other crops due to their content and high variability of secondary metabolites. Chemical characteristics of plants are very variable and their scientific value is difficult to appreciate. Different authors have reported different profiles of essential oil for the same species or even the same author have reported different composition for the same plant in different papers. This disparity may be caused by ecological and genetic variation, analytical techniques and methods or incorrect taxonomic identification. One of the most influenced agents on chemical composition of the same species is environmental factors which highly affect the results of chemical evaluation. The most reasonable compare of accessions of the same species is to move them to the field and cultivate on the same site.

The presented investigations accomplished in Lithuania were focused on the evaluation of *Acorus calamus* (sweet flag), *Achillea millefolium* (yarrow), *Hypericum perforatum* (St. John's Wort), *Origanum vulgare* (oregano), and *Thymus*

Table 1 Principle compounds of essential oils of medicinal and aromatic plants evaluated in Lithuania.

Plant name	Plant part	Compounds	References
Achillea milefolium	Inflorescences	β-pinene (6.5-30.2), α-pinene (2.7-16.4), α-phelandrene (2.3-16.0), sabinene (0.9-13.0), borneol (0.4-13.2), camphor (0.3-13.1), 1,8-cineole (5.4-21.6), terpinen-4-ol (0.9–8.6), β-caryophyllene (1.5-25.0), germacrene D (1.7-15.9), caryophyllene oxide (1.1-18.8), chamazulene (0-30.7), *trans*-nerolidol (0.3-13.5)	Judzentiene and Mockute 2002; Mockute and Judzentiene 2003, 2005
Acorus calamus	Leaves	β-caryophyllene (3.7-8.8), shyobunone (t-6.6), (*E*)-asarone (1.1-7.7), (*Z*)-asarone (15.5-45.5), (*Z*)-methyl isoeugenol (2.0-4.9), acorenones (0-6.6)	Venskutonis and Dagilyte 2003; Radušienė *et al*. 2006; 2007
	Rhizomes	Shyobunone (14.8-27.8), (*Z*)-asarone (4.3-9.6), acorenones (9.6-21.4)	Radušienė *et al*. 2006
Helichrysum arenarium	Inflorescences	1,8-cineole (0.8-7.0), β-caryophyllene (4.4-25.6), α-copaene (1.5-7.2), δ-cadinene (0.6-11.2), tetradecanoic acid (0-7.8), selina-3.7(11)-diene (0-8.6)	Judzentiene and Butkiene 2006
Hypericum perforatum	Inflorescences	β-caryophyllene (4.2-18.3), β-farnezene (t-8.2), germacrene D (t-31.5), caryophyllene oxide (6.1-35.8), spathulenol (3.9-11.0), viridiflorol (0-11.1), dodecanol (0.2-9.8), tetradecanal t-8.9, tetradecanol (0.9-11.2), heneicosane (1.5-32.1), nonadecane (0.3-12.2)	Mockute *et al*. 2003; Radušienė *et al*. 2005a
Origanum vulgare	Inflorescences	*cis* -β-ocimene (0.9-7.1), *trans*-β-ocimene (1.1-12.7), *p*-cymene (0-8.2), sabinene (0.3-25.1), 1,8-cineole (t-14.2), terpinen-4-ol (t-17.9), β-caryophyllene (4.7-25.0), germacrene D (1.7-20.1), vulgarone B (0-15.6), α-cadinol (0.3-9.6), caryophyllene oxide (0.3-18.8)	Mockute *et al*. 2001, 2003; Radušienė *et al*. 2005b, 2005c, 2006
	Leaves	sabinene (0.9-18.3), *cis*-β-ocimene (0.3-16.6), *trans*-β-ocimene (0.8-10.1), β-caryophyllene (5.4-24.5), germacrene D (1.5-15.7), caryophyllene oxide (0.7-24.4), spathulenol (1.9-9.8), α-cadinol (0-9.8)	
Tanacetum vulgare	Inflorescences/leaves	borneol (0-16.7), *iso*-borneol (0-13.0), camphor (0-61.8), 1,8-cineole (0.9-46.3), terpinen-4-ol (0-12.2), myrtenol (0-24.9), *cis*-thujone (0-43.9), *trans*-thujone (0-78.4), artenisia ketone (0-30.5), chrysanthenone (0-20.8)	Mockute and Judzentiene 2004; Judzentiene and Mockute 2005
Thymus pulegioides	Herb	γ-terpinene (0-21.4), *p*-cymene (0-16.7), carvacrol (0-25.5), geranial (0-16.1), geraniol (0-31.2), linalool (0-80.3), thymol (0-26.1), α-terpenyl acetate (0-70.4), β-caryophyllene (5.1-14.0)	Mockute and Bernotiene 1999, 2001; Ložienė *et al*. 2003
Thymus serpyllum	Herb	β-ocimene (0-36.2), borneol (0-27.1), 1,8-cineole (0-30.3), β-caryophyllene (0-27.2), germacrene D (0-20.4), germacrene B (0-25.4), α-cadinol (0-28.6), caryophyllene oxide (0-27.2), spathulenol (0-26.0), *cis*-*p*-menth-2en-1ol (0-24.1)	Ložienė *et al*. 1998; Ložienė and Venskutonis 2006
Thymus × *oblongifolius*	Herb	camphor (1.8-4.7), myrcene (5.7-21.0), β-caryophyllene (9.9-29.9), germacrene D (10.0-17.7), bisabolene (0.7-14.2), caryophyllene oxide (0.8-15.8)	Ložienė *et al*. 2002

ssp. (thyme). The choice of accessions aimed at covering the different habitats and morphological variability of evaluated species. The main objectives were: the search for inter- and intra-specific distribution of active compounds; the identification and selection of intra-specific chemotypes with desirable composition and with the absence of compounds that cause adverse effects; to check for biological activity of some species. The principal compounds of the essential oils of these species are summarized in **Table 1**.

Acorus calamus (sweet flag)

Acorus calamus has a long and rich history of ethnobotanical applications by many different cultures in many countries (Motley 1994). The plant is a regular item in the international drug market, whose raw material originates from natural populations. *A. calamus* has become extinct and endangered in several European countries due to the habitat loss of populations (Kozyuk 1997; Lange 1998).

In Lithuania rhizomes of sweet flag (*Rhizome Calami*) are used for treatment of diarrhea, dyspepsia, neuralgia, and hair loss. Leaves (*Herba Calami*) are used in baths to relieve arthritis, gout and rheumatism. Moreover, fresh leaves of sweet flag are used in bread bake. The use of leaves is more acceptable from the point of view of conservation of plant resources.

More recently, detailed analyses were performed on essential oils produced from leaves and rhizomes of wild populations (Radušiene *et al*. 2006b, 2007). The yield of essential oil isolated from different populations of sweet flag accounted for 0.21-0.64% (v/w) in leaves and for 2.4-4.4% (v/w) in rhizomes. Leaf essential oils were characterized by the presence of phenolic compounds with a highly complex mixture of compounds, many of which are common in other APs and responsible for their aroma. However, these odour compounds were found in low quantities. The most abundant constituent was (*Z*)-asarone which accounted from 15.7 to 29.0% of total oil. The content of (*Z*)-asarone limits the use of sweet flag for human purposes as it is considered as undesired mutagenic, carcinogenic and psychoactive constituent (Goeggelmann and Schimmer 1983; Motley 1994). Recent investigations demonstrated antiproliferative, immunosuppressive actions of *A. calamus* on human carcinoma cells (Graham *et al*. 2000; Mehrotra *et al*. 2003). Moreover, the high percentage of (*Z*)-asarone in sweet flag favoured its use as an insecticidal and fungicidal agent (Mungkornasawakul *et al*. 2002; Park *et al*. 2003).

Chemical characteristic of leaf essential oil may offer a possible new utilization of plant as a natural repellent agent in organic plant protection.

Rhizome essential oils were characterized by the presence of oxygenated sesquiterpenes. The most abundant sesquiterpenes were shyobunone isomers and acorenones, which were found in considerably higher concentrations than their content in leaf oils. (*Z*)-Asarone (4.3-9.6%) was detected in lower amounts than that in leaf oils.

Quantity differences of the most important constituent of essential oil (*Z*)-asarone have been considered to have taxonomic importance within the genus, as this constituent indicated polyploidy of *A. calamus* (Röst 1979; Keller and Stahl 1982; Lander and Schreier 1990). In accordance with (*Z*)-asarone content, present populations of *A. calamus*, widely distributed all over Europe, may result from the sterile triploid. The species is considered to be genetically homogenous due to asexual reproduction, though the results of an essential oil survey revealed differences among populations indicating the existence of chemical variability, which seems likely to arise from the influence of different habitats.

Achillea millefolium Agg. (common yarrow)

Achillea millefolium Agg. is a group of closely related species and subspecies widely distributed in central and northern, sparsely in southern Europe. The raw material of yarrow *Herba Millefolii* is one of the oldest and most important drugs used both in folk and official medicine (Chandler *et al*. 1982). Vulnerary, spasmolytic, hemostatic, anti-inflammatory and other pharmacological actions of milfoils mostly attributed to the presence of proazulenes, which produced by glandular trichomos in leaves and flowers and convert to chamazulenes during thermal effect (Hofmann *et al*. 1992). The presence of chamazulene has been regarded as a diagnostic character for recognition of *A. millefolium* group species. It was agreed that *A. millefolium* ssp. *millefolium* is hexaploid and does not contain proazulenes, while the tetraploid species (*A. collina* and *A. pratensis*) contain proazulenes (Hofmann and Fritz 1993; Michler and Arnold 1999). The raw material usually gathered from natural populations

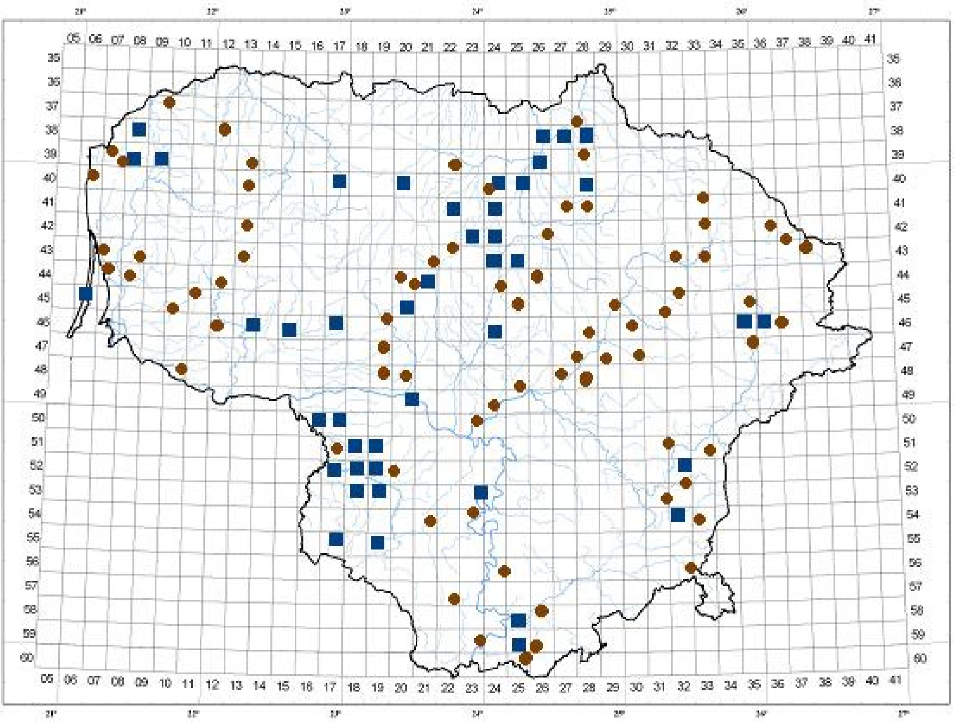

Fig. 3 Distribution of proazulene-containing (■) and proazulene-free (●) populations of *Achillea millefolium* in Lithuania.

and contains a mixture of different taxa or hybrids whose pharmaceutical value is uncertain. The analysis of morphological characters of wild populations allowed presuming intra-specific differences within this species in Lithuania (Gudaityte and Radušiene 2005). The testing of *A. millefolium* populations based on a total of 570 selected plants for the presence of proazulenes using colorimetric assay test (Stahl 1953) revealed high variations for these compounds in different habitats. Plants were grouped according to the presence of proazulenes into three groups: proazulene-free, and low and high amounts of proazulenes. On average 62% of all tested plants were proazulene-free while in others the content of these substances varied from low to high (Radušiene and Gudaityte 2005; **Fig. 3**). It was established that chamazulene chemotypes might be characteristic for particular growing habitats. In segetal and wasteland habitats which mostly used for gathering of raw material were found only proazulenes-free plants. The vast majority of plants gathered in woodland and scrubland habitats contained proazulenes. The results suggested that phytosociological dependence of *A. millefolium* may be important for the initial identification of proazulene-containing populations towards a meaningful selection of raw material. The results allow predicting the predominance of *A. millefolium* ssp. *millefolium* as a hexaploid species growing in Lithuania. On the other hand, chemical composition was related to some morphological traits. The study raised the possibility that selection, based on two morphological marker traits, i.e. node number and leaf width on the main stem, could be effective for the rapid identification of highly productive population(s) of pharmaceutical importance (Radušiene and Gudaityte 2005; **Fig**. **3**).

By evaluating the results of essential oil composition it could be concluded that *A. millefolium* showed considerable variation, a common phenomenon of *Achillea* plants showing intra- and inter-specific differences of the species. On the base of the proportion of main compounds the oils were attributed to several chemotypes whose majority differed in the quantities of main compounds. Some chemical profiles exposed qualitative differences related to the presence of chamazulene. Oils of a chamazulene-type were produced only by flowers (Mockute and Judzentiene 2002, 2003). The most general and main component of oils was the monoterpene β-pinene (0.33-62.29% w/v), which is considered as a taxonomically useful marker representing *A. millefolium* species (Hoffman 1993; Nemeth 2005). The most abundant and frequent other monoterpenes among the analysed oils were: α-pinene, 1,8-cineole, camphor and borneol, at a range of 0.37-16.67, 2.3-21.5, 0.08-7.2, and trace-13.2% (w/v), respectively. The most frequently identified sesquiterpenes were: β-caryophyllene (0.16-19.50%) and its oxide (0.13–13.08%), chamazulene (0.16–30.70%), *trans*-nerolidol (0.34–13.50%), and α-phelandrene (2.34-15.97%). The content of β-pinene decreased and *trans*-nerolidol increased in inflorescence oils with intensity of colour which varied from white to deep pink (Judzentiene and Mockute 2005).

Generally we could conclude that the *A. millefolium* group is highly variable in morphological and chemical traits which exposed intra-specific differences within species and in relation to its environment. In order to assume a high quality of raw material of yarrow we should focused on several markers: coenological, morphological, chemical and cytological.

Helichrysum arenarium (yellow everlasting)

Helichrysum arenarium is native to Middle and East Europe, South Sweden, Denmark and Caucasus (Clapham 1976). Everlasting is a clone perennial, semi-rosette herb growing on dry, poor sandy acid soils. The native populations of *H. arenarium* are becoming more and more endangered because of changes in its habitats and the gathering of raw material. The species is extinct in The Netherlands, fully protected in the Czech Republic, Hungary, Sweden

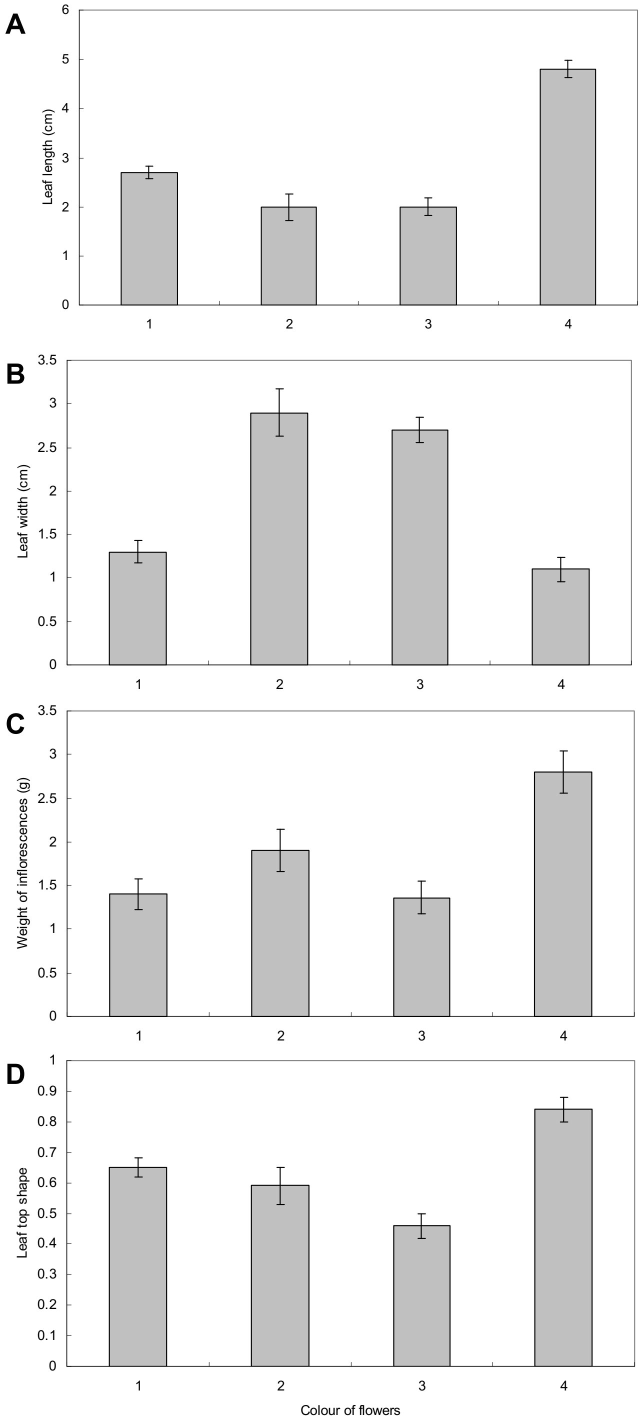

Fig. 4 Relationship between colour of flowers and morphological characters (± SE) of *Helichrysum arenarium*. Leaf length (**A**) and width (**B**), weight of raw material (inflorescences) (**C**) and top shape (sharp: 0, obtuse: 1) (**D**). Colour of flowers: 1, citric; 2, citric yellow; 3, yellow; 4, orange.

and Estonia and care is being demanded in other countries (Lange 1998). In Lithuania inflorescences of the plant (*Helichrysi flos*) have long been widely used for the treatment of gall-bladder, liver and urine-bladder disorders (Švambaris 1972). The harvest from the wild is legally regulated. The introduction of everlasting into cultivation could be the most acceptable mode of plant conservation and supplying of raw material.

Plants from the same population showed a wide rage of phenotypic variation, the most conspicuous of which is that in the colour of inflorescences which vary from citric to reddish-orange of various intensities. A morphometric statistical analysis ascertained a significant relationship between the colour of flowers and other morphological characters.

While selecting *H. arenarium* it is important to pay attention to the plants with orange inflorescences as they are distinguished by the longest and widest inflorescences which produce the highest amount of pharmaceutical material. It was revealed that 84% of plants with dark coloured flowers had an obtuse leaf top. The narrow (0.5-1.3 cm) and long (4.5-6.5 cm) leaves with obtuse top shape can be used as morphological markers to indicate orange flowers with a higher yield of raw materials (Radušiene 2002a, 2003; **Fig. 4**).

Evaluation of the volatile composition on the Lithuanian accessions of *H. arenarium* was estimated by differences in the colour of inflorescences (Judzentiene and Butkiene 2006). All investigated oils contained a similar array of constituents. Essential oils from orange inflorescences did not display any particular composition. No correlation was found between volatile chemistry and colour of inflorescences. *Trans*-caryophyllene was detected as one of the major constituents in all essential oils, whose amount varied from 4.4 to 25.6% (w/v) and seems to be the most consistent component of oils with other *Helichrysum* species (Roussis *et al*. 2000). The main difference between the percentages of volatile constituents detected in *H. arenarium* and other Mediterranean species seems to be a lower content of monoterpenes and linear hydrocarbons in inflorescence oils that characterized species as a low aromatic taxus of the genus.

Hypericum perforatum (St. John's Wort)

The genus *Hypericum* has received considerable interest from scientists, as it is a source of a variety of biologically active compounds including naphtodianthrones, phloroglucinols, flavonoids, xanthones, essential oils and others (Bombardelli and Morazonni 1995). *H. perforatum* is one of the best known and most frequently used herb in the treatment of mild and moderate depression in recent years (Nathan 2001). Pharmaceutical effects increased the importance of *H. perforatum* as a medicinal plant and have stimulated detailed biological and chemical evaluation of this species. There are some reports on the composition of essential oils of *Hypericum* species (Couladis *et al*. 2001; Gudzic *et al*. 2001; Bertoli *et al*. 2003). In the case of Lithuanian investigations, a range of ecological adaptation, morphological and chemical variation of *H. perforatum* was reported (Radušiene 2002b; Radušiene and Bagdonaite 2002; Radušiene *et al*. 2004).

In another report the compounds with a caryophyllane skeleton were prevalent in oils from flowers and leaves of *H. perforatum* (Radušiene *et al*. 2005a). The presence of some other components, namely heneicosane (1.5-32.1), spathulenol (3.9-11.0%), dodecanol (0.2-9.8%), tetradecanol (0.9-11.2%), tetradecanal (trace-8.9%), manool (trace-7.6%) in considerably large and variable amounts testified to the presence of chemotypes of essential oil. Data on the composition of essential oils from our plant material showed profound differences with those reported from other localities. The main distinction seems to be a higher content of oxygenated sesquiterpenes. It is not surprising that the volatile composition of *H. perforatum* reveals great differences, which probably depends on environmental factors.

Origanum vulgare (oregano)

Origanum vulgare is most widespread among all the species within the *Origanum* genus. It is distributed all over Europe, West and Central Asia up to Taiwan (Ietswaat 1980). *O. vulgare* plays a primary role among culinary herbs in world trade (Oliver 1997). Most commercial oregano comes from wild populations of Turkey and Greece (Arnold *et al*. 1993). A number of studies have shown that variation within species may occur in its morphological and chemical features (Chalchat and Pasquier 1998; Skoula *et al*. 1999; D'Antuono 2000). It has been ascertain that the variation follows its geographical distribution. Moreover, research on oregano germplasm is very limited outside the Mediterranean region. On the other hand, it is necessary to investigate and conserve the genetic diversity on a whole and not just that which might be of interest to the industrial sector.

Oregano in Lithuania is a threatened species traditionally used as a medicinal plant. On the basis of discriminative morphologic characters oregano growing in Lithuania was attributed to ssp. *vulgare*, which has inconspicuous leaf glands, purple bracts and pinkish to purple corollas. The species is considered to be a poor source of essential oil whose amount varies according to year and accession, ranging from 0.3 to 1.5% in flowers and from 0.1 to 1.0% in leaves (Radušienė *et al*. 2005c). A field collection of oregano demonstrated high variation in the chemical composition of the essential oil. Two groups of essential oils were

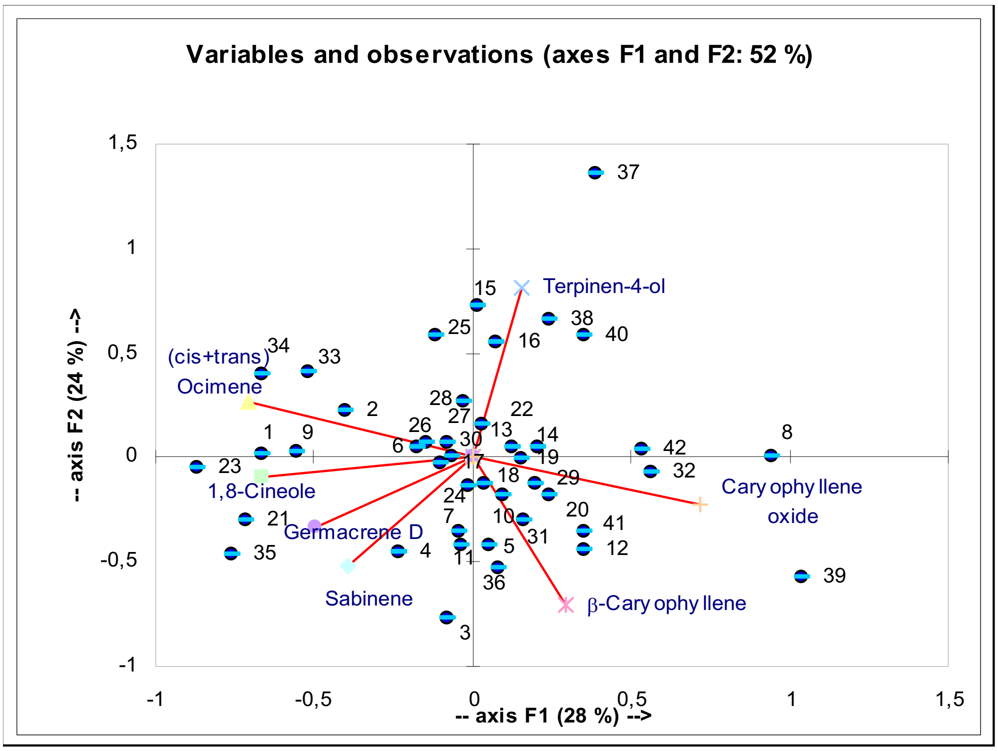

Fig. 5 Principal Component Analysis of essential oils isolated from inflorescences and leaves of *Origanum vulgare* subsp. *vulgare* based on the variation of seven constituents with quantity ≥10%. Inflorescences: odd figures, leaves: twin figures. (Reprinted from Radušiene *et al*. (2006a), *Acta Horticulturae* **723**, 393-398 with kind permission of the International Society for Horticultural Science, http://www.actahort.org)

distinguished according to the amounts of compounds from the same biosynthetic pathway. One group showed a high proportion of mono- and sesquiterpene hydrocarbons, while the other had high levels of oxygenated sesqiterpenes (Radušiene *et al*. 2005b). The results support previous findings, which showed that ssp. *vulgare* produces essential oils rich in acyclic compounds and sesquiterpenoids (Skoula *et al*. 1999). The definition of analysed oils variability was based on Factor Analysis showing the relative position of oils with respect to principal constituents originated in the Principal Component Analysis (Radušiene *et al*. 2006a; **Fig. 5**). The variation of essential oils was confirmed by the presence of a relatively high number of oils with individual character. Some authors (Lawrence and Reynolds 1984; Gounaris *et al*. 2002) reported that carvacrol together with *p*-cymene, γ-terpinene, sabinene, and *cis*-ocimene dominated the essential oil of ssp. *vulgare*. Carvacrol was essentially absent or present in trace quantities in oils of Lithuanian populations. Carvacrol-free oils were also found in *O. vulgare* plants from Italy (Melegari *et al*. 1995) and France (Chalchat and Pasquier 1998). Bernáth (2002) noted that there are intra-specific taxa of oregano having no "oregano" character that is based on the presence of carvacrol. The essential oil composition of oregano populations confirmed that synthesis of phenols like thymol, carvacrol, and eugenol is favoured in a warmer and drier climate, while nonphenolic compounds predominate in cooler and damper areas (Adzet *et al*. 1977).

Tanacetum vulgare (common tansy)

Tansy is traditionally used as a herbal remedy for healing migraines, neuralgia, rheumatism, and digestive ailments. The plant is cultivated in gardens and is widespread in wild habitats in Lithuania. Investigations on the essential oil composition revealed several groups of oils according to the dominant constituents: camphor (19.8-61.8%), 1,8-cineole (23.6-46.3%), *trans*-thujone (35.7-78.4%), myrtenol (13.1-24.9%) and artemisia ketone (30.5%) (Mockute and Judzentiene 2004; Judzentiene and Mockute 2005). Camphor chemotype of essential oils prevailed in wild populations of tansy in Lithuanian, whereas a review of data by Lawrence (2000) of about 100 oils testifies the predominance of the *trans*-thujone chemotype. The high toxicity of thujone limits the use of tansy while other chemotypes can be used more safely (Woolf 1999). The oil of the artemisia ketone-chemotype was found only in the inflorescence of one population. The myrtenol chemotype was determined in tansy for the first time (Mockute and Judzentiene 2005). The flower and leaf oils were of the same chemotype differing only in the amounts of the main constituents.

Thymus ssp. (thyme)

Two indigenous *Thymus* species, *T. pulegioides* (large thyme) and *T. serpyllum* (creeping thyme) and their interspecific hybrid *T.* × *oblongifolius* are common in Lithuania. Thyme collected from the wild is used for herbal teas and pharmaceuticals. Morphological and chemical polymorphism is characteristic of thyme growing in Lithuania and of other species of the genus from other localities (Stahl-Biskup 1991). Three subspecies and 11 varieties of *T. pulegioides* and two subspecies and four varieties of *T. serpyllum* were distinguished in Lithuania (Lekavičius and Jaskonis 1968, 1969). Both species of thyme showed remarkable variation in the chemical composition of essential oils. Seven chemotypes according to the principal constituents were defined for *T. pulegioides*, namely linalool, geranial/geraniol/neral, thymol, carvacrol/γ-terpinene/*p*-cymene, thymol/carvacrol/*p*-cymene/γ-terpinene, α-terpenyl acetate and citral/geraniol (Mockute and Bernotiene 1999, 2001; Ložienė *et al*. 2003) and five chemotypes for *T. serpyllum*: 1,8-cineole, germacrene B, (*E*)-β-ocimene, α-cadinol and *cis*-*p*-menth-2-en-1-ol (Ložienė and Venskutonis 2006). The oil of the hybrid showed an intermediate composition compared with the parent species and allows for predicting the interspecific origin of a plant. Considering the high variability of the composition of volatile oils studies of thyme are of great interest from a chemotaxonomic point of view.

Plants transferred to a field collection and then annually cloned revealed a stability in essential oil composition despite environmental changes. Corresponding morphological variation of thyme corresponded to the chemical composition of the essential oil. No relationship was found between varieties and chemotypes within investigated species (Ložienė and Venskutonis 2005).

The findings proved that morphological and chemical markers must be used for identification of intra-specific taxonomy of *Thymus* species.

CONCLUDING REMARKS

Environmental changes and wild-harvested plant material have impacts on natural populations, causing population sizes of common and widely distributed species to decrease. Information about recourses and distribution of MAP species and possible threats to their habitats are of great importance for their conservation. The establishment of projects for *in situ* conservation of threatened and vulnerable MAPs in protected areas could become an important strategy for further conservation activity. An integrated approach to conserve MAPs includes both conservation methods – *in situ* and *ex situ*, which encompassed an inventory and monitoring of wild populations, sampling of accessions from the wild, their identification, characterization/evaluation, establishing and cultivation in the field collection, and selection with a view to providing better material to pharmaceutics.

As for morphological characters and chemical composition of essential oils there is variation in/between species, indicating the existence of intra-specific (at the species level) diversity and chemical polymorphism. Wide intra-specific variations determine some uncertain definitions at the species and botanical variety levels, as a consequence of ecological and genetic effects. Morphological, chemical and ecological markers support the selection and taxonomical identification of intra-specific diversity and are essential for germplasm conservation and utilization. Morphological markers provide the primary identification of valuable accessions and sustainable use of plant recourses. An evaluation of bioactive constituents is of major importance when accessions of MAP species are considered for selection and cultivation. On the other hand, chemical studies provide an additional understanding of ecological-geographical role for the distribution of compounds found within the corresponding genus from the Baltic region. Chemical variability is determined by several factors as individual genetic, morphological and ecological factors. The question that remains to be answered based on this treatment is whether morphological and chemical traits are correlated with genetic variation and if these markers can consequently be used in intra-specific taxonomy of corresponding species. No serious research at the molecular level on MAPs has been conducted in Lithuania so far. Application of molecular genetic technologies could provide further possibilities for measuring the genetic divergence of wild species and identification of genotypes having value in breeding. Taking everything mentioned above into account, it is obvious that the conservation of MAPs in Lithuania is a multiple integrated process. Multidisciplinary scientific research on ecology, taxonomy, morphology, agronomy, chemistry and genetics taking into consideration demographic, trade market and legal aspects should be necessary to establish complimentary strategy of MAPs conservation.

ACKNOWLEDGEMENTS

The study was conducted within the framework of Plant Genetic Resources Conservation Programme supported by the Ministry of Education and Science of Lithuania and Lithuanian State Science and Studies Foundation.

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