

# Oregano

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*S. Padulosi,  
editor*



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## Preface

Oregano has always played an important role in our daily lives. According to estimates, more than 300 000 tons of oregano are consumed every year in the United States alone. Its flavour is almost irreplaceable in several food preparations (what would pizza be without the typical smell of oregano!). Oregano is used in traditional medicine to treat health disorders and has many other uses (natural insecticide, in land reclamation, etc.).

Oregano is still an underutilized species, in the sense that its genetic resources are not properly exploited, as the market concentrates only on a narrow part of its diversity. The reasons for this include the fact that little work has been done so far on its domestication or on crop improvement. Oregano species are neglected by conservationists: the amount of genetic diversity that is being collected and maintained in genebanks or in Botanic Garden collections around the world is very limited. This situation is in striking contrast with the degree of popularity of the crop and at the same time represents a great risk for the preservation of its genetic diversity.

Oregano is under serious threat of genetic erosion. This is most dramatic for those species of limited distribution like *Origanum dictamnus* which is over-harvested from the wild in Crete, Greece and risks disappearing altogether from this island. The exploitation from natural habitats of oregano is, however, more evident in countries like Morocco, Turkey or Albania, traditionally the largest oregano exporters in the world. In these countries, oregano is collected massively to meet the high market demand and very little is done to regulate these harvests. There is an urgent need to raise awareness on this unsustainable harvesting and studies are needed to investigate what should be done on the one hand to allow local people to continue their exploitation of these resources, and on the other to ensure the self-regeneration of these plants in their natural habitats.

A way to contribute to the fulfilment of these objectives is to enhance the collaboration among players involved at various levels with the conservation and use of oregano. IPGRI has taken up this challenge and in 1994 promoted the establishment of a collaborative network on oregano, the "Oregano Genetic Resources Network" whose objectives are (1) the rescuing and assessment of oregano genetic diversity, (2) the promotion of collaborative efforts in the Mediterranean region, (3) the rescuing of local knowledge along with germplasm, (4) the creation of a database for selected *Origanum* species, and (5) the promotion of a greater awareness at the public and decision-making level of the need to safeguard oregano genetic diversity. The Oregano Network initiative represents an effort of the Italian-supported project on Underutilized Mediterranean Species (UMS), whose overall goal is the better conservation and use of those species with recognised market potentials, indigenous to the Mediterranean region, which have yet to receive proper attention from genebanks and researchers alike.

Crop networks bring together germplasm collectors, curators, researchers, breeders and users into groups focused on individual crop genebanks. Experience has shown that the network concept is successful in promoting collaboration, ensuring wider use and better conservation of underexploited collections, including oregano, and providing good support to crop-improvement programmes. A key factor in networking is that the working-together approach yields greater benefits than any strategy. Yet the success of this formula lies in the fact that networks promote direct contacts between scientists from different countries who agree on doing something

together. It is our hope that this meeting will be instrumental in setting in motion an effective collaborative effort on oregano at an international level.

This Workshop – Oregano: safeguarding the diversity and promoting better uses of an important Underutilized Mediterranean crop – represents the first attempt at an international level to review the state of the art on the conservation, taxonomy, origin, ecogeographical distribution, uses, genetic resources, biology, agronomy, crop improvement and potentials of *Origanum* species. With this meeting, the organizers [IPGRI; the Centre International de Hautes Etudes Agronomique Méditerranéennes (CIHEAM) of Valenzano (IAM) and Chania, Greece (Mediterranean Agronomic Institute of Chania, MAICH); the University of Bari; the Germplasm Institute (National Research Council) of Bari] aimed particularly at the exchange of information on the conservation and utilization of the genetic resources of oregano species in the Mediterranean region and elsewhere and at the identification of gaps and constraints in these areas.

Relevant points raised at the workshop were then discussed further at the meeting of the Oregano Network which took place the following day. The report of this meeting is provided in Appendix VI.

This Workshop was jointly organized by the BMZ/GTZ-supported German Project on Neglected Species and the UMS Project.

Joachim Heller  
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International Plant Genetic Resources Institute

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We are also much indebted to the Department of Agriculture, Forests and Productive Activities of the Regione Basilicata, Potenza, Italy, the Comunità Montana of Valsamento, Noepoli (Potenza) and the Ente Parco Nazionale del Pollino, Rotonda (Potenza), for their kind generosity in hosting the visits to oregano experimental fields and natural habitats in southern Italy.

## **I. Taxonomy, Evolution, Distribution and Origin**

## Taxonomy, diversity and distribution of *Origanum* species

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### Abstract

The genus *Origanum* (tribe Mentheae, Labiatae family) is characterized by a large morphological and chemical diversity. Forty-nine taxa divided into 10 sections belong to this genus, most of them having a very local distribution around the Mediterranean. In particular, three taxa are restricted to Morocco and south of Spain, two occur in Algeria and Tunisia, three are endemic to Cyrenaica, nine are restricted to Greece, South Balkans and Asia Minor (six are local Greek endemics), 21 are found in Turkey, Cyprus, Syria and Lebanon (21 are local Turkish endemics), and eight are locally distributed in Israel, Jordan and Sinai Peninsula. The essential oils of the members of the *Origanum* genus vary in respect of the total amount produced by plants (ranging from traces to 8 ml/100 g of dry weight) as well as in their qualitative composition. *Origanum* essential oils are characterized by a number of main components which are implicated in the various plant odours. A wide chemical diversity is found even within a single *Origanum* species, like the widely used *O. vulgare*. The pattern of variation of quantitative and qualitative essential oils in the latter species follows its geographical distribution or depends on the time of plant collecting.

### Introduction

The *Origanum* species are subshrubs or perennial herbs with several stems, ascending or erect, subsessile or petiolate leaves and flowers in verticillasters aggregated in dense or loose spikes which are arranged in a paniculate or corymbiform inflorescence. *Origanum* plants are widely used all over the world as a very popular spice, under the vernacular name 'oregano'. They are of great economic importance which is not only related to their use as a spice. In fact, as recent studies have pointed out, oregano is used traditionally in many other ways as their essential oils have antimicrobial, cytotoxic and antioxidant activity (Lagouri *et al.* 1993; Sivropoulou *et al.* 1996). Knowledge of the large morphological and chemical diversity of the genus *Origanum* and the native distribution of its different taxa is essential for the better exploitation of this very promising crop.

### Variation within the genus

#### Morphology

The morphological variation within the genus results in the distinction of 10 sections consisting of 42 species or 49 taxa (species, subspecies and varieties) (cf. Ietswaart 1980; Carlström 1984; Danin 1990; Danin and Küne 1996). Following Ietswaart's classification (1980), with indications of the country of distribution within the Mediterranean regions, the following taxa occur.

**I. Section *Amaracus* (Gleditsch) Bentham**

It consists of seven species, all restricted in the east Mediterranean region. These species are mainly characterized by their usually purple bracts, 1 or 2-lipped calyces without teeth, and saccate corollas.

- |  |                |
|--|----------------|
| 1. <i>O. boissieri</i> Ietswaart                               | Turkey         |
| 2. <i>O. calcaratum</i> Jussieu                                | Greece         |
| 3. <i>O. cordifolium</i> (Montbret et Aucher ex Bentham) Vogel | Cyprus         |
| 4. <i>O. dictamnus</i> L. [Figs. 1a and 1b]                    | Crete (Greece) |
| 5. <i>O. saccatum</i> Davis                                    | Turkey         |
| 6. <i>O. solymicum</i> Davis                                   | Turkey         |
| 7. <i>O. symes</i> Carlström                                   | Greece         |

**II. Section *Anatolicon* Bentham**

It comprises eight species, presenting a very restricted distribution in Greece, Asia Minor, Lebanon and Libya. The plants have strongly bilabiate 5-toothed calyces.

- |   |                   |
|---|-------------------|
| 1. <i>O. akhdarensis</i> Ietswaart et Boulos          | Libya (Cyrenaica) |
| 2. <i>O. cyrenaicum</i> Beguinot et Vaccari           | Libya (Cyrenaica) |
| 3. <i>O. hypericifolium</i> Schwarz et Davis          | Turkey            |
| 4. <i>O. libanoticum</i> Boissier                     | Lebanon           |
| 5. <i>O. scabrum</i> Boissier et Heldreich            | Greece            |
| 6. <i>O. sipyleum</i> L.                              | Greece, Turkey    |
| 7. <i>O. vetteri</i> Briquet et Barbey                | Greece            |
| 8. <i>O. pampaninii</i> (Brullo et Furnari) Ietswaart | Libya (Cyrenaica) |

**III. Section *Brevifilamentum* Ietswaart**

This section includes six species which are steno-endemics mainly in the eastern part of Turkey. These species are characterized by bilabiate calyces and stamens strongly unequal in length, whose upper two are very short and included in the corolla.

- |  |               |
|--|---------------|
| 1. <i>O. acutidens</i> (Handel-Mazzetti) Ietswaart | Turkey        |
| 2. <i>O. bargyli</i> Mouterde                      | Syria, Turkey |
| 3. <i>O. brevidens</i> (Bornmüller) Dinsmore       | Turkey        |
| 4. <i>O. haussknechtii</i> Boissier                | Turkey        |
| 5. <i>O. leptocladum</i> Boissier                  | Turkey        |
| 6. <i>O. rotundifolium</i> Boissier                | Turkey        |

**IV. Section *Longitubus* Ietswaart**

There is only one species found in a few places in the Amanus Mountains. It is mainly characterized by the slightly bilabiate calyx, the lips of the corolla which are nearly at right angles to the tube and the very short staminal filaments.

- |                          |        |
|--------------------------|--------|
| 1. <i>O. amanum</i> Post | Turkey |
|--------------------------|--------|

**V. Section *Chilocalyx* (Briquet) Ietswaart**

It comprises four species which are steno-endemics of South Anatolia or of the island of Crete. The plants have slightly bilabiate, conspicuously pilose in throat calyces.

- |  |                |
|--|----------------|
| 1. <i>O. bigleri</i> Davis                 | Turkey         |
| 2. <i>O. micranthum</i> Vogel              | Turkey         |
| 3. <i>O. microphyllum</i> (Bentham) Vogel  | Crete (Greece) |
| 4. <i>O. minutiflorum</i> Schwarz et Davis | Turkey         |

### VI. Section *Majorana* (Miller) Bentham

Three species are characterized by 1-lipped calyces and green bracts. Among them *O. syriacum* is further subdivided into three geographically distinct varieties; these are recognised mainly from differences in their indumentum and leaf shape.

- |   |  |
|---|--|
| 1. <i>O. majorana</i> L.                      | Native plant of Cyprus and south Turkey. It has been introduced almost all over the Mediterranean. |
| 2. <i>O. onites</i> L. [Fig. 1c]              | Greece, Sicily (Italy), Turkey   |
| 3. <i>O. syriacum</i> L. var. <i>syriacum</i> | Israel, Jordan, Syria  |
| 4. var. <i>bevanii</i> (Holmes) Ietswaart     | Cyprus, Syria, Turkey, Lebanon   |
| 5. var. <i>sinaicum</i> (Boissier) Ietswaart  | Sinai Peninsula  |

### VII. Section *Campanulicalyx* Ietswaart

Six local endemic species belong to this section. The calyces of the plants have 5 (sub)equal teeth and are campanulate (even when bearing fruits).

- |                                       |             |
|---------------------------------------|-------------|
| 1. <i>O. dayi</i> Post                | Israel      |
| 2. <i>O. isthmicum</i> Danin          | North Sinai |
| 3. <i>O. ramonense</i> Danin          | Israel      |
| 4. <i>O. petraeum</i> Danin           | Jordan      |
| 5. <i>O. punonense</i> Danin          | Jordan      |
| 6. <i>O. jordanicum</i> Danin & Künne | Jordan      |

### VIII. Section *Elongatispica* Ietswaart

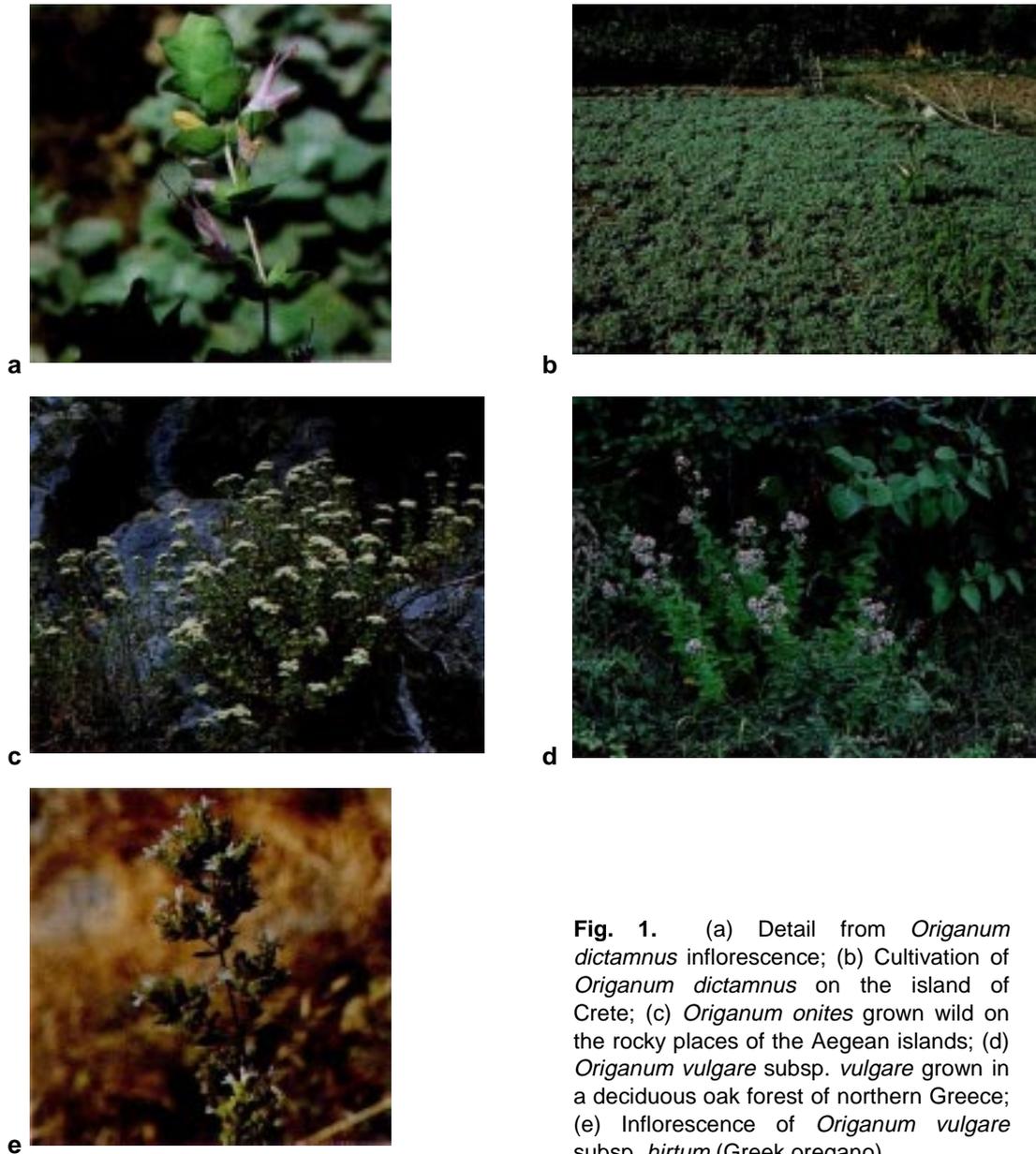
It comprises three steno-endemic species of North Africa, which are characterized by loose and tenuous spikes and tubular calyces with 5 equal teeth.

- |   |         |
|---|---------|
| 1. <i>O. elongatum</i> (Bonnet) Emberger et Maire | Morocco |
| 2. <i>O. floribundum</i> Munby                    | Algeria |
| 3. <i>O. grosii</i> Pau et Font Quer ex Ietswaart | Morocco |

### IX. Section *Origanum*

It is a monospecific section consisting of the species *O. vulgare*, widely distributed in Eurasia and North Africa. Introduced by humans, this species has also been encountered in North America (Ietswaart 1980). The plants of *O. vulgare* have dense spikes, and tubular 5-toothed calyces, never becoming turbinate in fruit. Six subspecies have been recognised within *O. vulgare* based on differences in indumentum, number of sessile glands on leaves, bracts and calyces, and in size and colour of bracts and flowers. The southernmost range of *O. vulgare* is occupied by the three subspecies 'rich' in essential oils, whereas those 'poor' in essential oils are found toward the northern part of the species' range of distribution (Fig. 2).

- |  |  |
|--|--|
| 1. <i>O. vulgare</i> L. subsp. <i>vulgare</i> [Fig. 1d]                      | Europe, Iran, India, China   |
| 2. <i>O. vulgare</i> L. subsp. <i>glandulosum</i> (Desfontaines) Ietswaart   | Algeria, Tunisia   |
| 3. <i>O. vulgare</i> L. subsp. <i>gracile</i> (Koch) Ietswaart               | Afganistan, Iran, Turkey, former USSR                                    |
| 4. <i>O. vulgare</i> L. subsp. <i>hirtum</i> (Link) Ietswaart [Fig. 1e]      | Albania, Croatia, Greece, Turkey   |
| 5. <i>O. vulgare</i> L. subsp. <i>viridulum</i> (Martrin-Donos) Nyman        | Afganistan, China, Croatia, France, Greece, India, Iran, Italy, Pakistan |
| 6. <i>O. vulgare</i> L. subsp. <i>virens</i> (Hoffmannsegg & Link) Ietswaart | Azores, Balearic Is., Canary Is., Madeira, Morocco, Portugal, Spain      |



**Fig. 1.** (a) Detail from *Origanum dictamnus* inflorescence; (b) Cultivation of *Origanum dictamnus* on the island of Crete; (c) *Origanum onites* grown wild on the rocky places of the Aegean islands; (d) *Origanum vulgare* subsp. *vulgare* grown in a deciduous oak forest of northern Greece; (e) Inflorescence of *Origanum vulgare* subsp. *hirtum* (Greek oregano).

#### X. Section *Prolaticorolla* Ietswaart

It comprises three species endemic to eastern or western parts of the Mediterranean. These species are characterized by dense spikes and tubular calyces becoming turbinate in fruiting.

- |                                   |                |
|-----------------------------------|----------------|
| 1. <i>O. compactum</i> Benth      | Morocco, Spain |
| 2. <i>O. ehrenbergii</i> Boissier | Lebanon        |
| 3. <i>O. laevigatum</i> Boissier  | Turkey         |

In summary, it appears that 46 *Origanum* taxa out of 49 present a very local distribution within the Mediterranean. Three taxa are restricted to Morocco and South Spain, two occur in Algeria and Tunisia, three are endemic to Libya, nine are restricted in Greece, South Balkans and Asia Minor (six are local Greek endemics), 21 are found in Turkey, Cyprus, Syria and Lebanon (21 are local Turkish endemics), and finally eight are locally distributed in Israel, Jordan and Sinai Peninsula (Fig. 3).

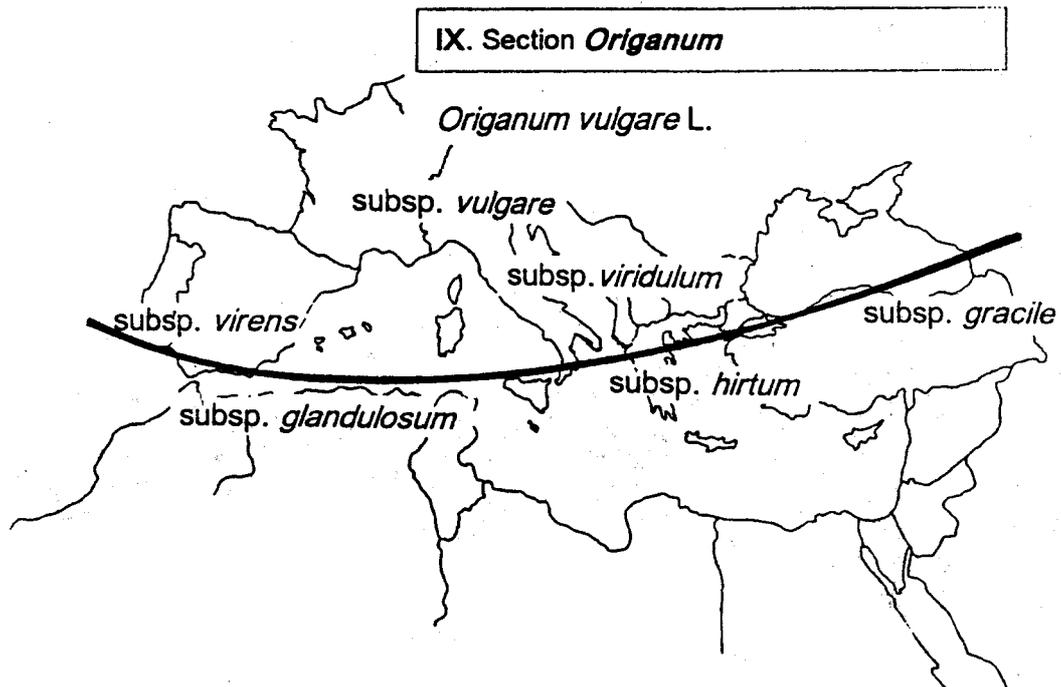


Fig. 2. Simplified presentation of the distribution of the six *Origanum vulgare* subspecies. Above the line, the taxa are poor in essential oil, whereas the essential oil rich subspecies of *O. vulgare* occur below the line.

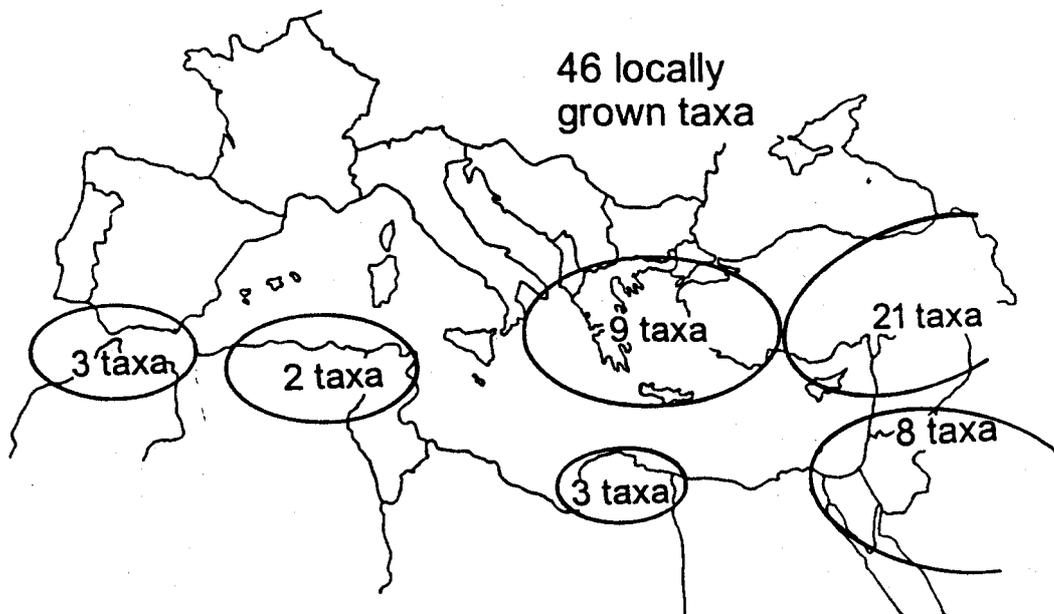


Fig. 3. Number of locally grown *Origanum* taxa in the different Mediterranean countries.

Besides the above-mentioned *Origanum* taxa, 17 hybrids between different species have been described. Some of them are putative and their occurrence in the natural populations needs further investigation, whereas four are known only from artificial crosses (Ietswaart 1980). The most widely distributed hybrid is *O. x intercedens* Rechinger (*O. onites* x *O. vulgare* subsp. *hirtum*) which forms extensive populations in the Aegean islands (Kokkini *et al.* 1991; Kokkini and Vokou 1993).

### Essential oils

The essential oils of *Origanum* members vary in respect of the total amount produced per plant as well as in their qualitative composition. Based on their essential oil content, the different taxa of the genus can be distinguished as three main groups:

1. Essential oil 'poor' taxa with an essential oil content of less than 0.5% (ml/100 g dry weight), e.g. the Greek endemic *O. calcaratum* (Karousou 1995);
2. Taxa with an essential oil content between 0.5 and 2%, e.g. the Cretan endemic taxon *O. microphyllum* known as 'Cretan marjoram' (Karousou 1995);
3. Essential oil 'rich' taxa with an essential oil content of more than 2%, as for example the two most well commercially known 'oregano' plants, *O. vulgare* subsp. *hirtum* (Greek oregano) and *O. onites* (Turkish oregano) (Kokkini *et al.* 1991; Vokou *et al.* 1988, 1993).

With reference to their essential oil composition, *Origanum* taxa may be characterized by the dominant occurrence of the following compounds:

- Linalool, terpinen-4-ol, and sabinene hydrate like the essential oil of *O. majorana* (syn. *Majorana hortensis* Moench.) (Fischer *et al.* 1987);
- The phenolic compounds, carvacrol and/or thymol, like the essential oils of *O. vulgare* subsp. *hirtum* (Kokkini and Vokou 1989; Kokkini *et al.* 1991; Vokou *et al.* 1993) and *O. onites* (Vokou *et al.* 1988; Ruberto *et al.* 1993);
- Sesquiterpenes, like the essential oil of *O. vulgare* subsp. *vulgare* (Lawrence 1984).

### Infraspecific variation

A number of studies have shown that variation within a single *Origanum* species may occur in its morphological and chemical features. Furthermore, it has been found that the pattern of variation of a single species follows its geographical distribution or it depends on the season of plant collecting.

### Geographical variation

A characteristic example of a noticeable infraspecific morphological variation is the geographical differentiation of *O. vulgare* in Greece. The range of the three subspecies found in this country is associated with the climatic conditions prevailing in each area. As can be seen in Figure 4, *O. vulgare* subsp. *hirtum* (syn. *O. hirtum* Link, *O. heracleoticum* auct. non L.), is mainly found on the islands and southern mainland, whereas toward the north it is mostly confined to the lowland coastal areas. Its distribution range in Greece is limited by the presence of the continental type of climate in the northern and central part of the mainland (Kokkini *et al.* 1991). From the morphological point of view, subsp. *hirtum* can be distinguished by its small green bracts and white flowers (Fig. 1e). Toward the northern parts of Greece, where a continental Mediterranean climate occurs, subsp.

*hirtum* is replaced either by subsp. *viridulum* [syns. *O. heracleoticum* L., *O. viride* (Boiss.) Halácsy] characterized by large green bracts or by subsp. *vulgare*. The latter is easily distinguished by the large purple bracts and pinkish to purple flowers (Fig. 1d). The number and the size of the sessile glands in leaves, bracts and calyces are remarkably reduced in samples from the southern to the northern part of the country. These glands which appear as small bladders are the peltate glandular hairs described by Bosabalidis and Tsekos (1984) and Werker *et al.* (1985). Since they contain the bulk of the secreted essential oil, the reduced number of sessile glands is connected with a low essential oil content (Bosabalidis and Kokkini 1996).

During our studies on the essential oil content of Greek *O. vulgare* plants, we have found a large variation within the species. In fact, the subsp. *hirtum* plants, though very variable in leaf and bract sessile gland number, but always characterized by densely glandular calyces, are in any case rich in essential oil (1.8-8.2 ml/100 g dry weight). On the other hand, plants belonging to the other two subspecies, having fewer and smaller (inconspicuous) sessile glands, contain a much lower amount of essential oil (traces up to 0.8%) (Kokkini and Vokou 1989; Kokkini *et al.* 1991, 1994).

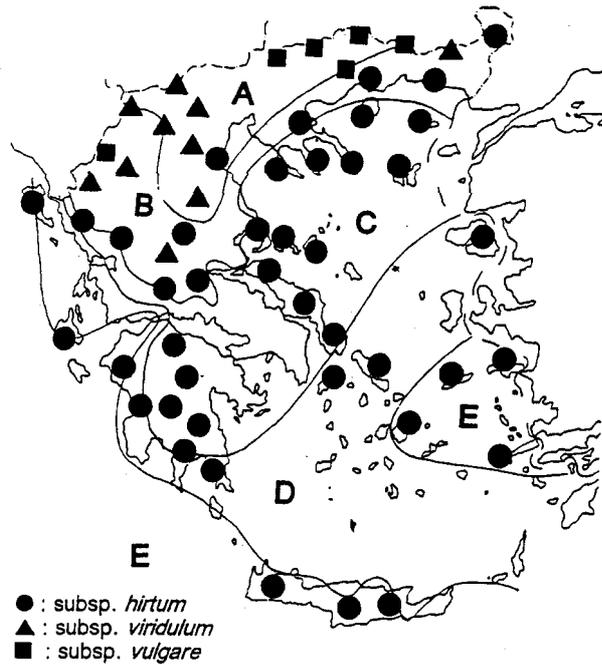
*Origanum vulgare* subsp. *hirtum* is widely used as a spice under the name 'Greek oregano'. Among the different Labiatae and Verbenaceae taxa, used all over the world and all known as 'oregano', it is generally accepted that the Greek oregano has the best quality (Calpouzios 1954; Fleisher and Sneer 1982; Fleisher and Fleisher 1988; Lawrence 1984). A study of its essential oils in the different Greek populations has demonstrated that these are very variable in quality and quantity. The essential oil content, as well as the ratio of carvacrol to thymol to the total oil amount in the different Greek populations, are shown in Figure 5. The extremely high values of essential oil yield (>7 ml/100 g dry weight) have been recorded on the islands of Crete (sample no. 2) and Amorgos (sample no. 4), as well as in Gythion (no. 5) and Athos Peninsula (no. 16). The highest yields correspond to plants growing at low altitudes, in Mediterranean ecosystems, as is common for the whole family of Labiatae (Kokkini *et al.* 1989). It should be noted that these values are the highest essential oil yields reported for any oregano plant.

Quantitative and qualitative essential oil analyses have shown that the major constituents are carvacrol and/or thymol, accompanied by p-cymene and  $\gamma$ -terpinene (Vokou *et al.* 1993). As can be seen in Figure 5, in some cases the essential oil consists of a high quantity of carvacrol, as in the South Peloponnese – more than 90% of the total oil – or in other cases the predominant phenol is thymol (island of Corfu sample no. 23). In these cases carvacrol, the compound responsible for characterizing a plant as of the oregano type, is a minor constituent. The dominance of thymol suggests that these should belong to the group of plants used as thyme.

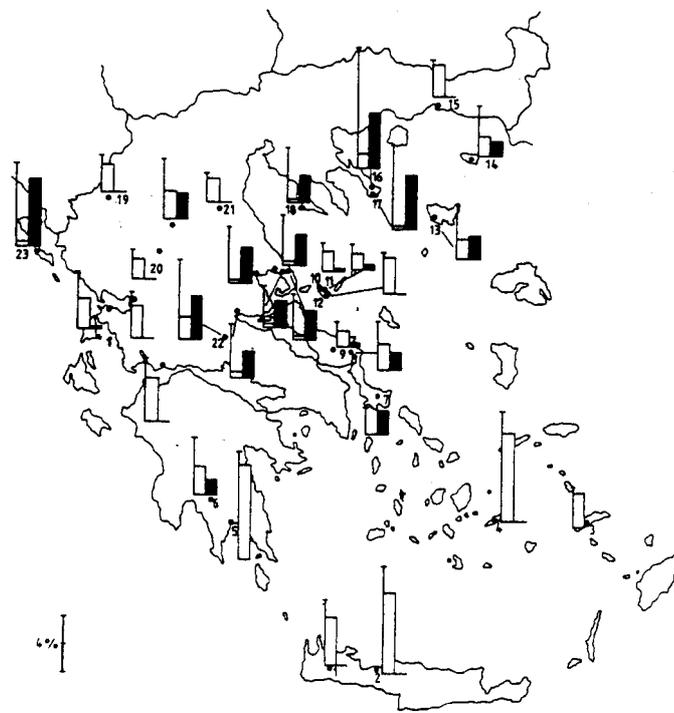
### Seasonal variation

The leaf characters of several taxa show strong variation during the different developmental stages of the plants. For example, *Origanum* plants have much smaller and hairier leaves during summer than in other seasons.

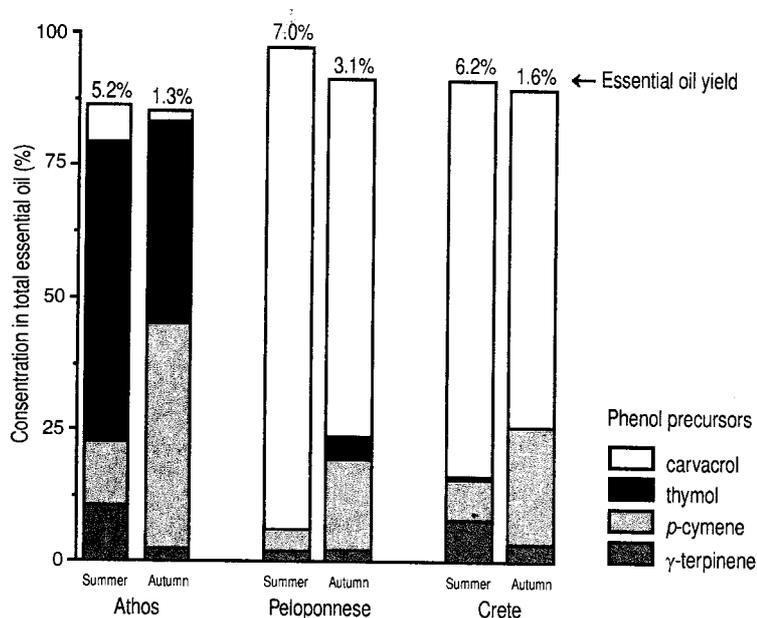
As mentioned earlier, the season of collecting may also strongly affect the essential oil yield of the plants and the concentration of its main components. The differences found in the total amount of *O. vulgare* subsp. *hirtum* essential oil and in each one of the four main oil components between the summer and autumn plants are shown in Figure 6. The essential oil content is much lower in autumn plants, ranging from 1.0 to 3.1% (ml/100 g dry weight), compared with those collected



**Fig. 4.** Distribution of the three *Origanum vulgare* subspecies in the five climatic zones of Greece: **A.** Continental - Mediterranean climatic zone; **B.** Transitional zone deviating to Continental-Mediterranean climate; **C.** Main Transitional climatic zone; **D.** Real Mediterranean climatic zone; **E.** Real Mediterranean climatic zone with higher atmospheric stability than zone D (climatic zones after Kotini-Zambaka 1983).



**Fig. 5.** *Origanum vulgare* subsp. *hirtum*. Essential oil yield, carvacrol (white bars) and thymol (black bars) contents (as percentages of the total oil) in different Greek localities (after Vokou *et al.* 1993).



**Fig. 6.** Concentration (%) of the four main components in total essential oil content of *Origanum vulgare* subsp. *hirtum* (Greek oregano) plants collected from three geographical areas of Greece in summer (S) and autumn (A) (after Kokkini *et al.* 1996).

from the same areas in summer (4.8-8.2%). The most impressive difference is the increased amount of p-cymene in autumn: its amount ranges from 17.3-26.9% of the total oil in plants from South Peloponnese and Crete (instead of 4.0-9.5% found in the summer plants) to 37.1-51.3% of the oil in plants from Athos peninsula (instead of 12.0-12.2% in the summer) (Kokkini *et al.* 1996).

In spite of the striking quantitative differences of the major oil components, their sum ( $\gamma$ -terpinene + p-cymene + thymol + carvacrol) is almost equal in the essential oils of different geographic origin as well as in the different seasons, ranging from 85.0 to 96.8%. These results suggest that the essential oils of *O. vulgare* subsp. *hirtum* are characterized by stability – irrespective of the season of plant collecting – with regard to (1) the high concentration of the sum of the four components involved in the phenolic biosynthetic pathway, and (2) the predominant phenol type. It should be noted that the two monoterpene hydrocarbons are very common constituents of all 'oregano' or 'thyme' type essential oils (Kokkini 1994; Lawrence 1984; Ravid and Putievsky 1986). However, high concentrations of p-cymene similar to those found in *O. vulgare* subsp. *hirtum* plants collected in autumn from Athos peninsula have not been found in any 'oregano' or 'thyme' oil. Thus, it should be taken into account that the Greek oregano may be devoid of its characteristic odour when the plants are collected in this period.

In conclusion, the high variability of essential oils suggests that *Origanum* plants may be exploited for a wide range of commercial applications, bearing in mind, however, the importance of always checking the quantity and quality of their active ingredients.

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## II. Conservation

## Conservation of oregano species in national and international collections: an assessment

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### Abstract

The genus *Origanum* L. comprises 49 taxa belonging to 10 different sections. Most *Origanum* species (ca. 75%) are found exclusively in the east Mediterranean subregion and only a few species occur in the western part of the Mediterranean. *Origanum vulgare* (section *Origanum*) is the most widespread among all the species within the genus, ranging from the Azores to Taiwan. Several *Origanum* species are nowadays cultivated as culinary herbs, as garden plants and, rarely, as medicinal plants. Despite the great commercial importance of this genus, there is a serious lack of information throughout the world on its cultivation, collecting and germplasm handling. Consequently, the degree of genetic erosion is not well known. However, many species of *Origanum* are on the list of rare, threatened and endemic plants of Europe. Many institutions throughout the world collect genetic resources of *Origanum*, especially for research purposes. A list of species preserved in genebanks is provided.

### Introduction

The genus *Origanum* L. (family Labiatae) includes dicotyledonous dwarf shrubs or annual, biennial or perennial herbs that occur mostly in warm and mountainous areas (from the Greek words: *oros* – mountain and hill, and *ganos* – ornament).

With the exception of pharmacological and phytotherapeutic aspects, there is little information on this genus, which includes aromatic, flavouring, oil and dye plants of big commercial value. This gap concerns all sectors of herbs, spices and medicinal plants which, although including plants used for thousands of years, have only recently attracted public and scientific interest. A recent survey of the Working Group for Herbs, Spices and Medicinal Plants of the American Society for Horticultural Science indicates only a limited number of locations with active plant screening and/or breeding programmes on these plants. Among these, the programme of the Delaware State College in Delaware, USA, is concerned with *Origanum* species (Craker 1989). Moreover, the research on germplasm conservation of these plants is also very limited: the absence of genetic resources inventory is partially due to concentration of most genetic diversity in collections of individual growers, who keep their collections as a hobby or for small business purposes.

### Geographic distribution in Italy and in the world

The large variability encountered in *Origanum* makes the classification of its different species and varieties a difficult task. Ietswaart (1980), in his revision of the genus *Origanum*, described 49 taxa belonging to 10 different sections (see Kokkini, elsewhere in this book). A section is understood to be a group of related species

which have more morphological characters in common with each other than with other species.

However, most intraspecific variation has not yet been named. A subspecies has been recognised only when all specimens from several local populations of a species were found to be different from the specimens in the 'type population'. With regard to *Origanum*, this has been the case in only one species (namely *O. vulgare*), of which many specimens were available for study. Also, varieties have been named in one case only (*O. majorana*).

Many species are found growing only in the wild, but many others, used as medicinal, culinary herbs and garden plants, are also found as cultivated plants.

Subsequent to Ietswaart's revision (1980), two new species have been described: *O. munzurense* Tan & Sorger (Tan and Sorger 1984) and *O. symes* Carlström (Carlström 1984), which are found in Turkey and the east Aegean Isles respectively (Pinner *et al.* 1987).

The distribution area of this genus is given in Figure 1. About 75% of the species are found exclusively in the east Mediterranean and only a few in the west Mediterranean subregion. Furthermore, most species occupy rather small areas: about 70% are endemic to one island or mountain group. Only *O. vulgare* has a very large distribution area, stretching not only across the Mediterranean, but in many areas falling within the Euro-Siberian and Irano-Turanian region.

The distribution in the world and in Italy of the most common *Origanum* species is reported in Table 1. These figures have been compiled from data taken from both Flora Europaea (Tutin *et al.* 1972) and Flora d' Italia (Pignatti 1982).

Five species are reported to occur in Italy; *O. majorana* and *O. vulgare* are the most widespread, *O. heracleoticum* is typically present in the south of the country, *O. onites* and *O. dictamnus* being very rare.

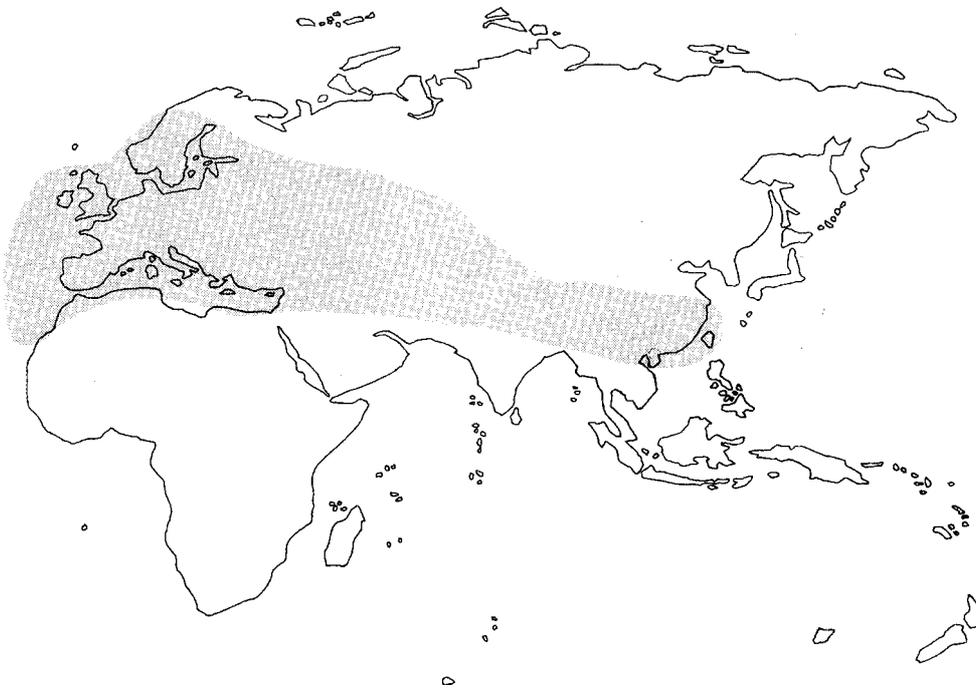


Fig. 1. Distribution area of the genus *Origanum*.

*Origanum heracleoticum* and *O. onites* are wild species. The first is very common in Sicily and Sardinia, the second occurs only in some areas in eastern Sicily. *Origanum majorana* and *O. vulgare* are both cultivated (the first, all over the peninsula and the second, mainly in north and central Italy); *O. dictamnus* is cultivated as a culinary herb occasionally in northern and central Italy.

**Table 1. Distribution of main *Origanum* species around the world and in Italy.**

| Species                                   | Distribution  |  |
|---|---|--|
|   | In the world (Tutin <i>et al.</i> 1972)                   | In Italy (Pignatti 1982)   |
| <i>O. compactum</i> Benth.                | southwest Spain, northern Africa                          | –  |
| <i>O. dictamnus</i> L.                    | Crete, England,   | north, centre (rare, cultivated)   |
| <i>O. heracleoticum</i> L.                | southeast Europe  | south, Sicily, Sardinia (common, wild)   |
| <i>O. lirium</i> Heldr.                   | mountains of southern Greece                              | –  |
| <i>O. majoricum</i> Camb.                 | southwest Europe, Balears,<br>Cyclades, Crete             | –  |
| <i>O. majorana</i> L.                     | southern Europe, northern<br>Africa, southwest Asia       | all over (common, cultivated/ wild)  |
| <i>O. microphyllum</i><br>(Benth.) Boiss. | Crete   | –  |
| <i>O. onites</i> L.                       | Mediterranean   | Sicily (eastern part, rare, wild)  |
| <i>O. scabrum</i> Boiss. &<br>Heldr.      | Mountains of southern Greece                              | –  |
| <i>O. tourneforti</i> Aiton               | Cyclades, Crete   | –  |
| <i>O. vetteri</i> Briq. & W.<br>Barbey    | Karpathos, Crete  | –  |
| <i>O. virens</i> Hoffmanns &<br>Link      | southwest Europe, Argentina,<br>Azores, Canaries, Morocco | –  |
| <i>O. vulgare</i> L.                      | most of Europe, central and<br>west Asia                  | north, centre, Corsica (common, wild);<br>south, Sicily, Sardinia (rare, wild) |

### ***Ex situ* conservation**

#### **Genetic resources**

About 20 European public institutions hold genetic resources of different species of *Origanum* L. (Marzi *et al.* 1992; Frison and Serwinsky 1995) (Table 2). Most hold very few accessions (from 1 to 21), only two (the Olomouc Gene Bank, Czech Republic and the Conservatoire des Plantes Médicinales, Aromatiques et Industrielles, Milly La Forêt, France) store a relatively high number of samples (37 and 95 respectively). The Aegean Agricultural Research Institute of Plant Genetic Resources Department in Izmir, Turkey and the Institute of Agronomy, University of Palermo, Palermo, Italy hold the majority of accessions (119 and 214 respectively). *Origanum vulgare* is the most represented species in these collections (141 accessions). Other relatively well-represented species are *O. majorana* (21), *O. dictamnus* (10) and *O. onites* (7); *O. rotundifolium*, *O. laevigatum*, *O. microphyllum*, *O. scabrum*, *O. album buckland* and the subspecies *vulgare* and *gracile* of *O. vulgare*, all represented by one accession only. In addition there are also 125 unclassified accessions.

**Table 2. European public institutions holding genetic resources of *Origanum* species.**

| Country   | Institution   | Species   | No. accessions <sup>†</sup> |
|---|---|---|-----------------------------|
| Albania   | Tirana, Plant Breeding and Seed Production Section, Department of Agronomy, Agricultural University of Tirana | <i>Origanum vulgare</i>                             | 5a                          |
| Czech Republic  | Olomuc-Holice, Vegetable Section Olomuch, Gene Bank Department, Research Institute of Crop Production         | <i>Origanum</i> spp.                                | 37                          |
| France  | Milly-La-Foret, Conservatoire des Plantes Medicinales, Aromatiques et Industrielles                           | <i>Origanum vulgare</i>                             | 95                          |
| Germany   | Braunschweig  | <i>Origanum vulgare</i>                             | 8a                          |
|   | Institute of Crop Sciences  | <i>Origanum majorana</i>                            | 3a                          |
|   | Federal Research Center for Agriculture   | <i>Origanum rotundifolium</i>                       | 1a                          |
|   | Gatersleben, Institute for Plant Genetics and Crop Plant Research   | <i>Origanum vulgare</i><br><i>Origanum majorana</i> | 15<br>6                     |
|   | Halle/Salle, Institute for Agricultural Research  | <i>Origanum</i> spp.                                | 1a                          |
| Greece  | Thermi-Thessaloniki   | <i>Origanum dictamnus</i>                           | 5a                          |
|   | Greek Genebank  | <i>Origanum onites</i>                              | 3a                          |
|   | Agricultural Research Center of Macedonia-Thrace, National Agricultural Research Foundation                   | <i>Origanum</i> spp.                                | 1a                          |
|   | Thermi-Thessaloniki   | <i>Origanum dictamnus</i>                           | 5a                          |
|   | Department of Aromatic and Medicinal Plant, Agricultural Research Center of Macedonia-Thrace                  | <i>Origanum onites</i><br><i>Origanum</i> subsp.    | 3a<br>3a                    |
| Italy   | Bari, Germplasm Institute, CNR, National Research Council   | <i>Origanum vulgare</i>                             | 4                           |
|   | Bari, Institute of Agronomy, University of Bari   | <i>Origanum vulgare</i>                             | 6                           |
|   |   | <i>O. vulgare</i> subsp. <i>vulgare</i>             | 1                           |
|   |   | <i>O. vulgare</i> subsp. <i>gracile</i>             | 1                           |
|   |   | <i>Origanum heracleoticum</i>                       | 5                           |
|   |   | <i>Origanum virens</i>                              | 2                           |
|   |   | <i>Origanum majorana</i>                            | 3                           |
|   | Bari, Experimental farm "E. Pantanelli", University of Bari   | <i>Origanum</i> spp.                                | 1                           |
|   |   | <i>Origanum vulgare</i>                             | 5                           |
|   |   | <i>Origanum majorana</i>                            | 1                           |
|   |   | <i>Origanum laevigatum</i>                          | 1                           |
| <i>Origanum album buckland</i>                        |   | 1   |                             |
| <i>Origanum microphyllum</i>                          |   | 1   |                             |
| Palermo, Institute of Agronomy, University of Palermo | <i>Origanum onites</i>  | 1   |                             |
|   | <i>Origanum scabrum</i>   | 1   |                             |
|   | Palermo, Institute of Agronomy, University of Palermo   | <i>Origanum heracleoticum</i>                       | 214c                        |
| Lithuania   | Babtai, Lithuanian Horticultural Institute  | <i>Origanum majorana</i>                            | 2                           |
|   | Vilnius   | <i>Origanum vulgare</i>                             | 1                           |
|   | Institute of Botany   | <i>Origanum vulgare</i>                             | 1a                          |
| Poland  | Poznan, Institute of Medicinal Plants   | <i>Origanum majorana</i>                            | 2                           |

| Country      | Institution  | Species                  | No. accessions <sup>†</sup> |
|--------------|--|--------------------------|-----------------------------|
| Portugal     | Mirandela, Regional Directorate of Agriculture for Tras-os-Montes                | <i>Origanum vulgare</i>  | *a                          |
|              | Vila Real, Department of Plant Protection University of Tras-os-Montes           | <i>Origanum majorana</i> | *                           |
|              |  | <i>Origanum virens</i>   | *                           |
|              |  | <i>Origanum vulgare</i>  | *                           |
| Slovakia     | Novè Zamky Research and Breeding Institute for Vegetable and Special Plants      | <i>Origanum majorana</i> | 3c                          |
| Slovenia     | Ljubljana Agronomy Department University of Ljubljana                            | <i>Origanum vulgare</i>  | 2b                          |
| Turkey       | Izmir Plant Genetic Resources Department, Aegean Agricultural Research Institute | <i>Origanum</i> spp.     | 119                         |
| Total number |  |                          | 569                         |

<sup>†</sup> \* = unknown; a = wild/weedy species; b = advanced cultivars; c = number of ecotypes.

Usually, the amount of information accompanying each sample is rather poor. Even the site of collecting may be unknown. In fact, only a few genebanks can provide some information on the origin of the sample and/or the name of the donor providing it. Among them is the IPK Genebank in Gatersleben, Germany which has supplied the authors with data on botanical classification, donor name, morphological description, site of collecting and other characters referring to the oregano material held in their collection. As with the origin of the material held in smaller collections, it is likely that this has been collected during local exploration and collecting activities. In fact, these collections are generally made up of wild species or advanced cultivars of *Origanum* mostly widespread in those countries whose institutions hold the collection (as is the case for *O. dictamnus*, in Greece).

Among the non-European *Origanum* germplasm holders we should mention the Agricultural Research Service of the United States Department of Agriculture (USDA-ARS), which currently holds 15 accessions of *O. vulgare*, two of *O. tyttanthum* (syn. *O. vulgare* subsp. *gracile*) and one of *Origanum* spp. (data taken from the USDA-ARS GRIN database) (Table 3).

Private and Botanic Garden collections also play an important role in the *ex situ* conservation of *Origanum* species (Ietswaart 1980) (Fig. 2 and Table 4). In particular the former preserve many rare and threatened species of the genus (Table 5). Although it is not always easy to obtain data from Botanic Gardens one would expect many of them to be preserving a high number of species and accessions. At present, according to our data, the number of *Origanum* accessions stored in public institutions, apart from genebanks, in the world is around 600 (Tables 2 and 3), while the number of collections preserved by private growers is nearly 500.

### Storage conditions

There is little information on the conditions in which the genetic resources of *Origanum* are being preserved. Most institutions hold seed collections and only a few maintain field collections. Seed collections of *Origanum* do not need particular conservation methods: seeds are preserved in the same controlled conditions used for any other orthodox-seeded plant, thus being maintained in short-, medium- or long-term storage rooms. As for most aromatic plants, also for *Origanum*, long-term storage (ca. -18°C) is a good conservation method, which ensures the safe seed conservation for at least a period of 8 years (Montezuma-De-Carvalho *et al.* 1984).

**Table 3. Conservation of *Origanum* spp. In United States Department of Agriculture (List of accessions found; Complete accession information).**

| Accession number | Name                       | Additional reference number |
|------------------|----------------------------|-----------------------------|
| Ames 13184       | <i>Origanum vulgare</i>    | Index Seminum 341           |
| Ames 1682        | <i>Origanum vulgare</i>    | Ames 1682                   |
| Ames 1683        | <i>Origanum vulgare</i>    | Ames 1683                   |
| Ames 1684        | <i>Origanum vulgare</i>    | Ames 1684                   |
| Ames 1685        | <i>Origanum vulgare</i>    | Ames 1685                   |
| Ames 1686        | <i>Origanum vulgare</i>    | Ames 1686                   |
| Ames 17764       | <i>Origanum vulgare</i>    | Ames 17764                  |
| Ames 20036       | <i>Origanum vulgare</i>    | 3104                        |
| Ames 21076       | <i>Origanum vulgare</i>    | No. 66                      |
| Ames 21199       | <i>Origanum vulgare</i>    | No. 376                     |
| Ames 22109       | <i>Origanum vulgare</i>    | Index Seminum 335           |
| Ames 7471        | <i>Origanum</i> sp.        | H 6802                      |
| NSL 6410         | <i>Origanum majorana</i>   | SWEET MARJORAM              |
| PI 325450        | <i>Origanum vulgare</i>    | Chebret                     |
| PI 325451        | <i>Origanum vulgare</i>    | BN-18692-70                 |
| PI 383835        | <i>Origanum vulgare</i>    |                             |
| PI 384485        | <i>Origanum vulgare</i>    |                             |
| PI 440579        | <i>Origanum tyttanthum</i> |                             |
| PI 440580        | <i>Origanum tyttanthum</i> |                             |

/USDA/ARS/GRIN/NPGS/New Search/

Data as at Friday 8 March 1996. Data extracted from the USDA-ARS GRIN database.

Please send comments to the Database Management Unit at: [dbmu@ars-grin.gov](mailto:dbmu@ars-grin.gov)

**Table 4. Main herbaria of the world holding (genetic resources of) *Origanum* spp.**

- Biologisch Laboratorium. Afdeling Plantensystematiek. Vrije Universiteit. Amsterdam, The Netherlands.
- Botanisches Museum. Berlin, Germany.
- British Museum (Natural History). London, United Kingdom.
- Museum of Natural History (Department of Botany). Budapest, Hungary.
- Private Herbarium of Dr Buttler. Munich, Germany.
- Botanical Museum and Herbarium. Copenhagen, Denmark.
- Department of Botany (Faculty of Sciences). Cairo, Egypt.
- Istituto di Botanica. Orto Botanico. Catania, Italy.
- Botanical Institute of the University. Coimbra, Portugal.
- Royal Botanic Garden. Edinburgh, Great Britain.
- Herbarium Universitatis Florentinae (Istituto Botanico). Florence, Italy.
- Conservatoire et Jardin botaniques. Geneva, Switzerland.
- Private Herbarium of Dr Huber-Morath. Basel, Switzerland.
- Department of Botany. Hebrew University. Jerusalem, Israel.
- Institut für Spezielle Botanik und Herbarium Haussknecht. Jena, Germany.
- The Herbarium and Library. Kew (Richmond), London.
- Rijksherbarium. Leiden, The Netherlands.
- The Linnean Society. London, United Kingdom.
- Istituto "Antonio José Cavanilles". Jardín Botánico. Madrid, Spain.
- Institut de Botanique. Montpellier, France.
- Fielding Herbarium. Druce Herbarium (Department of Botany). Oxford, United Kingdom.
- Muséum National d'Histoire Naturelle. Laboratoire de Phanérogamie. Paris, France.
- Istituto Orto Botanico dell'Università. Padova, Italy.
- Universitatis Carolinae. Facultatis Biologicae Scientiae Cathedra. Prague, Czech Republic.
- Private herbarium of Dr Sorger. Vienna, Austria.
- Naturhistoriska Riksmuseum (Botanical Department). Stockholm, Sweden.
- Instituut voor Systematische Plantkunde. Utrecht, The Netherlands.
- Naturhistorisches Museum. Vienna, Austria.
- Botanisches Institut und Botanischer Garten der Universität. Vienna, Austria.
- Claude E. Phillips Herbarium, Delaware State College. Delaware State, USA.

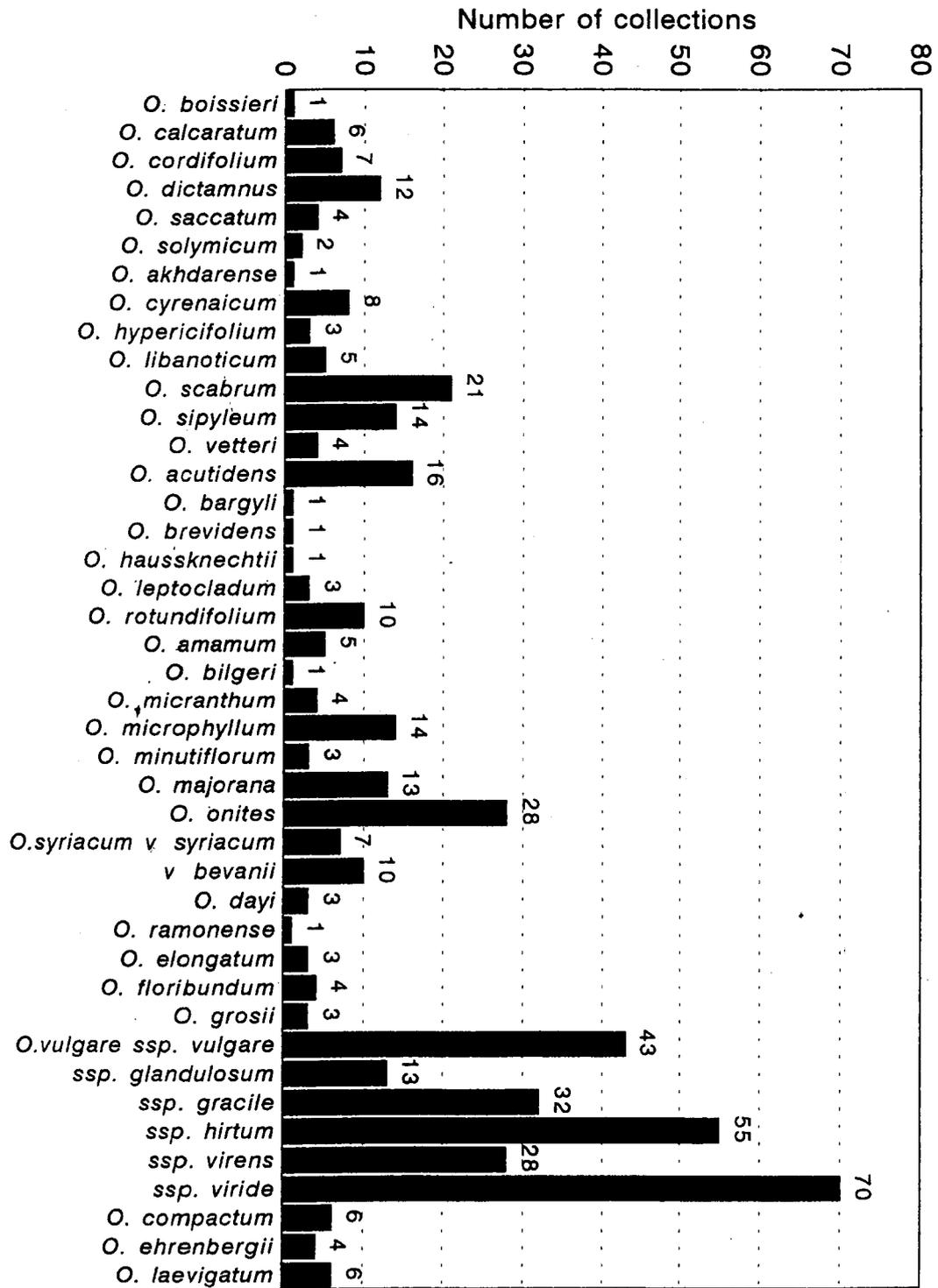


Fig. 2. Ex situ conservation of *Origanum* spp. in private collections.

**Table 5. Conservation of rare and threatened species of the genus *Origanum* in the world.**

| Species   | Distribution      | Status <sup>†</sup> | Conservation <sup>‡</sup> |
|---|-------------------|---------------------|---------------------------|
| <i>O. amanum</i> Post                             | Turkey            | R                   | PC                        |
| <i>O. bargyli</i> Mout.                           | Syria             | vR                  | PC                        |
| <i>O. bilgeri</i> Davis                           | Turkey            | R                   | PC                        |
| <i>O. boissieri</i> letsvaart                     | Turkey            | K                   | PC                        |
| <i>O. brevidens</i> (Bornm.) Dinsm.               | Turkey            | K                   | PC                        |
| <i>O. calcaratum</i> Juss.                        | Greece            | R                   | PC                        |
| <i>O. compactum</i> Benth.                        | Spain (southwest) | V                   | PC                        |
| <i>O. cordifolium</i> (Auch. Eloy & Montbr Vogel) | Cyprus            | V                   | PC                        |
| <i>O. dictamnus</i> L.                            | Greece            | V                   | PC, GB                    |
| <i>O. floribundum</i> Munby                       | Algeria           | R                   | PC                        |
| <i>O. haussknechtii</i> Boiss.                    | Turkey            | R                   | PC                        |
| <i>O. hypericifolium</i> O. Schwarz & Davis       | Turkey            | R                   | PC                        |
| <i>O. isthmicum</i> Danin                         | Egypt             | R                   | NT                        |
| <i>O. leptocladum</i> Boiss                       | Turkey            | R                   | PC                        |
| <i>O. micranthum</i> Vogel                        | Turkey            | R                   | PC                        |
| <i>O. minutiflorum</i> O. Schwarz & Davis         | Turkey            | R                   | PC                        |
| <i>O. munzurensense</i> Kit Tan & Sorger          | Turkey            | R                   | ?                         |
| <i>O. ramonense</i> Danin                         | Israel            | V                   | PC                        |
| <i>O. saccatum</i> Davis                          | Turkey            | R                   | PC                        |
| <i>O. scabrum</i> Boiss & Heldr.                  | Greece            | V                   | PC, FC                    |
| <i>O. solymicum</i> Davis                         | Turkey            | R                   | PC                        |
| <i>O. vetteri</i> Briq. & Barbey                  | Greece            | R                   | PC                        |

<sup>†</sup> R: Rare; vR: very rare; V: vulnerable; K: insufficiently known; NT: neither rare or threatened.

<sup>‡</sup> PC: private collections; GB: genebanks; FC: field collections; ?: no data.

Studies on germination of different species of *Origanum* (Putievsky 1983; Thanos *et al.* 1995) confirmed, however, their poor germinative ability. This fact was previously observed by the writer Theophrastus (4th century BC) in his "Enquiry into Plants" (*Historia Plantarum*) who noticed that the maximum percentage and speed of germination were obtained at a day/night regime of 24/19°C (62% after 5 days). As also observed by Theophrastus, old seeds germinate at a higher percentage than fresh ones, possibly as a result of the volatilisation of the essential oils present on the nutlet coat. Besides, there is indirect evidence that the seeds of *O. majorana* and *O. vulgare* may exhibit dormancy (Ellis *et al.* 1995). These characteristics can be advantageous for seed conservation, dormancy determining a condition of metabolic quiescence which holds unvaried the vigour and quality of seed thus reducing seed deterioration rate during conservation, even in adverse environmental conditions. However, the direct relation between dormancy and seed longevity and viability, studied in different species (Tran and Cavanagh 1984), also should be investigated for *Origanum*.

Conservation of genetic resources of *Origanum* in field collections is not particularly problematic, as their cultivation is rather easy, especially if the species held at the genebank originated in the same or nearby areas.

On the other hand, when dealing with multiplication and/or rejuvenation of *Origanum* seed collections, it is important to bear in mind that in these plants the gynodioecy is rather frequent, particularly in the *Chilocalyx*, *Elongatispica*, *Majorana*, *Origanum* and *Prolaticorolla* sections. It has been estimated that in populations of *O. vulgare* in western and northern Europe, 30-50% of the plants have female flowers (Lewis and Crowe 1956). Consequently, for these sections, the occurrence of outbreeding, determining loss of genetic identity, is very high during multiplication.

## Conclusions

A survey of the European *Origanum* collections has revealed that only a little more than one-fourth of those species mentioned in Ietswaart's work are actually being conserved. On the other hand, if we were to follow Tutin *et al.* (1972) and Mansfeld (1986) classifications [they have recognized only 13 (wild and cultivated) and 5 (cultivated) main *Origanum* species respectively], nearly all of them are present in these collections. In any case, according to our data, the total number of accessions present in the form of seeds and field collections held by public institutions is certainly not adequate enough to represent the wide genetic diversity of this genus.

We should also mention, however, that private growers are preserving quite a reasonable number of collections, amounting to nearly half of the species classified by Ietswaart.

In conclusion, although this paper does not provide a full inventory of all germplasm of *Origanum* preserved in the world, it has indicated that:

- the degree of diversity within the genus is very high and still little investigated;
- a greater collaboration between taxonomists, genebank managers and private collectors is very much needed to achieve a better safeguarding and gain a better knowledge of the genetic diversity of this important group of plants.

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## Conservation of *Origanum* spp. in botanic gardens

**Etelka Leadley**

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This short communication reports on the survey carried out by Botanic Gardens Conservation International (BGCI) on the level of erosion and threat to *Origanum* species in the world. Information is provided with regard to some collections of *Origanum* species from those Botanic Gardens which have answered to the BGCI survey at the time of the Workshop.

### Conserved taxa of the genus *Origanum*

The following table, prepared on 22 April 1996, reports data extracted from the database of the BGCI. This information has been compiled by BGCI on the basis of data provided by the World Conservation Monitoring Centre (WCMC), Cambridge, United Kingdom.

| Plant name and author  | Distribution (Area, Cons. status) <sup>†</sup> | World |
|--|--|-------|
| <i>Origanum acutidens</i> (hand-Mazz.) Ietswaart                   | Turkey, nt                                     | nt    |
| <i>Origanum amanum</i> Post  | Turkey, R                                      | R     |
| <i>Origanum bargyli</i> Mout                                       | Syria, ?                                       | ?     |
| <i>Origanum bilgeri</i> Davis                                      | Turkey, R                                      | R     |
| <i>Origanum boissieri</i> Ietswaart                                | Turkey, K                                      | K     |
| <i>Origanum brevidens</i> (Bornm.) Dinsm                           | Turkey, K                                      | K     |
| <i>Origanum calcaratum</i> Juss                                    | Greece, R                                      | R     |
| <i>Origanum compactum</i> Benth                                    | Spain (southwest), V                           | V     |
| <i>Origanum cordifolium</i> (Auch. Eloy & Montbr.) Vogel           | Cyprus, V                                      | V     |
| <i>Origanum dictamnus</i> L.                                       | Greece, V                                      | V     |
| <i>Origanum elongatum</i> (Bonnet) Emberger & Maire                | Morocco, nt                                    | nt    |
| <i>Origanum floribundum</i> Munby                                  | Algeria, R                                     | R     |
| <i>Origanum grosii</i> Pau & Font Quer                             | Morocco, nt                                    | nt    |
| <i>Origanum haussknechtii</i> Boiss                                | Turkey, R                                      | R     |
| <i>Origanum hypericifolium</i> O.Schwarz & Davis                   | Turkey, R                                      | R     |
| <i>Origanum isthmicum</i> Danin                                    | Egypt, R                                       | R     |
| <i>Origanum leptocladum</i> Boiss                                  | Turkey, R                                      | R     |
| <i>Origanum micranthum</i> Vogel                                   | Turkey, R                                      | R     |
| <i>Origanum microphyllum</i> (Benth.) Boiss                        | Greece, nt                                     | nt    |
| <i>Origanum minutiflorum</i> O.Schwartz & Davis                    | Turkey, R                                      | R     |
| <i>Origanum munzurense</i> Kit Tan & Sorger                        | Turkey, R                                      | R     |
| <i>Origanum pau</i> Martinez                                       | Spain, I                                       | I     |
| <i>Origanum ramonense</i> Danin                                    | Israel (Ramon Hills, C. Negev), V              | V     |
| <i>Origanum saccatum</i> Davis                                     | Turkey, R                                      | R     |
| <i>Origanum scabrum</i> Boiss. & Heldr.                            | Greece, V                                      | V     |
| <i>Origanum sipyleum</i> L.  | Turkey, nt                                     | nt    |
| <i>Origanum solymicum</i> Davis                                    | Turkey, R                                      | R     |
| <i>Origanum syriacum</i> L. var. <i>bevanii</i> (Holmes) Ietswaart | Cyprus, ?                                      | I     |
| <i>Origanum vetteri</i> Briq. & Barbcy                             | Greece, R                                      | R     |

<sup>†</sup> WCMC Threatened Categories: I – Indeterminate; K – Insufficiently known; V – Vulnerable; R – Rare; nt – neither rare or threatened; ? – not known.

**Rare and threatened species of *Origanum* in botanic garden cultivation**

The following table reports on the known occurrences of rare and threatened species of *Origanum* maintained in botanic gardens. The table has been built upon with data extracted from the databases of BGCI.

| <i>Origanum</i> species | WCNC distribution with known conservation category <sup>†</sup> (include subspecies) | Prov. <sup>‡</sup> | Botanic garden  |
|-------------------------|--|--------------------|---|
| <i>amanum</i>           | Turkey (R)   |                    | Royal Botanic Garden, Edinburgh, UK   |
| <i>calaratum</i>        | Greece (R)   | W<br>G             | Royal Botanic Gardens, Kew, UK<br>The Royal Horticultural Society's Garden, Wisley, UK  |
| <i>dictamnus</i>        | Greece (V)   | W                  | Royal Botanic Garden, Edinburgh, UK   |
|                         |  | G                  | Botanische Tuin Elsloo, Elsloo, Netherlands   |
|                         |  | G                  | Royal Botanic Gardens, Kew, UK  |
|                         |  | G                  | University of Aarhus Botanical Institute, Aarhus, Denmark   |
|                         |  | G                  | Botanischer Garten der Universität Bonn, Germany  |
|                         |  | G                  | National Botanic Gardens, Glasnevin, Ireland  |
|                         |  | G                  | The Berry Botanic Garden, Portland, USA   |
| <i>microphyllum</i>     | Greece (nt)  | G                  | The Royal Horticultural Society's Garden, Wisley, UK  |
|                         |  | W                  | Royal Botanic Garden, Edinburgh, UK   |
| <i>scabrum</i>          | Greece (V)   | W<br>G             | Royal Botanic Garden, Edinburgh, UK<br>Botanischer Garten der Philipps Universität, Narburg, Germany<br>Royal Botanic Garden, Edinburgh, UK |

<sup>†</sup> WCMC threatened categories: V – Vulnerable; R – Rare; nt – neither rare or threatened.

<sup>‡</sup> WCMC origin abbreviation: G – Plants not of known wild source; W – Plants from a known wild source; Z – Propagule/propagated from a wild source in cultivation.

***Origanum dictamnus* L. and *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart: Traditional uses and production in Greece**

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**Abstract**

*Origanum dictamnus* and *O. vulgare* subsp. *hirtum* are the most important *Origanum* species in Crete (Greece), in relation to their utilization. Traditional therapists in Crete suggest the infusion of leaves of *O. dictamnus* and flowers for treating several health disorders. *Origanum vulgare* subsp. *hirtum* is the most commonly used spice in the island, its essential oils being recommended against rheumatism and toothache whereas leaves and flower infusions are used against cold and diarrhoea. Essential oils of both species are rich in carvacrol,  $\gamma$ -terpinene and p-cymene. The biological properties of these compounds may justify some of the reported traditional uses. Both species are cultivated for commercial exploitation, some production features are reported.

***Origanum dictamnus* L.**

**Traditional uses**

Thirteen common names have been given by local populations to *Origanum dictamnus* L., a Cretan endemic species with pungent odour which grows on calcareous rocks, debris and cracks, usually in shady places from 300 to 1500 m asl (Ietswaart 1980). *Dictamos* (and its related words *adictamos*, *dictamnus*, *ditamo*, *atitamos*, *titamos*) is the most common name and refers either to one of the localities where the species grows, mount Dicti, or to the Cretan goddess Dictinna who governed the mountains and helped women during childbirth. The Greek Artemis and Roman Diana goddesses are also related to this plant, which explains why Artemis is often represented with a dictamos crown on her head. The following names are related to various therapeutic plant uses:

- *stamatohorto* (lit. meaning 'stopping herb') refers to the plant property to stop bleeding;
- *stomachohorto* (lit. 'stomach herb') refers to its property to cure stomach-ache;
- *stomatohorto* (lit. 'mouth herb') refers to its property to refresh the mouth.

Other names such as *malliarohorto* (lit. 'hairy herb') and *gerontas* (lit. 'old man') refer instead to the plant morphology, as its aerial parts are covered by dense hairs. The most interesting name is certainly *erontas* (and *erotas*), meaning love, which probably refers to the difficulties encountered in collecting the plant from the wild, where it thrives in rather inaccessible sites, which often lead to hard and sometimes even fatal collecting trips. Euripides (480-406 BC), Hippocrates, Aristotle, Theophrastus, Cicero (106-43 BC), Virgil (70-19 BC), Pliny (23-79 AD), Plutarch (46-127 AD), Dioscorides, Galen and other philosophers, poets and doctors of antiquity talked about a plant occurring only in Crete, known as *dictamnus*, that is said to help childbirth, cure wounds from arrows, snake bites and skin diseases. They also suggest the use of an infusion to treat various ailments made of wine extract and

crude leaves (Platakis 1951). Faure (1987) reports that essential oil from *dictamos* in olive oil was offered to the Minoan kings and priests of Crete.

Today, as in the past, *dictamos* is still widely used in Crete, to cure almost any disease and to maintain good health. The plant parts used in these preparations are leaves and flowers, which are collected in late summer during the species' flowering period. The following are reported to be the most common current uses of this plant in Crete, from interviews with local old villagers (Skoula 1996) and from reviews of ethnographic literature (Fragaki 1969; Havakis 1978):

- infusions in hot water are used against tonsillitis, cold, cough and sore throats;
- infusions or chewed crude plant parts are used against gingivitis and toothache;
- infusion is also considered diuretic, digestive, spasmolytic and relieves stomach-ache and kidney pains;
- infusion is recommended against liver diseases, diabetes and obesity;
- crude plant or its infusion induces menstruation and delivery, while it is thought to be abortifacient too;
- it lessens abdominal pains;
- plant parts crushed with water are used externally for wound healing and headaches;
- plant decoction helps to relieve rheumatism pains.

It is speculated that some of the above-mentioned medicinal properties could be related to the plant's essential oil compounds which include mainly  $\gamma$ -terpinene (4.5%), p-cymene (7.5%), caryophyllene (2.1%), carvacrol (73%) and borneol (1.7%) (Harvala *et al.* 1986). The following summarises the full range of reported activities attributed to these constituents according to data from Duke (1992) and Harborne and Baxter (1993).

|                     |  |
|---------------------|--|
| $\gamma$ -terpinene | insectifuge  |
| p-cymene            | analgesic, antifu, antirheumatic, bactericide, fungicide, herbicide, insectifuge, vermifuge  |
| caryophyllene       | anti-edemic, anti-inflammatory, insectifuge, perfumery, spasmolytic, termitifuge   |
| carvacrol           | anaesthetic, anti-inflammatory, antiplaque, antiseptic, bactericide, carminative, expectorant, fungicide, nematocide, prostaglandin inhibitor, spasmolytic, tracheorelaxant, vermifuge |
| borneol             | analgesic, anti-inflammatory, febrifuge, hepatoprotectant, herbicide, insectifuge, spasmolytic.  |

It should be noted that there are no research data referring to the presence of other active substances in the water extract.

### Production

*Origanum dictamnus* used to be a species with good economic significance. It was intensively collected from wild populations, a habit which is still common now despite the fact that the species is considered under threat and thus protected by the

Bern Convention. Unfortunately, such an excessive exploitation of wild *O. dictamnus* populations has resulted in the dramatic reduction of population sizes and has even caused its complete extinction from some areas. The great difficulties encountered in the collecting of the plant induced Cretan communities at the beginning of this century to pursue cultivation of the species. First records of this activity date back to 1920, cultivating sites being Cretan villages located around mount Dicta. Farmers became involved in the cultivation of dittany without any special technical or scientific know-how, and this situation remains unchanged to this day. Weeding and harvesting are done by hand and watering is necessary to be able to yield two harvests in a year (May and September). It is worth noting that farmers distinguish different varieties (or types) of the plant such as the 'black' and the 'white' referring to green (less hairy) and hairy plants respectively and plants with narrow or large leaves. These types occur in several locations and seem not to be related to any particular environmental conditions. The narrow-leaved type is more aromatic than the large-leaved one, but it usually requires more harvesting efforts as it is more woody. On the other hand, the narrow type might be more interesting as it yields more biomass per plant, although unfortunately it is at the same time more susceptible to pests during storage.

Figure 1 shows the production of cultivated *O. dictamnus* whereas Figure 2 shows the production of *O. dictamnus* collected from wild populations. Harvests from the wild do of course contribute very little to the total harvest of this species.

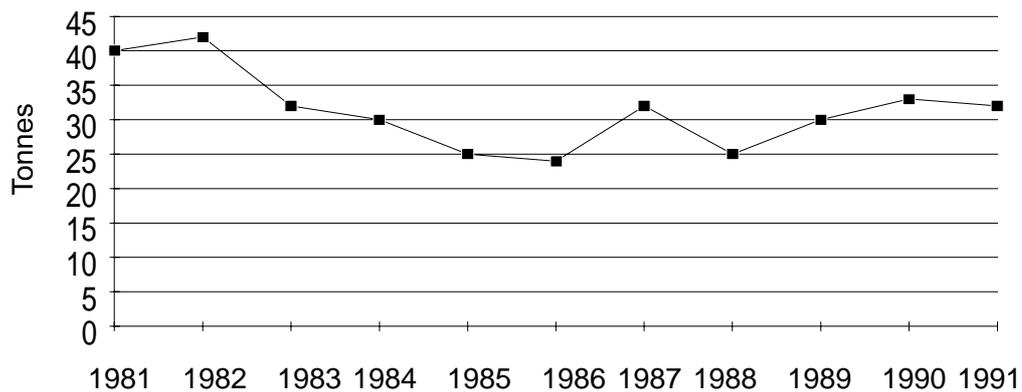


Fig. 1. Production of cultivated *Origanum dictamnus* L.

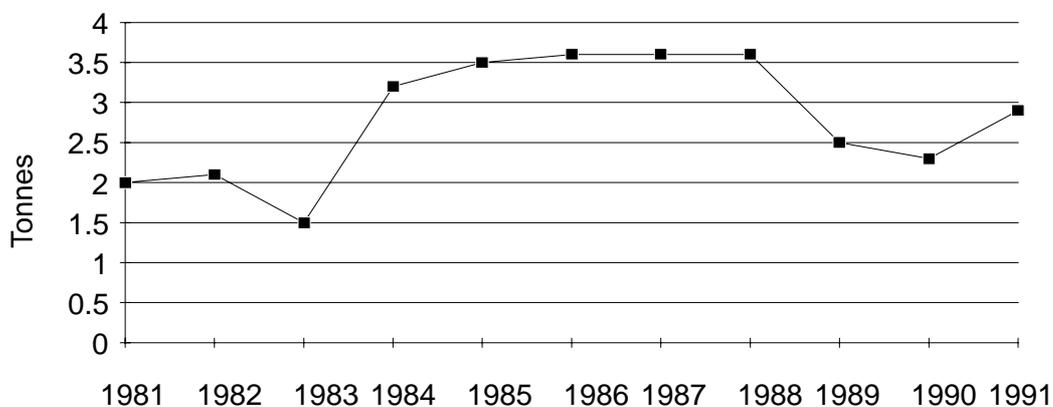


Fig. 2. Production of *Origanum dictamnus* L. collected from wild populations.

The comparison of cultivated and wild dittany market prices, over the last decade (Fig. 3), shows an increasing trend for both. Moreover, the price of wild dittany is much higher in every year. This difference may imply the presence of appreciable qualitative differences between cultivated and wild material, although no scientific results have ever been obtained to confirm this.

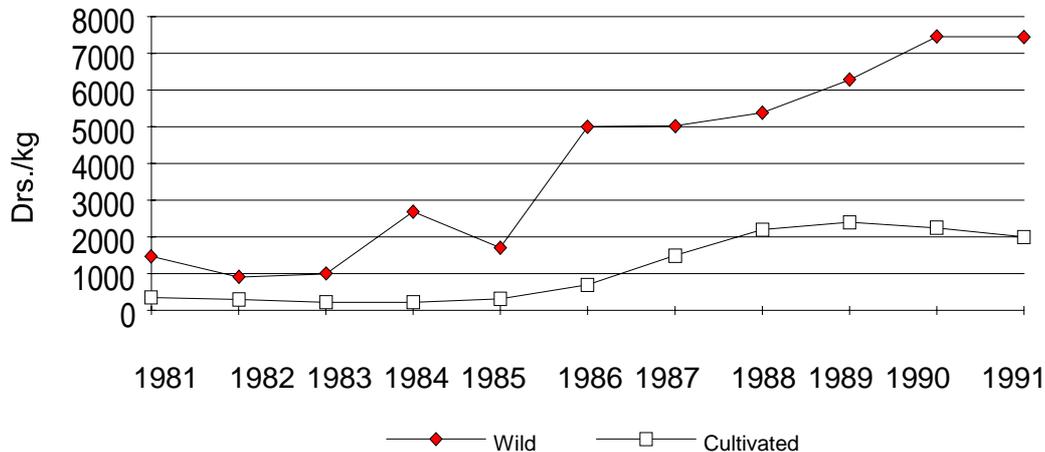


Fig. 3. Comparison of prices of cultivated and wild *Origanum dictamnus* L.

The production of dittany reached its peak from 1980 to 1990 and is now in decline. In 1991 a portion of 85% of the product was exported (mainly to Italy, France, Germany and Japan), while 15% of the total production was absorbed by the Greek market. Abroad, main users of the product are distillery industries. At present, the intensive cultivation of *O. dictamnus* has ceased and production has dropped to minimum levels with the price fluctuating between 800 and 900 GR.DR./kg (ca. US\$3.30-3.70/kg). Only a few farmers still harvest *O. dictamnus* today and they do so mainly from wild populations as this offers them an additional income, albeit low. Of the several reasons for the drop in dittany cultivation, the most important is the lack of a properly organized marketing system for such a crop.

### *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart

#### Traditional uses

*Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart (*O. heracleoticum* sensu) has less impressive common names and properties; however, it is the most widely used spice all over Greece. Its common name is *rigani* and accounts of its utilization have been reported by Theophrastus and Dioscorides. The plant parts used are leaves and flowers, collected in summer during the flowering period. Information gathered from aged local people (Skoula 1996), and from literature sources (Fragaki 1969; Havakis 1978) has revealed the following uses for this crop:

- its distilled essential oil (red thyme oil) was used in the past for the preparation of soaps with antiseptic properties;
- inhalation of the essential oils is reported to cure chronic pneumonia;
- essential oil placed on aching teeth relieves pain (a similar effect is caused by chewing leaves);

- essential oil – pure or dissolved in olive oil – is used externally against rheumatism; however, as it gives a burning effect it is recommended to be used with care;
- the infusion in hot water is used against cold, cough and diarrhoea.

The plant is extremely rich in essential oils (up to 7%) with carvacrol as a major constituent present in very high quantity (75-95%) followed by p-cymene (4-14%) and  $\gamma$ -terpinene (1-10%) (Skoula 1996). As with dittany, it seems possible that the known biological properties of p-cymene and carvacrol (Duke 1992; Harborne and Baxter 1993) could justify some of the uses of the plant in traditional therapies.

### Production

The area under cultivation of *O. vulgare*, in Greece, is reported in Figure 4, showing its significant increase during the last decade. Figures 5 and 6 show the production of cultivated and wild oregano, respectively. The production of cultivated oregano increases over time though with some fluctuations, whereas the production of wild oregano declines drastically throughout the decade 1981-91. It is important to note that in 1981 the production from cultivation comprised less than 2% of the total oregano production, while in 1991 the production from cultivation was almost half of the production from the wild. However, the total oregano production in the country was reduced to one-third compared with that recorded for 1981.

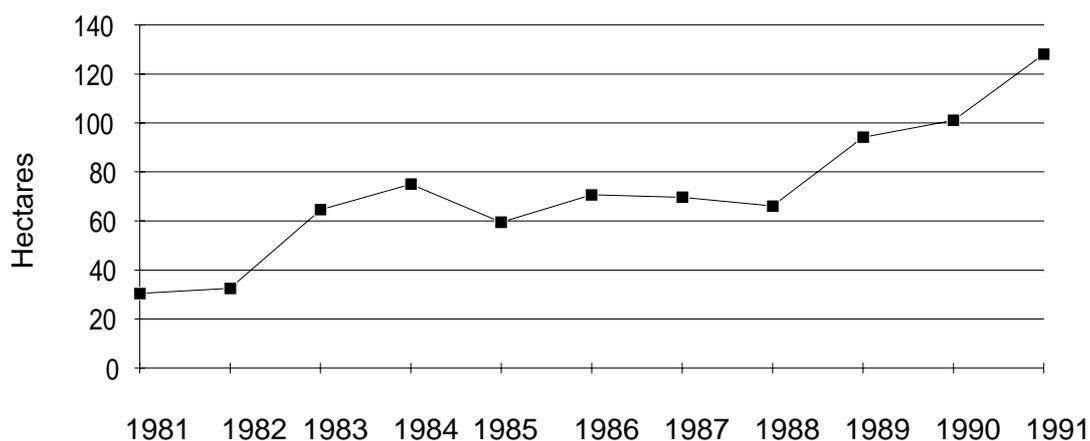


Fig. 4. Cultivated area of *Origanum vulgare* L.

Price comparison (Figure 7), indicates that generally the price of wild oregano is lower than cultivated oregano with a few exceptions. This could be attributed to the high heterogeneity of the material harvested from the wild due to its high interspecific diversity, presence of other plants in the harvest, bad storage conditions and other reasons. On the other hand, it is unlikely that cultivated material comes from proper selection procedures.

At the moment it is rather difficult to distinguish the species and subspecies that are cultivated or harvested from wild populations. *Origanum vulgare* L. includes three subspecies: *hirtum* (Link) Ietswaart, *viridulum* (Martin-Donos) Nynan and *vulgare*. Among them only subsp. *hirtum* is considered an essential oil-rich plant, while the other two subspecies are relatively poor (Kokkini *et al.* 1991).

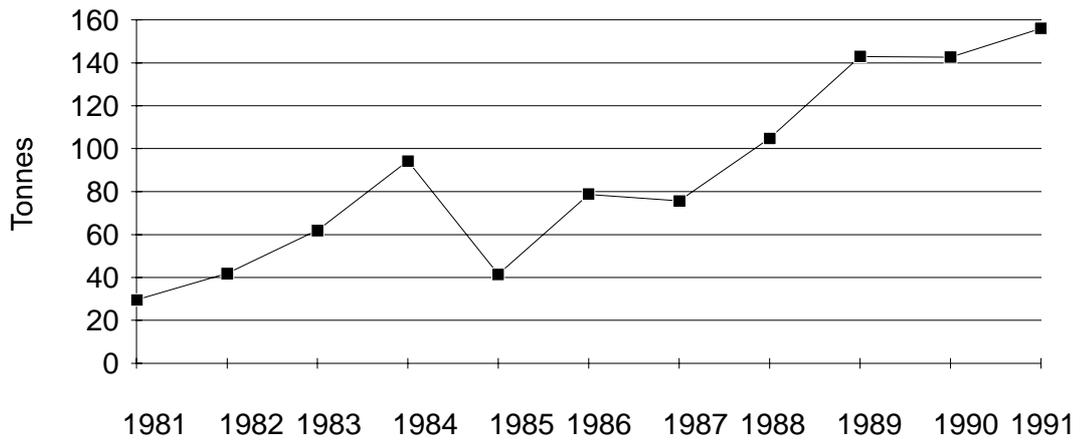


Fig. 5. Production of cultivated *Origanum vulgare* L.

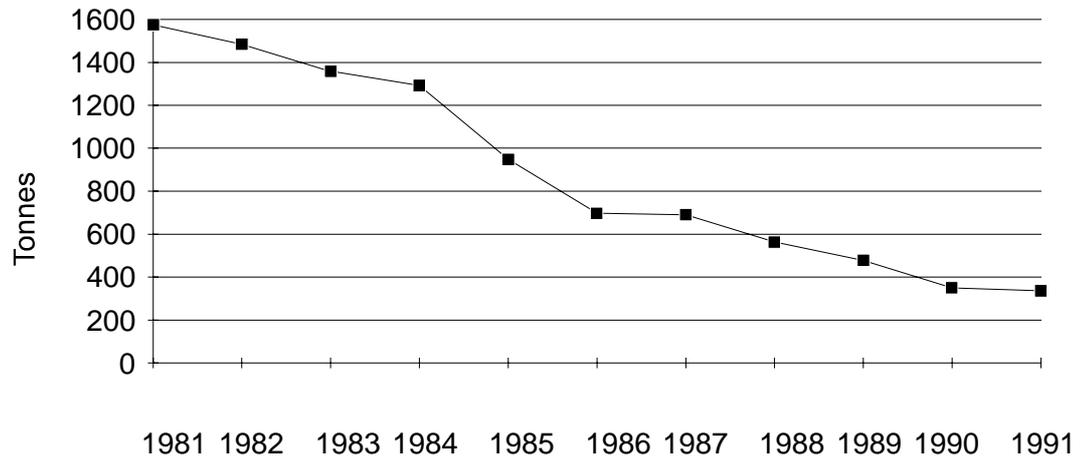


Fig. 6. Production of wild *Origanum vulgare* L.

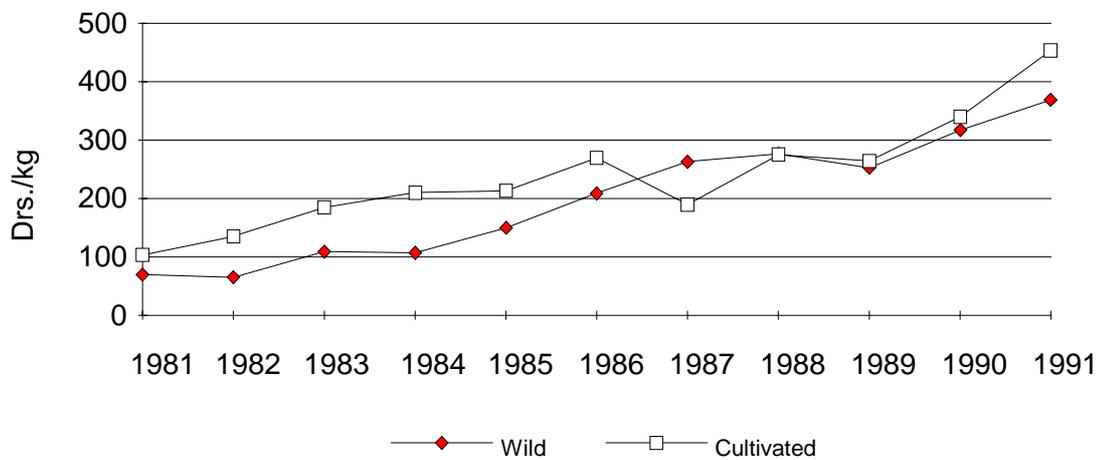


Fig. 7. Comparison of prices between cultivated and wild oregano.

Furthermore, *Origanum onites* L. is another essential oil rich species, with an essential oil profile very similar to that of *O. vulgare* subsp. *hirtum* (Skoula 1996). *Origanum onites* is a species very abundant in the Aegean Islands and eastern Crete, where it is being used as oregano. In addition, *Coridothymus capitatus* (L.) Reichenb. fil. and *Satureja thymbra* L. also are essential oil rich plants with high carvacrol contents (Kokkini and Vokou 1989) which might be included in oregano harvests. However, the most common type of oregano in Greece is likely to be *O. vulgare* subsp. *hirtum*.

### Acknowledgements

The present work has been financially supported through the research project "Identification, Preservation, Adaptation and Cultivation of Selected Aromatic and Medicinal Plants, suitable for Marginal Lands of the Mediterranean Region" (EU CAMAR, No. 8001 CT91 0104) and the concerted action project "Towards a model for technical and economic optimisation of specialist minor crops: aromatic and medicinal plants" (EU AIR3 CT92 2076).

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### **III. Biology, Agronomy and Crop Processing**

## Crop domestication and variability within accessions of *Origanum* genus

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### Abstract

*Origanum* accessions originating from various countries all over the world were gathered by the Agronomy and Field Crop Institute of the University of Bari to undergo evaluation trials on some agrobotanical and biochemical traits. The study has been carried out on 70 accessions, grown out at the "E. Pantanelli" research station of the University of Bari in Policoro (Matera, Basilicata region), southern Italy. First results obtained in these trials, herewith reported, generally appear very promising for crop improvement purposes.

### Introduction

The genus *Origanum*, belonging to the family of *Labiatae* (from the Greek οροξ = mountain and γαρνοξ = ornament for the beauty of some Greek hilly landscapes covered with oregano), includes many species, commonly found in the Mediterranean as wild plants. Within the *Origanum* biotypes there is wide variability that determines some uncertain definitions at both the species and botanical variety levels. This made the taxonomic revision of the genus *Origanum* provided by Ietswaart in 1980 particularly useful.

Although a notable contribution of studies has been given to the botanical insight of the genus *Origanum*, knowledge of its biochemical aspects is still incomplete and not sufficient for a proper assessment of the different species. Taking this into account, the Agronomy and Field Crop Institute of the Faculty of Agriculture, University of Bari, decided to pursue a research investigation to throw more light on this area. The study was conducted in the framework of the project "Cultivation and genetic improvement of medicinal and aromatic plants" carried out under the auspices of the Italian Ministry of Agriculture. The initial step consisted of gathering adequate genetic diversity of *Origanum*. This was achieved by contacting a number of botanical gardens, research institutes and local growers. This search resulted in 70 germplasm accessions of oregano, which were classified using the donor institute's classification.

The material consisted of the following species (Table 1): 25 accessions of *O. vulgare*, nine of *O. heracleoticum* and *O. vulgare* subsp. *vulgare*, seven of *O. vulgare* subsp. *hirtum*, six of *O. majorana*, four of *O. vulgare* subsp. *virens*, two of *O. onites* and *O. vulgare* subsp. *glandulosum*, and one accession respectively for *O. tythanthum*, *O. creticum*, *O. vulgare* subsp. *gracile*, *O. compactum*, *O. vulgare* subsp. *vulgare* var. *vulgare* and *Origanum* spp.

The study was basically designed to assess the morphophysiological diversity as well as essential oil content and composition of the collected germplasm, ultimate goals being the selection of the best material to use in breeding purposes, as well as the identification of the most appropriate agrotechniques required for growing the species.

Table 1. Sources of the investigated material.

| Accession   | Research institutes  | Seed companies                                    | Local populations   |
|---|--|---|---|
| <b><i>Origanum vulgare</i> L.</b>   |  |   |   |
| Bari, Berlino, Bonn, Bratislava, Budakalaski II, Budakalaski III, Budapest, Clujl; Kew II, Ljubljana; Meise, Nancy II, Parigi, Siena, Zagabria, Zalec, Zurich | Gatersleben Genebank, Germany<br>Sukhumi Exp. Station, Georgia | Battele, Blumen, Ingegnoll, Puget, Spagna, Taroni |   |
| <b><i>Origanum heracleoticum</i> L.</b>   |  |   |   |
| Bari, Zagabria, Zalec   |  |   | Bitonto, Castellaneta, Grecia 7.3; Grecia 7.1; Grecia 7.2; Policoro |
| <b><i>Origanum vulgare</i> L. subsp. <i>vulgare</i></b>   |  |   |   |
| Liegi I, Liegi II, Liegi III, Liegi IV, Liegi V, Liegi VI, Liegi VII, Liegi VIII, Liegi IX  |  |   |   |
| <b><i>Origanum vulgare</i> L. subsp. <i>hirtum</i></b>  |  |   |   |
| Bari, Zagabria, Zalec   |  |   | Bitonto, Castellaneta, Creta 7.2, Policoro                          |
| <b><i>Origanum majorana</i> L.</b>  |  |   |   |
| Bari, Dresda, Parigi  |  | Ingegnoll, Puget                                  | Carovigno   |
| <b><i>Origanum vulgare</i> L. subsp. <i>virens</i></b>  |  |   |   |
| Lisbona, Parigi, Zalec  | Gatersleben Genebank, Germany                                  |   |   |
| <b><i>Origanum onites</i> L.</b>  |  |   |   |
| Berlino   | Gatersleben Genebank, Germany                                  |   |   |
| <b><i>Origanum vulgare</i> L. subsp. <i>glandulosum</i></b>   |  |   |   |
| Portoroz, Zalec   |  |   |   |
| <b><i>Origanum tythanthum</i>, <i>O. creticum</i>, <i>O. vulgare</i> L. subsp. <i>gracile</i></b>   |  |   |   |
| Zalec   |  |   |   |
| <b><i>O. compactum</i>, <i>O. vulgare</i> subsp. <i>vulgare</i> var. <i>vulgare</i></b>   |  |   |   |
|   | Gatersleben Genebank, Germany                                  |   |   |
| <b><i>Origanum</i> spp.</b>   |  |   |   |
| Portoroz  |  |   |   |

The seedlings of the 70 accessions were obtained from seeds and grown out in the nursery alveolate containers until they reached 10 cm in height. In spring they were then transplanted to the field where they were planted at 50 cm and 40 cm distance between rows and within row, respectively. Frequent irrigation was ensured after transplanting, to allow a good vegetative state. Plants were fertilized with nitrogen, phosphorus ( $N_2PO_5$ ) and potassium ( $K_2O$ ).

Data on plant height, branches, height of flower-bearing branches, yield of leaves and inflorescence were gathered at full blooming time on two plants per accession. Within the collection, blooming period started in the second half of June and continued until the first half of July, according to the earliness degree of each accession.

## Results and discussion

The data gathered show considerable variation within the *Origanum* material studied. With regard to the flowering time, the earliest accessions were those of *Origanum* subsp. *hirtum* which were in full bloom around 19 June, whereas among *O. creticum* and *O. vulgare* spp. full bloom was reached on 10 July (Fig. 1).

Considerable variation in blooming time was observed in each of the studied species. Such variation was particularly high in *O. vulgare* (probably due to the wider range of accessions studied for this species), *O. heracleoticum*, *O. vulgare* subsp. *hirtum*, *O. vulgare* subsp. *vulgare* and *O. majorana*.

Within *O. vulgare* accessions, full blooming was observed between 22 June and 20 July. The earliest accessions were those provided by the Gatersleben Genebank, Germany and the Botanical Garden of Ljubljana and Bratislava, whereas accessions provided by the Botanic Garden of Meise and Zurich were the latest (Fig. 2).

Within *O. heracleoticum* (Fig. 3) the local populations from Bitonto, Castellaneta and Policoro (all situated in southern Italy) were the first that experienced full blooming (on 24 June) whereas accessions Grecia 7.1, 7.2, 7.3 and Zalec were the latest (10 July) (Fig. 4).

Among the accessions of *O. vulgare* subsp. *hirtum*, those from Crete, Castellaneta, Bitonto and Policoro were quite early flowering, whereas the accession from the Botanical Garden of Zagabria was particularly late with full blooming observed only on 6 July.

Within *O. vulgare* subsp. *vulgare* material (Fig. 5) received from the Botanical Garden of Liegi, 12-day differences in earliness were observed and three groups were eventually identified (the earliest ones being Liegi II, VI and IX, the intermediate Liegi I and III and the latest Liegi IV, V, VII and VIII, whose blooming dates were on average 28 June, 3 July and 10 July respectively).

*Origanum majorana* accessions (Fig. 6) showed a lower variability with regard to this character, with a 10-day difference in earliness between the earliest and the latest ones.

Within *O. majorana* accessions the earliest flowering were those from Bari and Desdra (Germany), which reached full blooming on 18 June; accessions from a USA seed company (PUGET) and from the Botanical Garden of Paris, France were the latest, reaching full blooming on 28 and 29 June respectively.

With regard to plant height, great variability was observed both among and within species. In general, among the species (Fig. 7), values above 70 cm were found in *O. vulgare* subsp. *vulgare*, *O. vulgare* subsp. *vulgare* var. *vulgare*, *O. virens*, *O. tythantum* and *O. vulgare*; smaller values (below 55 cm) were observed in *O. onites*, *O. majorana* and *O. vulgare* subsp. *hirtum*.

The tallest plants (about 88 cm) were recorded from *O. vulgare* subsp. *vulgare* material. Moreover, most accessions showed an erect habit that, combined with height, is an extremely important character for mechanical crop mechanical harvesting.

Within *O. vulgare* (Fig. 8) considerable variability was found among the 25 studied accessions; height ranged from 50 cm (Bari) to 110 cm (Budakalaski III).

As a whole, the tallest accessions within this species were Badakalaski II, Kew II, Nancy II and those received from a Spanish Seed Company (all characterized by values above 80 cm). It should be noted that on average, 36% of *O. vulgare* accessions showed higher values than the field average (equivalent to 71.9 cm).

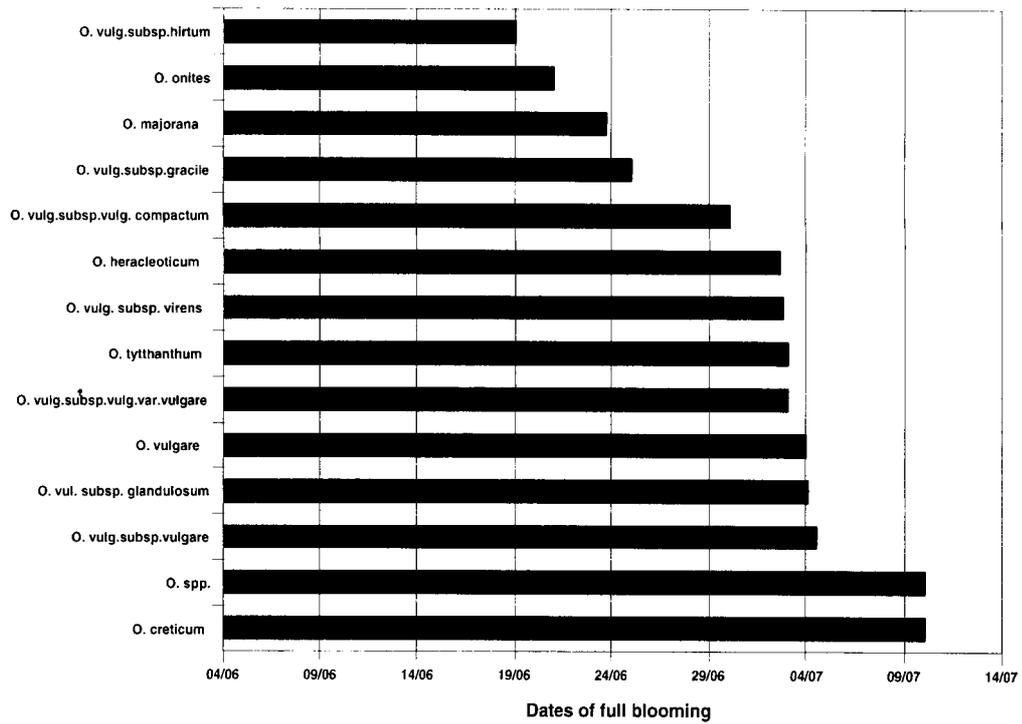


Fig. 1. Dates of full blooming within the tested *Origanum* taxa.

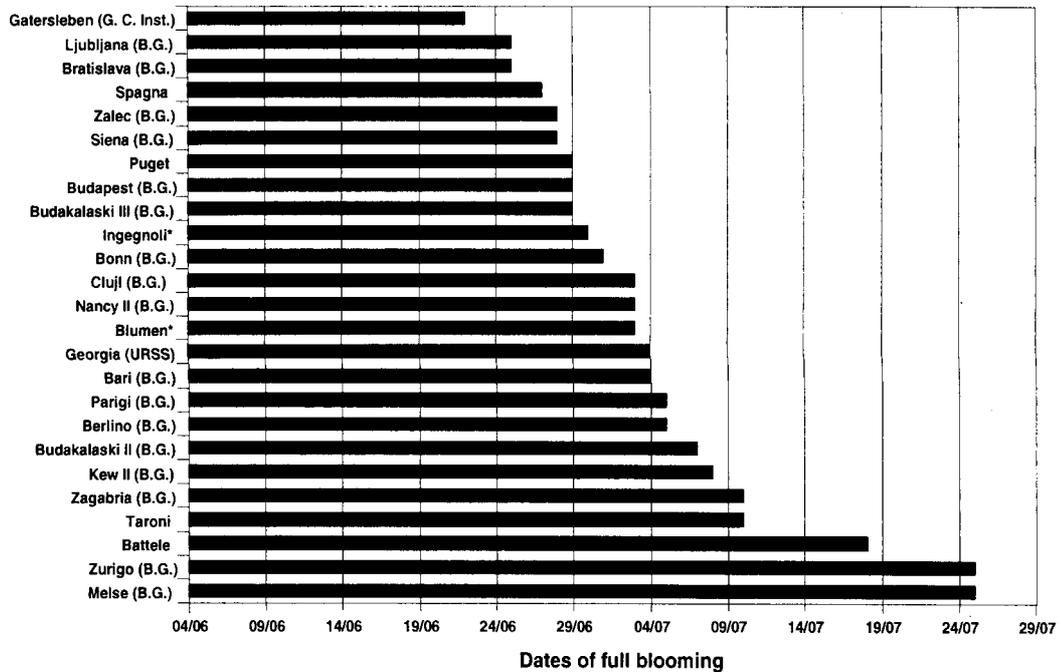


Fig. 2. Dates of full blooming within *Origanum vulgare* accessions.

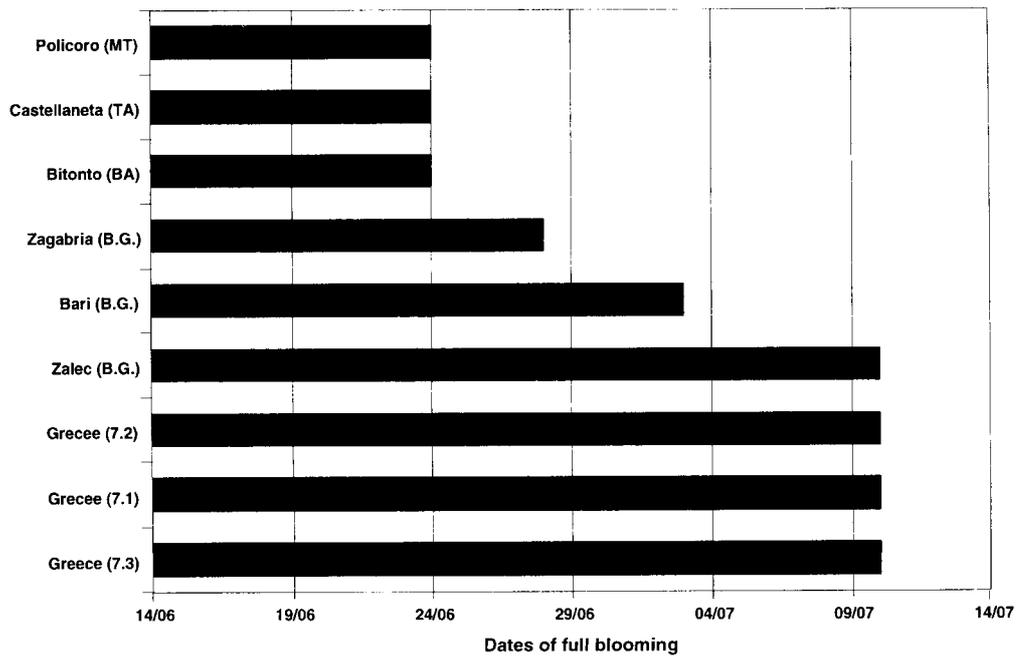


Fig. 3. Dates of full blooming within *Origanum heracleoticum* accessions.

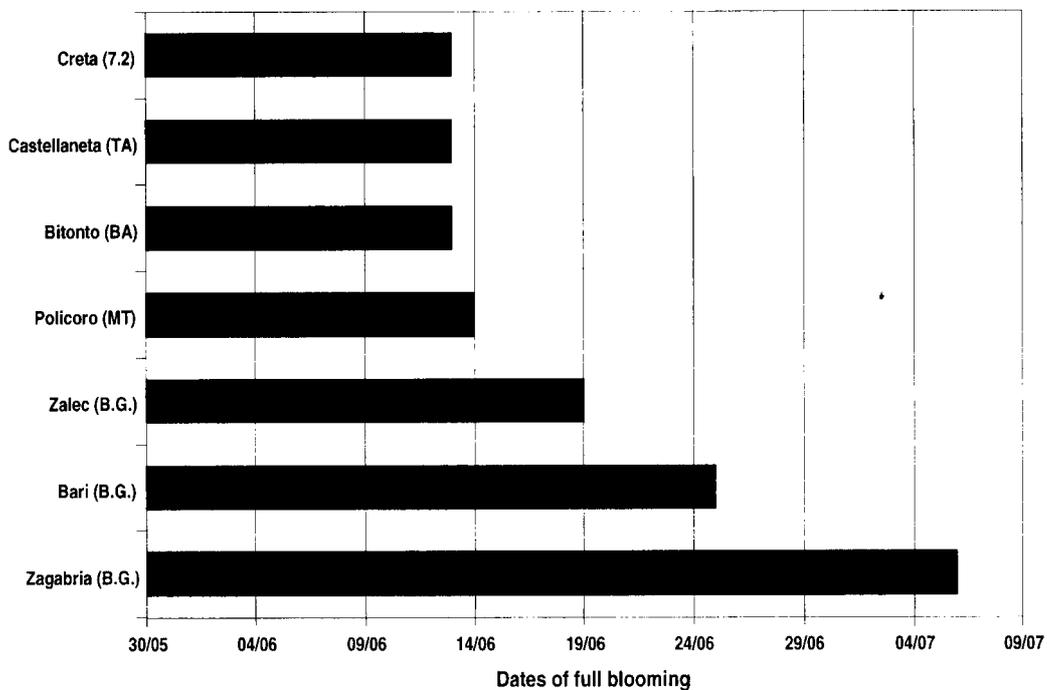


Fig. 4. Dates of full blooming within *Origanum vulgare* subsp. *hirtum* accessions.

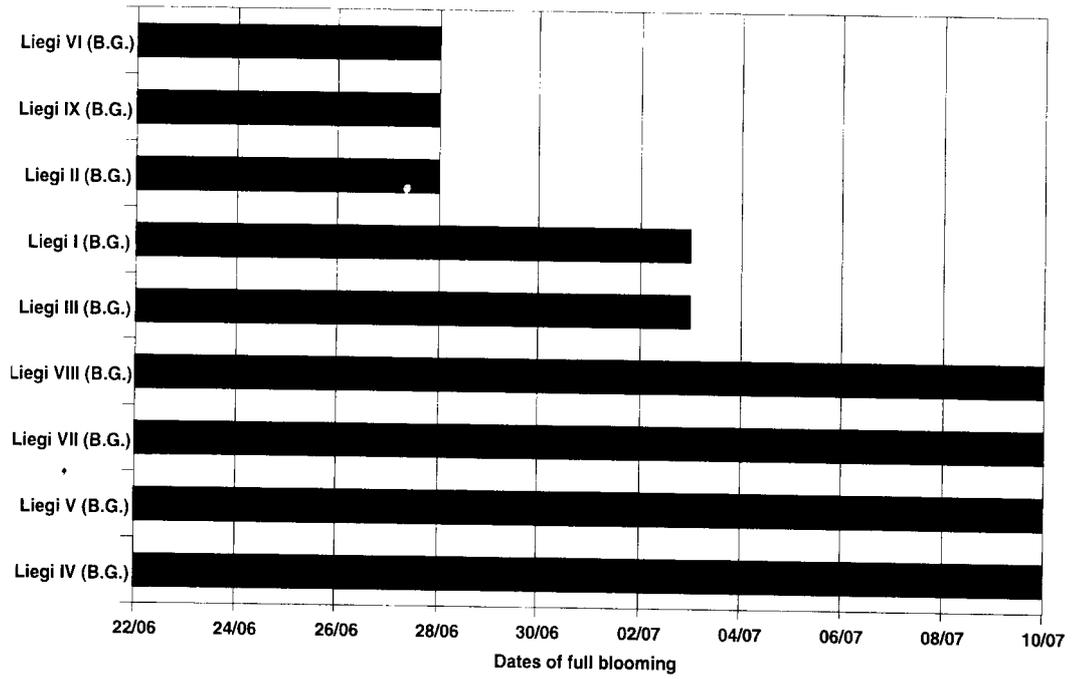


Fig. 5. Dates of full blooming within *Origanum vulgare* subsp. *vulgare* accessions.

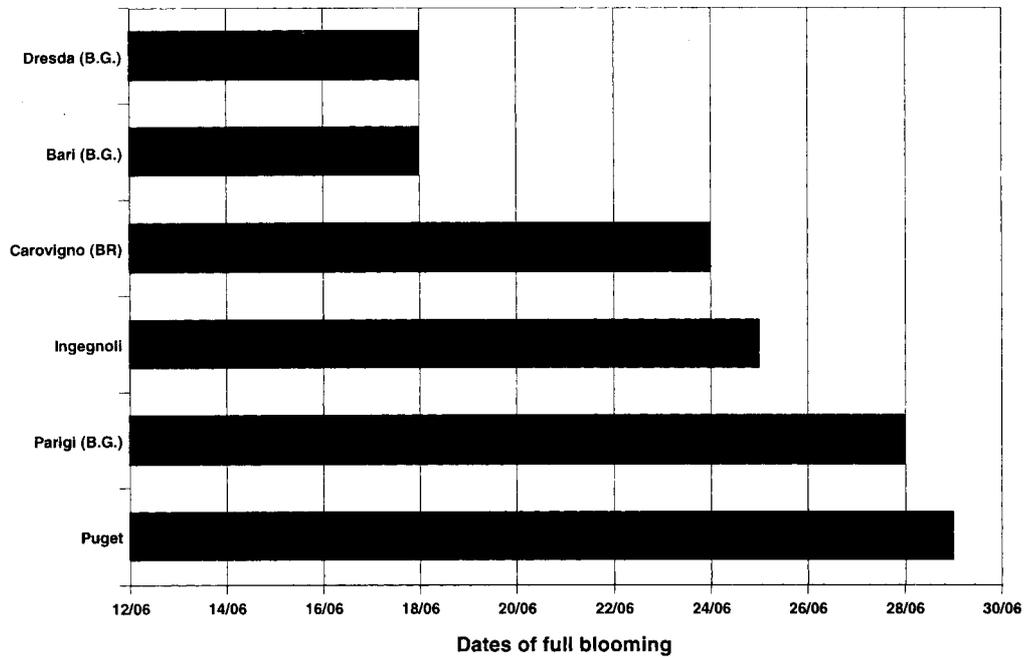


Fig. 6. Dates of full blooming within *Origanum majorana* accessions.

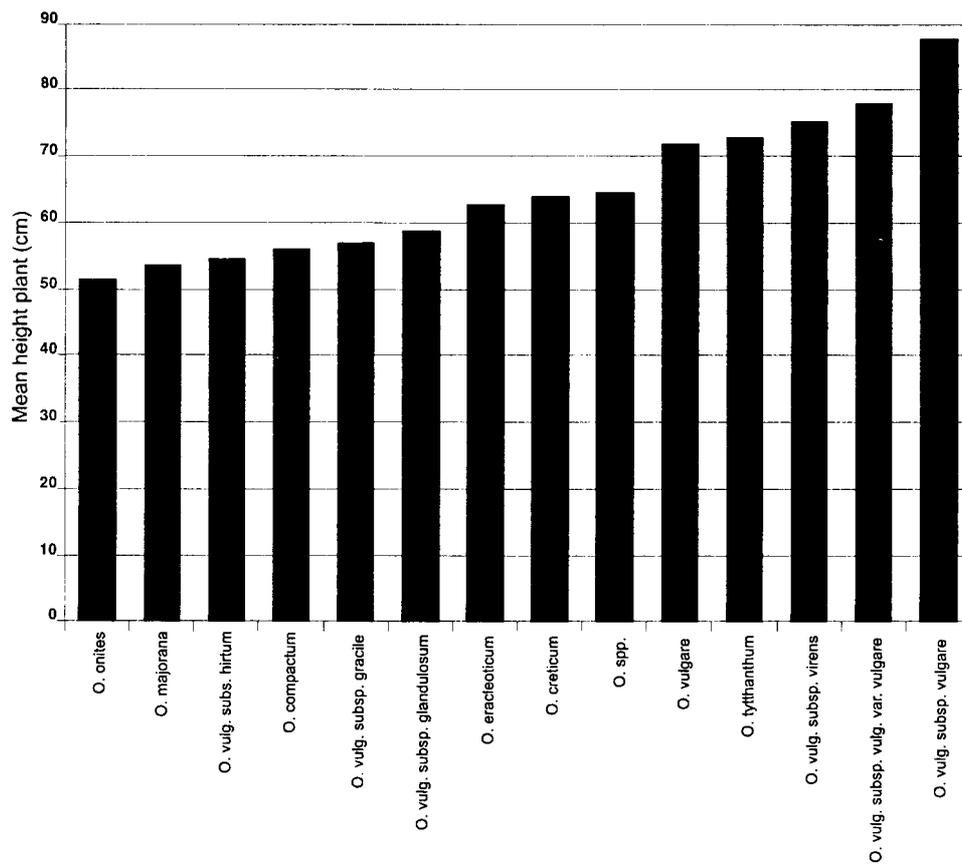


Fig. 7. Mean plant height within the species and subspecies tested.

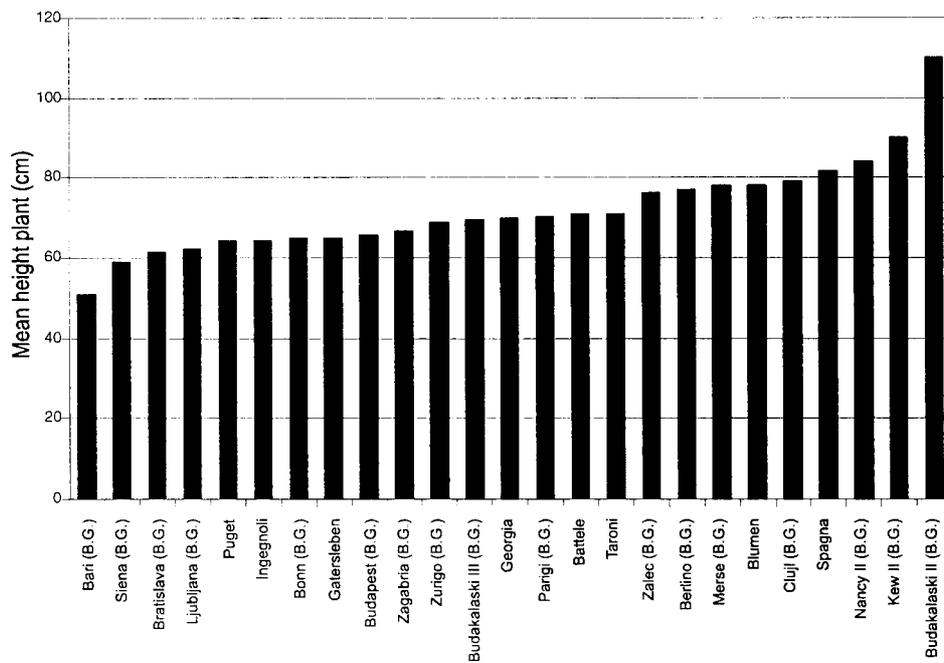


Fig. 8. Mean plant height within the *Oreganum vulgare* accessions.

Lower variability was observed in accessions belonging to other species. In particular, in *O. heracleoticum* plant height ranged from 49.2 cm in Castellaneta to 76.3 cm in Grecia 7.3 (7.3 cm), although most accessions showed values close to the field average (about 63 cm).

In *O. vulgare* subsp. *hirtum* plant height values ranged from 44.7 cm in Bari material to 66.1 cm in samples from Zagabria; the southernmost material – Creta 7.2, Castellaneta, Policoro and Bitonto – was lower than the northernmost – Zalec, Zagabria and Gatersleben.

Although *O. vulgare* subsp. *vulgare* accessions were characterized by a relatively higher plant height, they also showed some variability between 78 cm (Liegi II and VII) and 92 and 99 cm (Liegi V and IV, respectively).

The average number of branches per plant in all investigated material was about 140 cm and seemed correlated with plant height. A particularly branched material was the *Origanum* spp. accession with 224 branches per plant, whereas *O. vulgare* subsp. *gracile* showed a very low branching (only 33 branches per plant) (Fig. 9).

In about 38% of the accessions studied, the number of branches per plant ranged between 100 and 150 (Fig. 10).

High variability for this trait was observed, in particular in *O. vulgare* material, with values ranging from 50 to over 300 and in *O. vulgare* subsp. *hirtum* and *O. vulgare* subsp. *vulgare* with fluctuations between 35 and 200.

The height of the flower-bearing canopy, which is an important factor for herb yield, showed values ranging between 8.8 cm in *O. vulgare* subsp. *vulgare compactum* and 28.5 cm in *O. vulgare* subsp. *glandulosum* (Fig. 11).

The average height of flower-bearing branches was 19 cm, 39% of the analysed material showing values above average (Fig. 12).

Among yield-related characters, leaf and inflorescence yield (as percentage of the total plant biomass) also were investigated. They were found to be on average about 64%. Fluctuations from this value were observed in *O. creticum* and *Origanum* spp. (73% in both) and in *O. vulgare* (54.6%) (Fig. 13). Variability within accessions was highest in *O. heracleoticum*, leaf and inflorescence percentage in relation to the total biomass ranging between 53% (Zalec) and 77% (local population from Castellaneta).

Forty-five accessions out of the 70 tested showed leaf and inflorescence yield values ranging between 55 and 65% (Fig. 14).

A noteworthy result of this study was the observed correlation between leaf + inflorescence yield / total fresh biomass with the decrease in plant height (Fig. 15).

Average weight of fresh biomass was remarkably variable among the surveyed species, corresponding to around 540 g on average, with fluctuations between 160 g in *O. vulgare* subsp. *gracile* and over 1000 g in *O. vulgare* subsp. *vulgare* (Fig. 16). Within the latter species, fresh biomass weight per plant of accessions Liegi IX and VI were 1451.3 and 1596.3 g, respectively.

The biomass values per plant observed in the accessions of *O. vulgare* (Gatersleben and Budakalaski II) were 1195.3 and 1056.3 g respectively, whereas in *O. vulgare* subsp. *glandulosum* (Zalec) and *O. hirtum* (Zagabria) they were 1269.0 and 1133.0 g, respectively. Among the 70 accessions tested, 27.5% showed a fresh biomass weight ranging between 200 and 400 g and 29% between 400 and 600 g (Fig. 17).

Analyses of the oil yield were carried out on most representative accessions belonging to seven species (viz. *O. vulgare*, *O. heracleoticum*, *O. vulgare* subsp. *virens*, *O. majorana*, *O. creticum*, *O. tythantum* and *Origanum* sp.); whereas for the oil composition five *O. vulgare* subspecies (*hirtum*, *glandulosum*, *gracile*, *virens* and *vulgare*), represented by a more limited number of accessions than those used in the morphological characterization, were analysed.

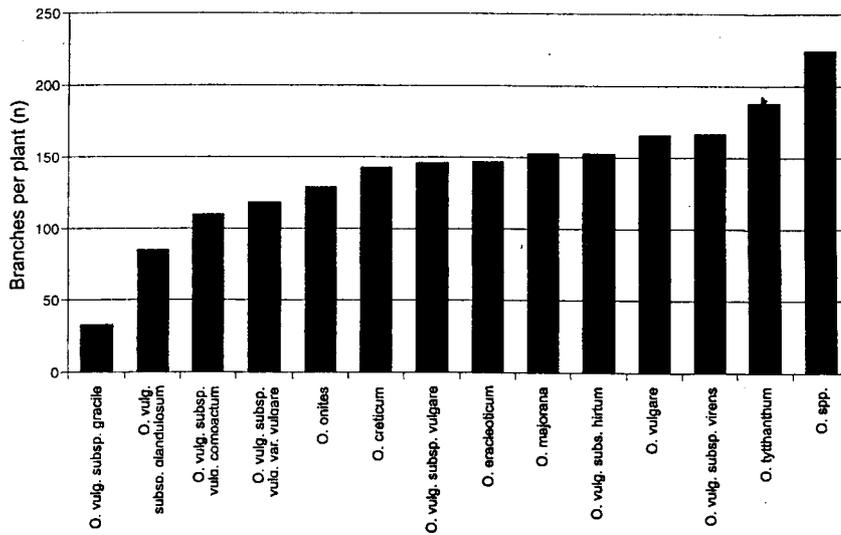


Fig. 9. Number of branches per plant within the species and subspecies tested.

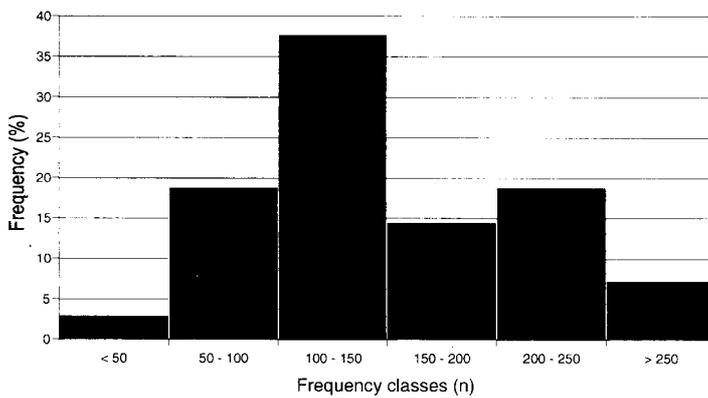


Fig. 10. Number of branches per plant, distribution within the tested material.

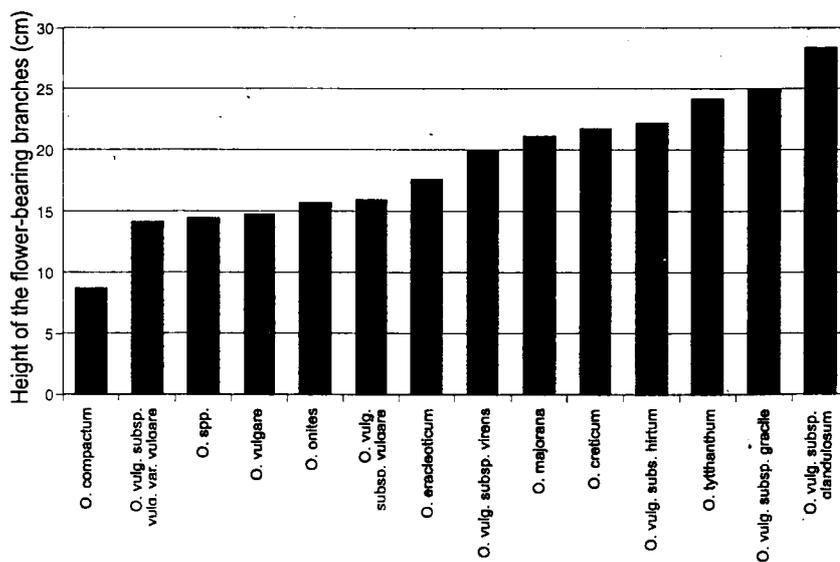


Fig. 11. Height of the flower-bearing branches within the species and subspecies tested.

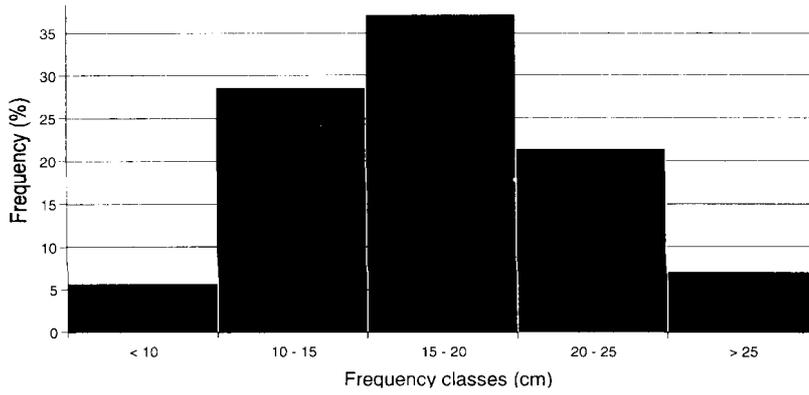


Fig. 12. Distribution of height of flower-bearing branches.

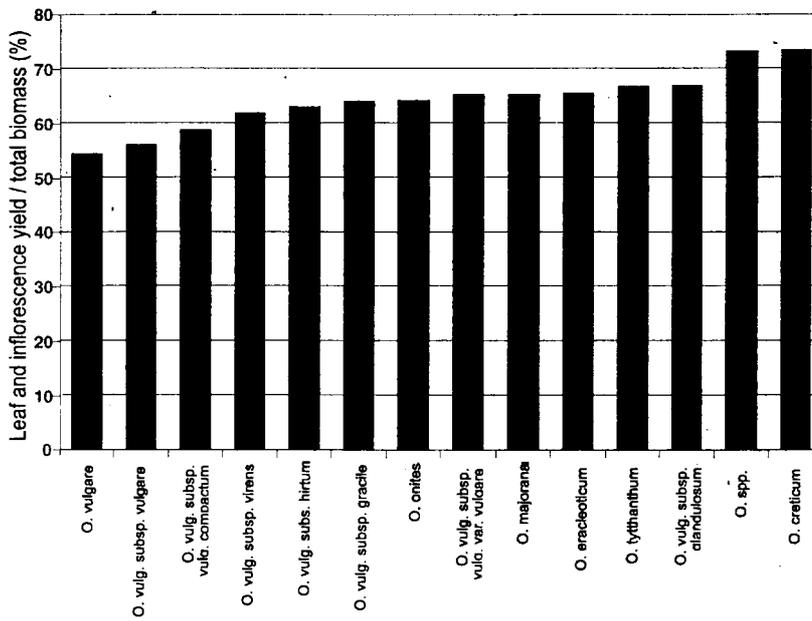


Fig. 13. Leaf and inflorescence yield in relation to fresh total biomass within each species.

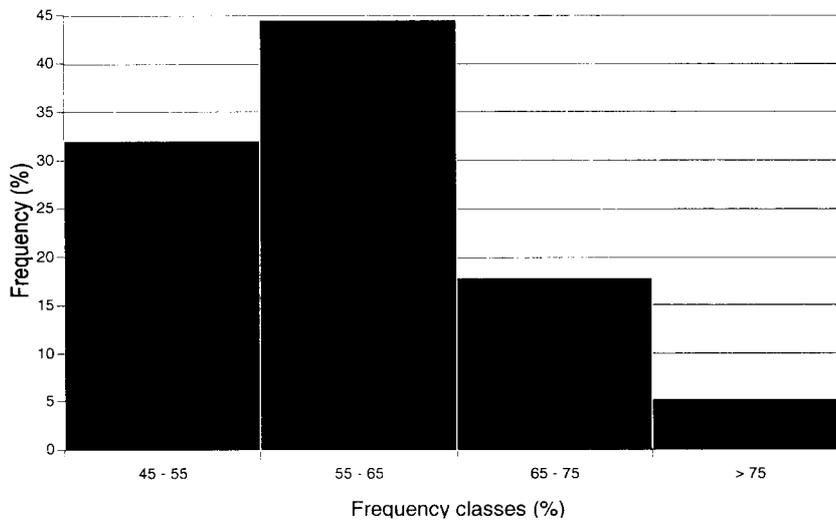


Fig. 14. Leaf and inflorescence yield in relation to total fresh biomass distribution.

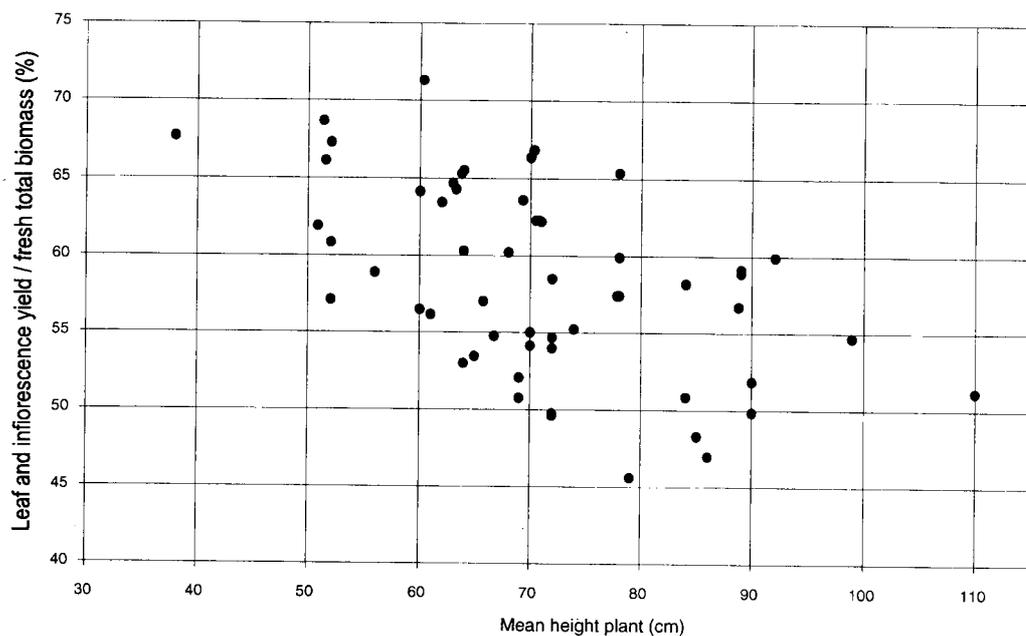


Fig. 15. Relation between the leaf and inflorescence yield and the mean plant height.

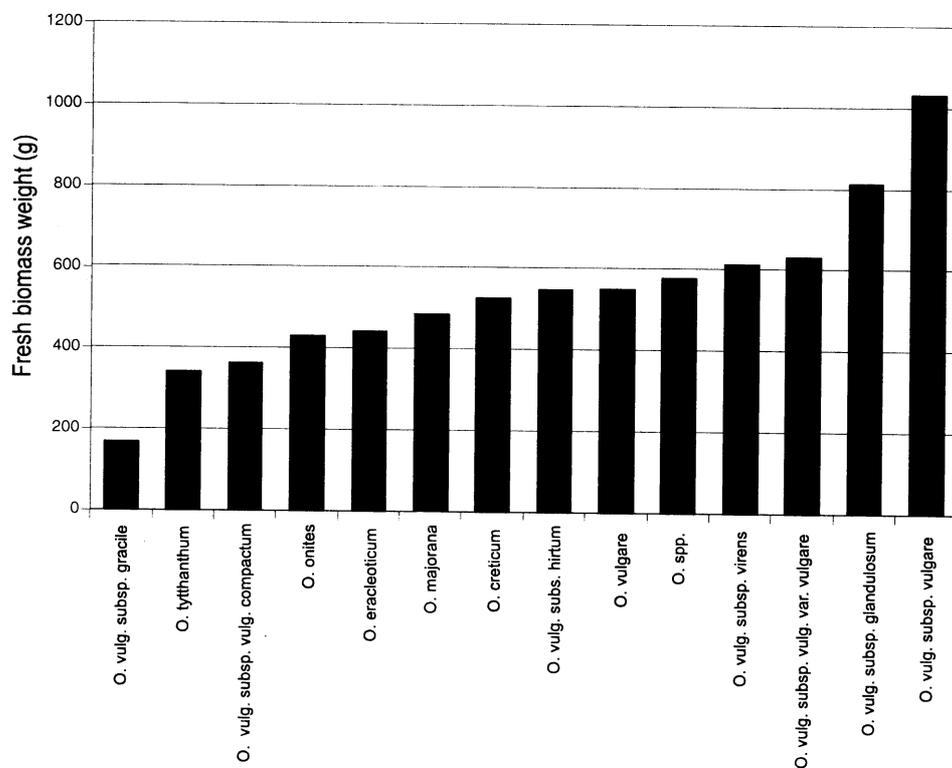


Fig. 16. Fresh biomass weight within the species and subspecies tested.

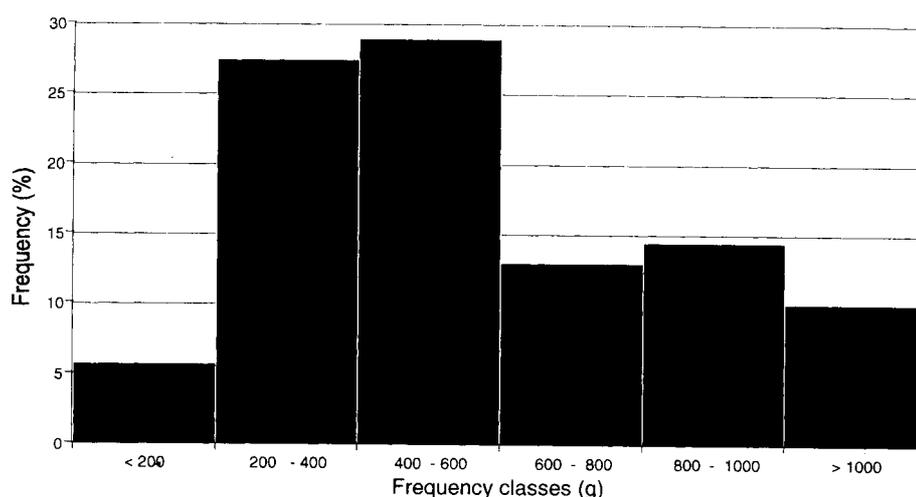


Fig. 17. Distribution of fresh biomass weight.

As to oil yield, the analyses carried out revealed a higher essential oil content in inflorescences than in the leaves.

In particular, among species (Table 2), the highest essential oil yields were found in *Origanum* spp. where 60.7 ml/kg of dry matter (DM) were obtained in inflorescences and 48.7 ml/kg of DM in leaves; in *O. creticum* with 45.8 ml/kg of DM in inflorescences and 27.3 ml/kg of DM in leaves, and in *O. heracleoticum* with 42.8 ml/kg of DM and 24.2 ml/kg of DM respectively in inflorescences and in leaves.

Table 2. Essential oil contents of some *Origanum* genus accessions.

| Species and subspecies                             | Accessions      | Essential oil content (ml/kg of DM) |        |       |
|--|-----------------|-------------------------------------|--------|-------|
|  |                 | Inflorescence                       | Leaves | Total |
| <i>O. vulgare</i> L.                               | Zalec (B.G.)    | 6.8                                 | 0.3    | 7.1   |
| <i>O. vulgare</i> L.                               | Siena (B.G.)    | 2.5                                 | 2.3    | 4.8   |
| <i>O. vulgare</i> L.                               | Battele (S.C.)  | 2.9                                 | 3.5    | 6.4   |
| <i>O. vulgare</i> L.                               | Bluemen (S.C.)  | 1.8                                 | 2.2    | 4.0   |
| <i>O. vulgare</i> L.                               | Parigi (B.G.)   | 8.2                                 | 1.4    | 9.6   |
| <i>O. vulgare</i> L.                               | Puget (S.C.)    | 2.1                                 | 1.8    | 3.9   |
| Mean   |                 | 4.1                                 | 1.9    | 6.0   |
| <i>O. heracleoticum</i> L.                         | Grecia (7.1)    | 53.4                                | 37.3   | 90.7  |
| <i>O. heracleoticum</i> L.                         | Grecia (7.3)    | 55.1                                | 28.0   | 83.1  |
| <i>O. heracleoticum</i> L.                         | Grecia (7.2)    | 53.3                                | 35.4   | 88.7  |
| <i>O. heracleoticum</i> L.                         | Zalec (B.G.)    | 15.9                                | 11.2   | 27.1  |
| <i>O. heracleoticum</i> L.                         | Bari (B.G.)     | 35.3                                | 9.1    | 44.4  |
| Mean   |                 | 42.6                                | 24.2   | 66.8  |
| <i>O. vulg.</i> subsp. <i>virens</i> Offm. et Link | Parigi (B.G.)   | 2.9                                 | traces | 2.9   |
| <i>O. vulg.</i> subsp. <i>virens</i> Offm. et Link | Zalec (B.G.)    | 2.0                                 | 0.4    | 2.4   |
| Mean   |                 | 2.5                                 | 0.4    | 2.9   |
| <i>O. majorana</i> L.                              | U.S.A. (S.C.)   | 14.7                                | 9.6    | 24.3  |
| <i>O. majorana</i> L.                              | Brindisi (L.P.) | 27.8                                | 29.1   | 56.9  |
| <i>O. majorana</i> L.                              | Parigi (B.G.)   | 14.3                                | 16.2   | 30.5  |
| Mean   |                 | 18.9                                | 18.3   | 37.2  |
| <i>O. creticum</i> L.                              | Zalec (B.G.)    | 45.8                                | 27.3   | 73.1  |
| <i>O. tythanthum</i> Gontsch.                      | Zalec (B.G.)    | 5.5                                 | 3.6    | 9.1   |
| <i>Origanum</i> spp.                               | Portoroz (B.G.) | 60.7                                | 48.2   | 108.9 |

A low oil yield was observed in both *O. vulgare* and *O. virens* with values ranging between 2 and 8 ml/kg of DM in the inflorescences and between 0.3 and 3.5 ml/kg of DM in the leaves. Best results were those found in Grecia 7.1, 7.2 and 7.3 (belonging to *O. heracleoticum*), in Portoroz (*Origanum* spp.) and Zalec (*O. creticum*).

The chemical composition of the essential oils in the tested accessions was very variable, depending on the origin of the material. However, it should be noted at this point that, according to literature available on this topic, the environment does not seem to have a strong effect on oil characteristics.

With reference to *O. vulgare* subsp. *hirtum* accessions, Bitonto showed a high content of the two isomers, thymol and carvacrol (41 and 38% respectively); the predominant oil component of Grecia was carvacrol (ca. 81%) whereas thymol was nearly absent. Oils obtained from accessions Zagabria, Castellaneta and Policoro show similar characteristics because the major oil component is thymol with values of 79, 55 and 40% respectively (Fig. 18). The essential oils extracted from the two accessions of *O. vulgare* subsp. *glandulosum* are quite similar (Fig. 19): their predominant component is carvacrol (79-83%) with the only difference being in thymol content (almost absent in Zalec). *Origanum vulgare* subsp. *gracile* seem to be a genuine thymol type of oregano, having 79% thymol content in its oil.

The accessions of *O. vulgare* subsp. *virens* (Fig. 20) showed a quite complex and uncertain chemical composition; essential oils seem, in fact, comparable in some respects but similar in others.

In general, with the exception of accession Lisbona, essential oils of all other samples have been found to be characterized by low thymol (<4%) and carvacrol (<6%) and high linalool contents (23-70%). Moreover, in Parigi, Zalec and Lisbona, terpineol content varied between 26 and 68%, a rather unusual value, given the fact that this component was virtually absent in all other subspecies of *O. vulgare*.

Another interesting result was the appreciable amounts of terpinen-4-ol (about 16%) found in Gatersleben accessions since this component is usually absent in subsp. *virens* and in other subspecies of *O. vulgare*, except the subsp. *vulgare* which has large amounts. With regard to accession Lisbona, its oil shows a special feature that distinguishes it from all the others: it contains about 14% carvacrol, which is quite a high value if compared with a mean value (not exceeding 5%) observed in oils from other accessions.

Based on the results obtained from the analysis of the oils of some *O. vulgare* accessions (Fig. 21) it may be observed that, with the exception of Budakalaski, the composition of these oils is characterized by a  $\beta$ -caryophyllene content of about 17-18% and other sesquiterpenes.

Accession Spagna showed a terpinen-4-ol content of about 14%, which might suggest the need for a further investigation on the taxonomic status of this sample, which seems to be chemically closer to *O. vulgare* subsp. *vulgare* than to *O. vulgare*.

A separate analysis was required for the Budakalaski accession, characterized by a considerable linalool presence (>28%) in its oil, which is almost absent in the other accessions.

As found in previous works, the main oil component in subsp. *vulgare* is terpinen-4-ol; in fact these studies found a content of about 37% in Liegi VII.

In general, the results of the chemical tests showed the complex chemistry in some of them. In particular, some unique oil contents were recorded in some accessions, i.e. a high carvacrol content in Grecia 7.1, a high thymol content in Zagabria and a carvacrol and thymol content in Bitonto accessions.

The oils of subsp. *glandulosum* and *gracile* are also interesting for their high carvacrol and high thymol contents, respectively.

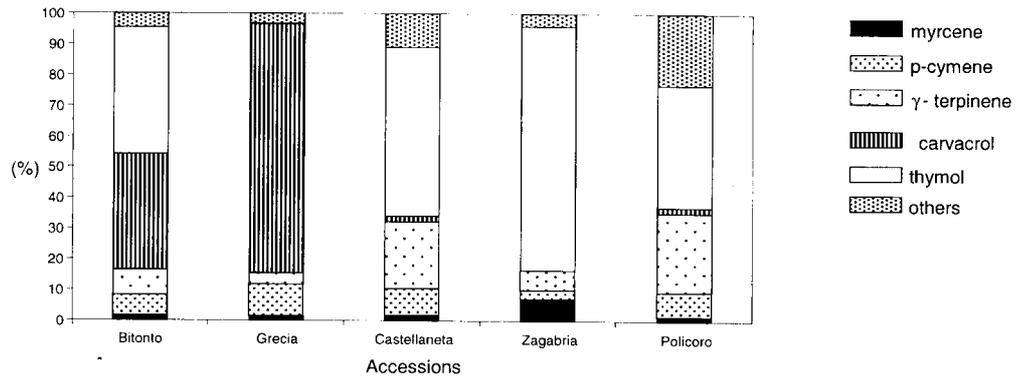


Fig. 18. Essential oil composition of *Origanum vulgare* subsp. *hirtum*.

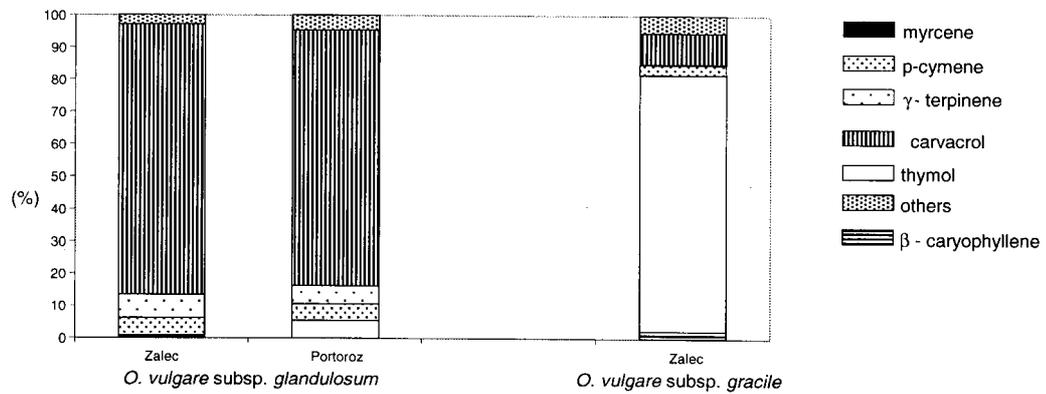


Fig. 19. Essential oil composition of *Origanum vulgare* subsp. *glandulosum* and subsp. *gracile*.

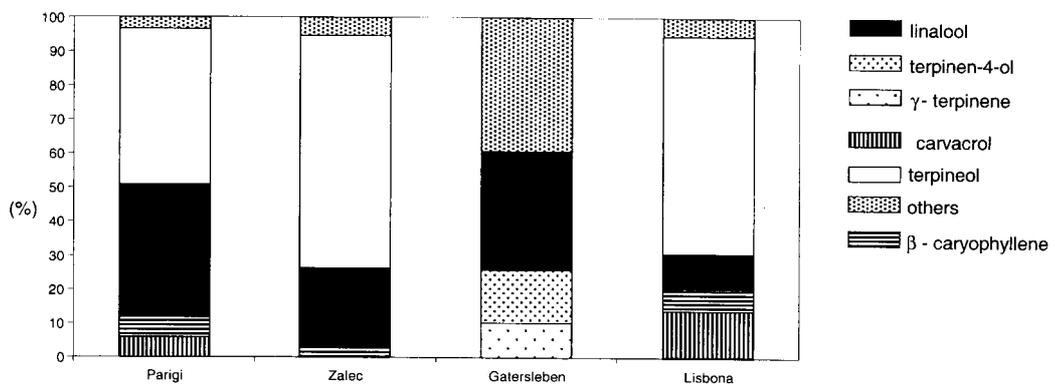


Fig. 20. Essential oil composition of *Origanum vulgare* subsp. *virens*.

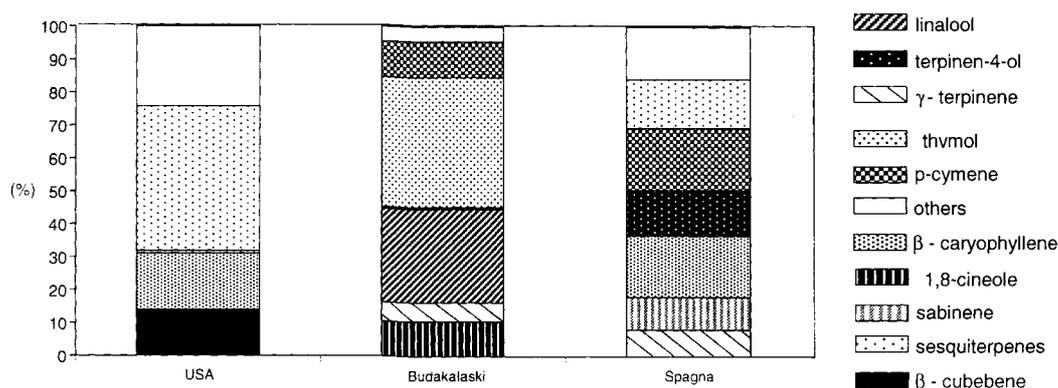


Fig. 21. Essential oil composition of *Origanum vulgare*.

### Conclusion

The results of these chemical analyses have revealed a wide variability in the composition of the essential oils. These results might be also used to spot possible incorrect taxonomic identification among the material studied.

These preliminary assessments on the possibility of domesticating *Origanum* material seem to be interesting for the relatively high variability encountered in morphological and qualitative traits of the accessions being tested only in the collection field.

Although these are just preliminary results, we should not fail to notice that the reported data stress the good potential of this crop, presently only partly exploited, while also representing a sound basis for further oregano varietal breeding initiatives.

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## Breeding of *Origanum* species

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### Abstract

Oregano and marjoram are crops for which genetic improvement is most necessary because of their high chemical and physiological heterogeneity. Crop improvement is highly recommended in consideration of their widespread use and of the great difficulties that non-uniform material may cause to the commercial sector. Taking into consideration both producers' and users' needs, efforts of any oregano breeding programme should be directed to the improvement of the following targets: yield-related parameters, e.g. growth habit, ramification, leaf/stem ratio, stress (salt, cold) tolerance, resistance to diseases and quality-related parameters, e.g. better aromatic characteristics, essential oil content and composition, antioxidant and antimicrobial properties. To achieve these goals, selection and hybridisation methods, combined with analytical controls on the variability encountered in the material, are the most appropriate tools for crop improvement. Local strains of *Origanum vulgare* subspecies and *O. majorana* (*Majorana hortensis*), as well as spontaneous hybrids (*O. x majoricum*, *O. x intercedens*), are traditionally cultivated in many countries. In addition, several ornamental varieties are also present on the market. Breeding of oregano has started in relatively recent times. Breeding work has focused mainly on *O. majorana*, *O. syriacum*, *O. virens*, *O. vulgare* subsp. *hirtum* and some hybrids, by using chemogenetic results and male sterility as tools for controlled crossings. Results so far are promising, as shown by the good results obtained in trials made with some new varieties. Further research is, however, still needed, particularly to investigate the species' genetic background and the possible application of biotechnology in this area.

### Introduction

From the users' point of view the genus *Origanum* consists of two main groups:

- 'Oregano' with many different species, mainly collected from the spontaneous flora, very rich in genetic diversity and characterized by high morphological variability. The flavour of these species is generally strong phenolic due to the presence of thymol, carvacrol or a mixture of the two as main compounds in their essential oil (Fleisher and Sneer 1982).
- 'Marjoram' [*Origanum majorana* L. (syn. *Majorana hortensis* Moench.)], species native to Cyprus and the adjacent part of southern Turkey, spontaneous in Mediterranean countries (Ietswaart 1980), but usually cultivated. Its essential oil consists mainly of cis-sabinene-hydrate, flavour ranging from sweet to fruity.

Owing to the extremely large morphological and chemical variability encountered in *Origanum* species, and taking into account the market demand for homogeneous raw material, selection and breeding activities represent an important part of the quality-assurance system.

### Breeding targets

Since almost none or only very few cultivars of oregano and marjoram exist, the first step in breeding these species consists in the proper definition of crop improvement direction and breeding targets. To accomplish this initial task, the breeder has to understand well all those factors involved in users' and producers' demands.

#### Important factors for quality

- Composition of essential oils (in oregano high carvacrol content, in marjoram high cis-sabinene-hydrate content);
- quantity of essential oils (in marjoram more than 2% is desired);
- colour of the dried herb (green is preferred over grey);
- sensorial (vs. analytical) qualities [e.g. *O. majorana*: cis-sabinene hydrate as typically 'marjoramy', terpinen-4-ol as unpleasant, potato-like compound, etc. (Franz 1990); *Origanum syriacum* var. *syriacum* of Mount Sinai: geraniol and esters, ethylcinnamate as "tender desert note" (Fleisher and Fleisher 1991)].

Regarding quality parameters, in *Origanum* there is a very large variability, which represents at the same time a challenge for the breeder in search of homogeneity and an excellent basis for selection activities.

#### Agronomical factors

- Yield of dry matter;
- upright growth (to avoid soil contamination and spoilage of leaves);
- ratio of leaves to stem (of special interest for herb-processing companies);
- quick development of young plants (especially for marjoram which is a slowly establishing crop and is rather weak when facing weed problems);
- resistance to pathogens (for example, *O. majorana* is severely affected by *Alternaria* and *Fusarium*);
- salt and drought tolerance (a much desired trait in Mediterranean areas);
- winterhardiness (desired for biennial/perennial production in Central Europe).

Because of the relevant economic importance of *O. majorana* in Germany, the German Professional Board for Medicinal and Aromatic Plants has indicated precise breeding targets for the improvement of this crop (Pank 1993, 1996; Bundessortenamt 1996):

- ratio of leaf to stem >60%;
- essential oil content >2 ml/100 g dry matter;
- content of cis-sabinenehydrate >45% (after buffered distillation);
- resistance against *Alternaria*;
- homogeneous and high field emergence;
- drought resistance, especially in early summer.

## Methods

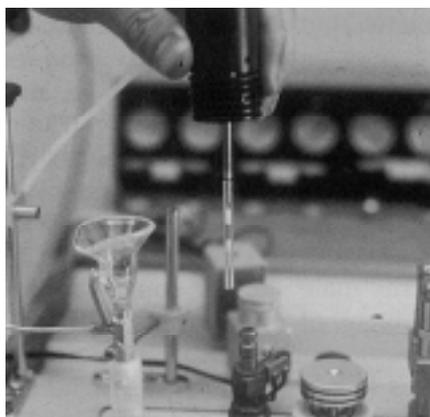
### Analytical and sensorial methods

The most common analytical method used today for determining essential oil composition is gas chromatography (GC). The development of special techniques such as GC, coupled with an olfactorial test (use of a sniffing detector) (Fig. 1) for the examination of single compounds and head-space techniques (Fig. 2) for the analysis of the genuine oil, proved to be very useful (Bicchi and Joulain 1990; Franz 1990; Neuner-Jehle and Etzweiler 1991).

**Fig. 1.** Gas chromatograph equipped with a sniffing detector.



**Fig. 2.** Gas chromatograph equipped with headspace and sniffing detector.



### Biosynthesis

Knowledge on the essential oil compounds biosynthesis and their inheritance is useful for a more effective selection of the breeding method. In *Mentha* the biosynthesis and the inheritance of the essential oil components is known in detail (Croteau and Gershenzon 1994). For the aromatic components of *Thymus* and *Origanum*, only some key enzymes have been identified so far (Poulouse and Croteau 1978; Croteau and Karp 1991). In general, the biosynthesis of monoterpenes is widely known, but not all steps could be connected with the genes encoding for the enzymes. In oregano, many different chemotypes are described such as terpinen-4-ol-type ('marjoram'), thymol-type, carvacrol-type (Fleisher and Sneer 1982), linalool-type (Carmo *et al.* 1989). In marjoram, cis-sabinene-hydrate seems to be responsible for the typical aroma of marjoram, although the exact responsibility of single compounds is still not known (Oberdieck 1981; Fischer *et al.* 1987).

### Selection (positive mass selection)

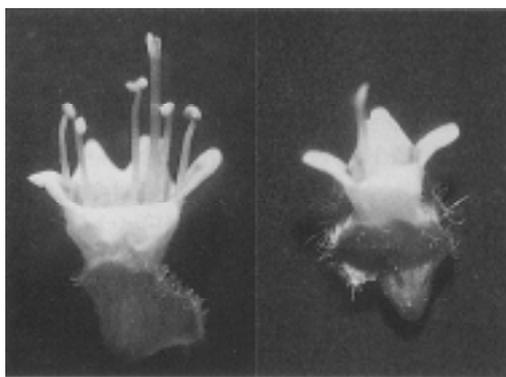
The quantity of essential oils is a trait characterized by high heritability (see the case of chamomile in Massoud and Franz 1990), though this fact has not been yet proven for oregano. The composition of essential oils, on the other hand, is also influenced

by the environment (Voirin *et al.* 1990; Letchamo *et al.* 1994; McGimpsey *et al.* 1994; Circella *et al.* 1995). Taking these facts into account and considering the variability existing within original populations, (positive) mass selection represents the most recommended selection method. This method seems to be also best suited for a rather fast crop improvement in *Origanum*, though for the essential oil content it has been rarely applied on a single-plant basis owing to the lack of fast screening methods using small amounts of plant material.

### Crossings

Naturally occurring hybrids provide important information on the degree of crossability among different species within the genus. Gene flow between species of different sections is also reported (Ietswaart 1980). In the case of the natural *Origanum x intercedens* hybrid (Kokkini and Vokou 1993), an interesting increase in the biomass yield has been detected, thus suggesting a particular economic importance for this plant. In most cases, however, the hybridisation is accompanied by complete sterility and therefore further genetic improvement of the material is not possible. On the other hand, if the hybrid is an exceptionally interesting plant, its economic cultivation could still be possible by using vegetative propagation methods (stem cuttings or *in vitro* techniques).

Controlled crossings by use of flower emasculation techniques are very expensive in *Origanum*, if not completely impossible to make, owing to the extremely small size of the flowers and type of inflorescence (Putievsky, pers. comm.; authors' own observations). The well known gynodioecy in *Origanum* (Appl 1932; Lewis and Crowe 1952; Kaul 1988 and references therein) can give us a tool for controlling crosses (Figs. 3 and 4). Male sterility – well examined in *Origanum vulgare* subsp. *vulgare* (Lewis and Crowe 1952; Kheyr-Pour 1980, 1981) – has a complex genetic background. This male sterility could be used either for heterozygotic breeding with the advantages of higher yields of dry matter and improved homogeneity, or for interspecific hybridisation for combining desired characteristics found in different species (e.g. transferring the winterhardiness of *O. vulgare* subsp. *vulgare* to *O. majorana*: Dzevaltov'skii and Polishchuk 1975) or for creating unusual oil compositions.



**Fig. 3.** Flowers of *Origanum majorana* L.: (left) 'normal'; (right) male sterile.



**Fig. 4.** Crossings made using male sterile flowers.

### Oregano varieties

A number of commercial varieties of oregano exist on the market, many of them used as horticultural crops, particularly suited for rockeries. Table 1 lists cultivars and populations of oregano, with a brief description for each of them.

**Table 1. Cultivars or local varieties of oregano.**

| Name   | Description   |
|--|---|
| <sup>†</sup> Greek Oregano   | Bright green leaves; white flowers. Strong, aromatic spicy flavour. Excellent culinary oregano.   |
| <sup>†</sup> White Oregano   | Culinary type with excellent flavour.   |
| <sup>†</sup> White Anniversary   | Bright green leaves, broadly margined in white. Spring growth in a white ground-hugging mat, changing to a pale cream by autumn.  |
| <sup>‡</sup> Kaliteri Oregano  | Specially selected for its high oil content, this strain is grown commercially in Greece for the high-quality oregano market. Spicy, silver-grey foliage.   |
| <sup>†</sup> Italian Oregano ( <i>O. x majoricum</i> )   | Somewhat narrow leaves. Aromatic, strong, resinous flavour. More bushy and upright than Greek oregano.  |
| <sup>†</sup> Golden Oregano ( <i>O. vulgare</i> subsp. <i>vulgare</i> 'Aureum'; Golden Creeping Oregano) | Compact, creeping habit, to 6 inches high. Attractive golden coloured foliage. Good groundcover for rock gardens and edges of flower beds. Mild, thyme-like oregano flavour.  |
| <sup>†</sup> Silver (Silver Oregano)   | Ornamental silver leaves. Mild oregano flavour. Can be used in cooking. Tender perennial.   |
| <sup>†</sup> Jim Perry's   | Small-leaved, deep green, sweet oregano with excellent flavour.   |
| <sup>†</sup> Seedless Oregano (oregano x marjoram?)  | Leaves resemble sweet marjoram; the flowers resemble a combination of sweet marjoram flowers and oregano flowers. An excellent culinary herb, as strong and distinct as Greek oregano but sweeter and less biting. Hardy to 10°F. Likely to be a natural hybrid between oregano and marjoram. |
| <sup>†</sup> Variegated Oregano  | Attractively streaked with golden variegation that contrasts prominently against the deep green background. Mildly flavoured. Excellent for edging or in the rock garden.   |

<sup>†</sup> Marketed according to Facciola 1990.

<sup>‡</sup> From RICHTER's seed catalogue, 1994.

In addition to this information, it has to be said that several countries have commenced research activities on the genetic improvement of oregano, mainly using indigenous wild material in their programmes. In France, for instance, *O. virens* is the species mainly used; in Israel, *O. vulgare* subsp. *hirtum* and *O. syriacum*, whereas in Greece *O. vulgare* subsp. *hirtum* and *Origanum x intercedens* are preferred.

In marjoram there are two groups of varieties, the 'Knospenmajoran' (flower bud marjoram) or 'German marjoram' and the 'Blattmajoran' (leaf marjoram) or 'French marjoram' (Heeger 1956; Bundessortenamt 1996). In Germany three varieties have been registered so far, namely Francia from Hungary (the oldest one – since 1956 – selected from French marjoram), Miraz from Poland and Marcelka from Czech Republic. All other marjoram material present at the market originates from local varieties (seeds as by-products of herb production). In Germany the following candidate varieties are being tested in official trials according to Heine (1995) (breeder's name in brackets) (see also Fig. 6):

- Typ P (Wagner)
- NLC 91 (Chrestensen)
- NLC 93-56 (Chrestensen)
- NLC 93-58 (Chrestensen)
- (Mauser)
- (Nutting, UK)
- (Sperling).



Fig. 6. Official varietal trials of *Origanum majorana* in Quedlinburg, Germany, 1993.

### Future aspects

Today evaluation work should be focusing on the genetic diversity of *Origanum* species with special regard to their antioxidant (Kizuzaki and Nakatani 1989; Lamaison *et al.* 1990, 1993; Lagouri *et al.* 1993) and antimicrobial (Deans and Svoboda 1990; Yadava and Saini 1991; Biondi *et al.* 1993) properties. In the event that these evaluation activities indicate the presence of a strong genetic basis for such desirable traits, breeding work could then be the right way to reach natural antioxidants or antimicrobial agents of high quality in the material we would like to improve.

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## Flower biology in *Origanum majorana* L.

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### Introduction

The growing interest in underutilized species like oregano has stimulated the present study, which intends to provide new insight into the problems related to the production of oregano seeds. The material used in this work was selected at the University of Wien (Austria) and tested under Mediterranean growing conditions. Seed was characterized by low 1000-seed weight (0.06 g) and a low germination rate (2%). A histological analysis was carried out on flower organs to identify the most critical periods for seed development and ripening and thus better the understanding of the flower biology in these species.

### Materials and methods

The trial was run in 1995-96. The material used consisted of 1-year-old oregano (*Origanum majorana* L.) lines (A2, A6 and A12), selected at the Institut für Botanik und Lebensmittelkunde of the University of Wien, and cultivated in open fields at the Pantanelli Research Station in Policoro (Matera, southern Italy) of the University of Bari. The plants were subject to free pollination.

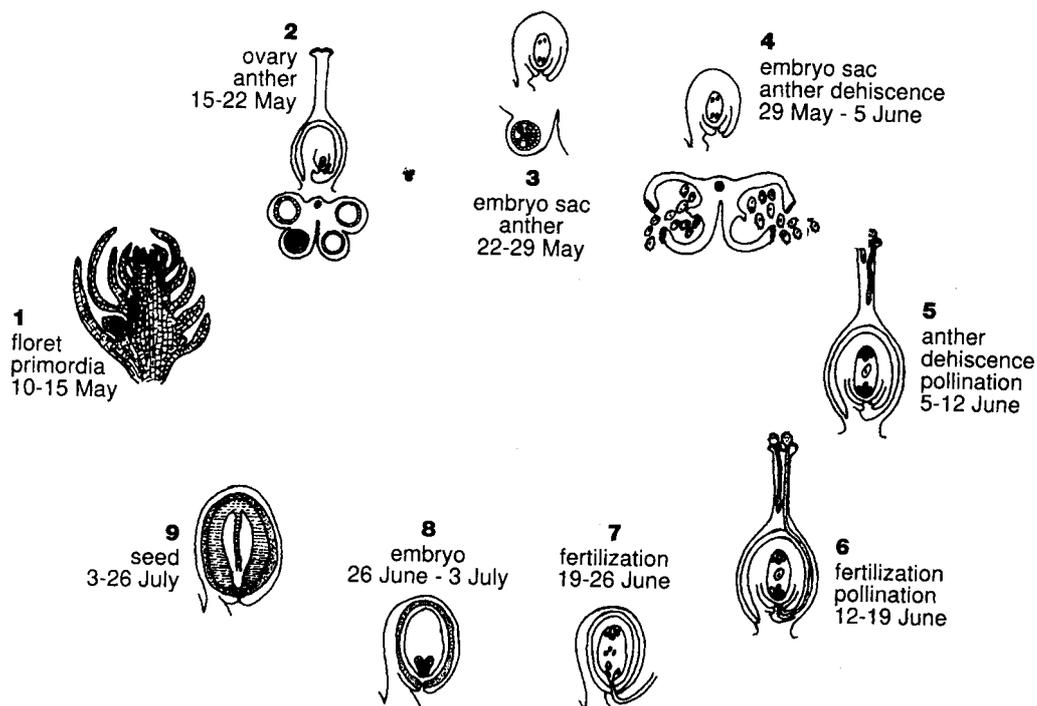
The histological analysis was carried out during the entire period of flower differentiation until seed-formation and ripening stages. Sampling started at the bud stage (on 10 May) and was made every 7 days until full seed-ripening stage (seed harvest took place on 26 July).

In oregano, spikes are terminal and closely spaced, forming more or less elongated racemes (verticillasters). Those terminal spikes that showed two flowers per verticil were sampled in this study. The histological technique employed consisted of sealing the sample with paraffin, cutting it using the sliding microtome, colouring with safranin fast-green, preparing slides and examination with an optical microscope (Johansen 1940; Sass 1958; Cutter 1969; Fahn 1979).

### Results

The rate of development of the various flower organs and time of development of differentiation, pollination, seed fertilization and ripening stages were determined. The times required for the inflorescence development until the seed formation and ripening are shown in Figure 1.

Pollination commenced in early June, followed by fertilization (mid-June) and seed ripening which started and ended in July. Figures 2 to 10 illustrate the most representative conditions of flower differentiation detected during the investigation. Figure 2 shows the evolution from floret primordia to the differentiation of microsporangii with sporogenic tissue, whereas Figs. 3 and 4 show ovaries with anatropous ovules and nucellar tissue, the mother cells of pollen (divided by meiosis) with the tetrad of microspores, the four-loculi and assile placentation ovary. Six-loculi ovaries also were detected during the investigation (Fig. 5).



**Fig. 1.** The diagram shows the inflorescence evolution until formation and ripening of seeds. Pollination starts early in June and continues to fertilization (starting in mid-June) and seed development. Full seed filling occurs in July.

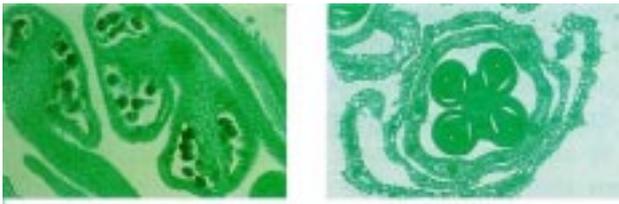
Dehiscence of anthers is displayed in Figure 6 where the embryo sac is also distinguishable in the ovaries. In Figure 7, fertilization is visible and in Figure 8 the first phases of embryo formation (remaining in this condition although ripening continues) can be seen. The seed appear to consist mostly of teguments and contains a globular type of embryo. From a careful look at the results of the investigation the difficulty encountered by the seed in germinating (Fig. 9) can be easily seen. In fact only a few seeds were able to reach a proper ripening stage, becoming well formed and able to germinate (Fig. 10).



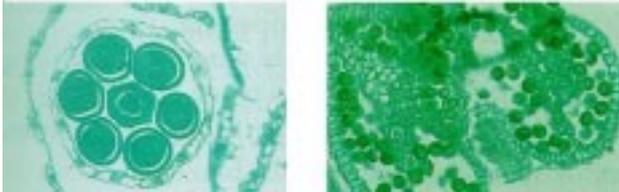
**Fig. 2.** Inflorescence apices (spikelets with floret primordia are visible).



**Fig. 3.** (left) Anther: microsporangia showing esothecium, endothecium, tapetum, sporogenous tissue/pollen mother cells; (right) Ovary: anotropous ovules and nucellus.



**Fig. 4.** (left) Anther: pollen mother cells is dividing itself by meiosis into the tetrad of microspores; (right) Ovary: four cavities/locules with axile type of placentation.



**Fig. 5 (left).** A six-loculi ovary.

**Fig. 6 (right).** Dehiscent anther; pollen grains in the pollen sacs and endothecium.



**Fig. 7.** Fertilization in the embryo sac.



**Fig. 8.** Globular and globular late stages of the embryo.



**Fig. 9 (left).** The seed is unable to complete its development (globular, late globular and torpedo stages of the embryo).

**Fig. 10 (right).** Full seed development.

### Discussion and conclusions

The histological analysis has pointed out the normal development of flower from floret primordia differentiation to gametophyte germination and fertilization. The first stages of seed formation evolve normally but once the globular and/or torpedo stage is reached not all embryos are able to carry on their normal development. This results in the formation of poorly formed seeds that are unable to germinate.

There could be various reasons behind this disturbance in the physiological development of the embryo. Among them the likely cause could be the harsh climatic conditions encountered by the sampled plants in the month of July. It is

known that the storage of reserve material in seeds and their ripening stage are both greatly affected by environmental conditions. Optimal plant water conditions ensure the correct transfer of storage materials to the seeds. If the water balance indicates a deficit due to either soil dryness (decline in uptake) or low air humidity (high transpiration), seed ripening is always abnormal. Indeed, the mobilisation and transfer of photosynthates from the leaves to the storage organs is wholly affected by the plant water status. These inconveniences contribute dramatically to seed malnutrition. In unfavourable water conditions the seed can in fact regress and be unable to complete its dehydration and physiological ripening.

In the case of oregano, even a single day of unfavourable climatic conditions, which are quite common in southern areas of Italy as well as other Mediterranean countries, can halt the correct seed development and jeopardise its proper ripening.

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## Agricultural practices for oregano

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### Abstract

Several agricultural practices were investigated to define the most appropriate for the cultivation of oregano in southern Italy. For growing oregano successfully it is necessary to transplant seedlings obtained in the nursery. In the field, the best plant density is about 8-10 plants/m<sup>2</sup>. Selective herbicides are available for weed control. To obtain a second harvest it is necessary to irrigate during the summer. Highest yields are those obtained in the second year with two cuts (June-July and end of October).

### Introduction

*Origanum* is a subshrub which grows spontaneously throughout the whole Mediterranean area. In many regions of southern Italy it is harvested by hand and sold dry in bunches as a condiment plant. Harvesting is carried out in June.

Given the climatic conditions of southern Italy, the growing period for oregano begins in March and ends in November. In the coldest period, November-February, no growth takes place. Flowering occurs from June to July. After summer cutting, the plant may have vegetative regrowth until October. To ensure this regrowth it is necessary to irrigate the crop during the dry period, particularly from June to September (Fig. 1).

*Origanum* is a long-day plant. The photoperiod does influence the growth of the plant and the floral differentiation. Plants grown under conditions of 16-12 light-hours/day enter the full floral differentiation stage around the sixtieth and the ninetieth day of cultivation, respectively. Plants grown in 12-hour daylength conditions are more vigorous, with a larger leaf area and a greater plant total dry weight (Table 1).

The oil glands' density does not change significantly in different photoperiodic conditions, although an increase in density is observed when moving from short to longer days (e.g. 7.0 to 8.6 and 9.7 glands/mm<sup>2</sup> have been recorded when moving from 12 to 16 light-hours/day (Table 1). The number of oil glands also seems to increase along with the plant growth from 3-5 glandular scales/mm<sup>2</sup> in the very early growth phases to 6-10/mm<sup>2</sup> after 150-180 days of growth (Fig. 2).

### Seedbed preparation, transplanting, plant density

Because *Origanum* seeds are very small (the 1000-seed weight is only 0.20-0.25 g) it is recommended to carry out seedling transplanting. This is done in March-April on seeds planted in October in the seedbed. It is possible to carry out this operation also in autumn; however, as weather remains cold until March, frost damage could easily occur to the young plants. Distances of 50-60 cm between rows are advised, to permit hoeing and mechanical weeding. Plant density influences the yield and the weight of the plant.

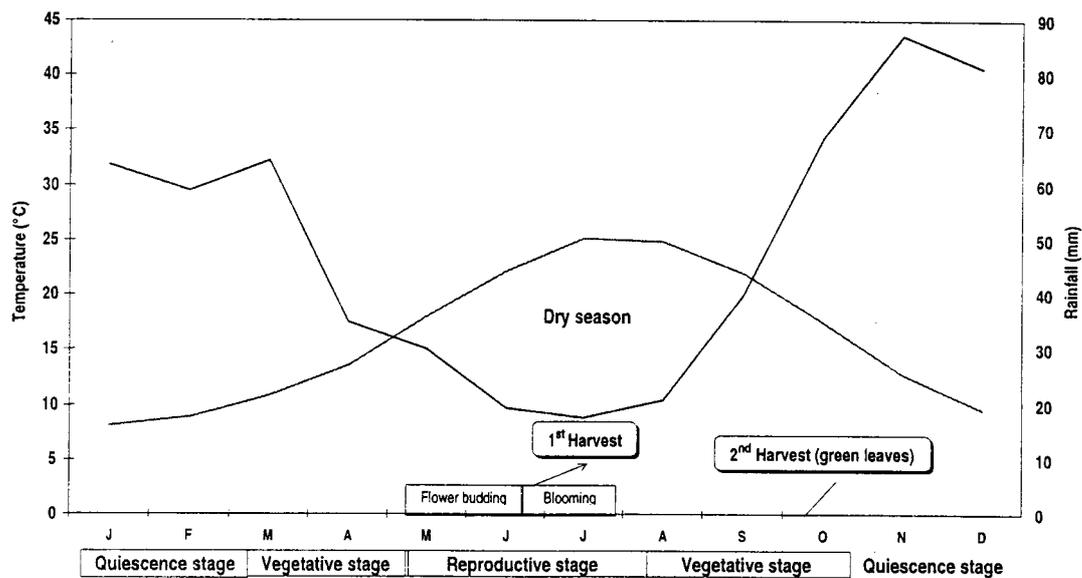


Fig. 1. Physiological stages in relation to the climatic trend.

Table 1. Effect of daylength on *Origanum vulgare* subsp. *hirtum*.

| Day-length<br>(h) | Shoot system   |                   |                |  |  |       |       | Root system    |                |                                  |
|-------------------|----------------|-------------------|----------------|--|--|-------|-------|----------------|----------------|----------------------------------|
|                   | Height<br>(cm) | Dry weight<br>(g) | Nodes<br>(no.) | Leaf surface<br>of plant<br>(cm <sup>2</sup> ) | Oil gland density on the<br>leaf epidermis (no./m <sup>2</sup> ) |       |       | Length<br>(cm) | Dry wt.<br>(g) | Apex differ-<br>entiation<br>(%) |
|                   |                |                   |                |  | upper  | lower | total |                |                |                                  |
| 8                 | 35.1b          | 0.8B              | 22.3b          | 130B   | 3.4  | 3.6   | 7.0   | 16.8b          | 0.1b           | –                                |
| 12                | 60.5ab         | 7.3A              | 27.3ab         | 1376A  | 4.1  | 4.5   | 8.6   | 27.8a          | 0.9a           | 90                               |
| 16                | 73.3a          | 5.2AB             | 31.7a          | 871A   | 4.5  | 5.2   | 9.7   | 16.0b          | 0.4ab          | 60                               |
| Means             | 56.3           | 4.4               | 27.1           | 792  | 4.0  | 4.4   | 8.4   | 20.2           | 0.5            |                                  |

Means with the same letter are not significantly different at  $P=0.05$  (small letters) and at  $P=0.01$  (capital letters).

With regard to the interplant distances in the field, the results of an experiment (randomized block design with four replications) have indicated an increase in plant biomass from 6.3 to 5.6 and 4.7 t/ha when using 40, 60 and 80 cm distances between rows respectively and from 6.3 to 5.3 and 5.0 t/ha when using 20, 30 and 40 cm within row respectively (Table 2). Furthermore, plant weight changes from 350 g to 366 and 403 g with 40, 60 and 80 cm between-row distances and from 306 to 361 and 452 g with 20, 30 and 40 cm within-row distances (Table 3). This explains why by increasing plant density the mean of plant weight decreases (Fig. 3). The best plant density is about 8-10 plants/m<sup>2</sup> (60 x 20 cm or 50 x 20 cm). Number of branches is also influenced by plant density (Table 4). No significant difference is observed, however, in plant height when changing plant density in the field (Table 5).

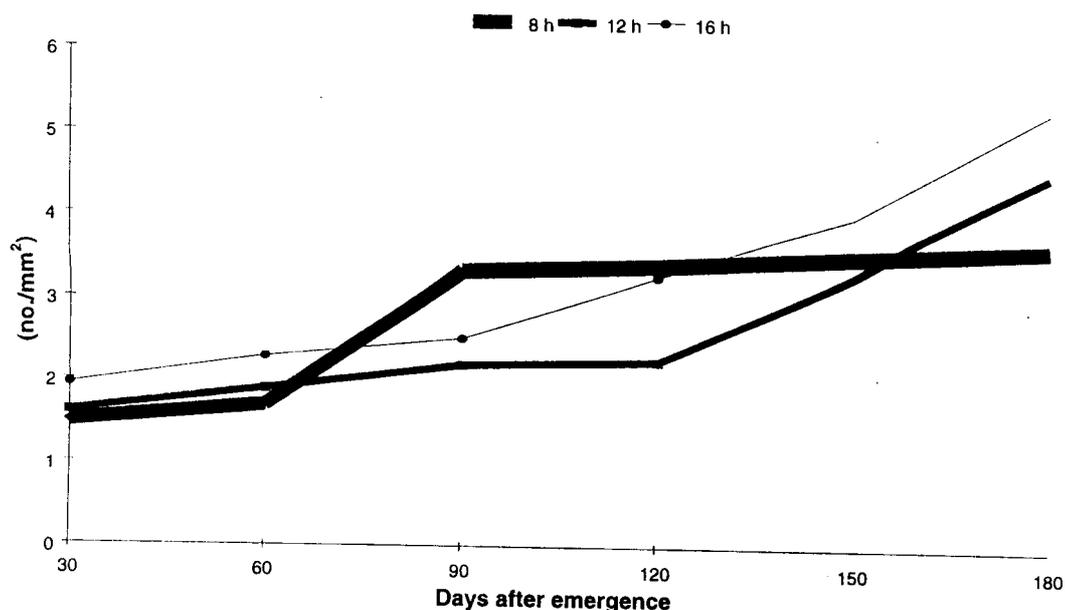


Fig. 2. Trend of oil glands differentiation on lower leaf epidermis in relation to the daylength.

Table 2. Influence of planting distances in *Origanum*.

| Space between rows<br>x within rows (cm) | Plant density<br>(no./m <sup>2</sup> ) | Biomass (t/ha) |          |          |       |
|--|--|----------------|----------|----------|-------|
|  |  | 1st year       | 2nd year | 3rd year | Mean  |
| 40x20                                    | 12.5                                   | 3.0            | 10.4     | 6.9      | 6.8   |
| 40x30                                    | 8.3                                    | 2.7            | 10.5     | 5.6      | 6.3   |
| 40x40                                    | 6.3                                    | 2.4            | 10.3     | 5.2      | 6.0   |
| Mean                                     |  | 2.7            | 10.4     | 5.9      | 6.3 A |
| 60x20                                    | 8.3                                    | 2.8            | 11.0     | 6.1      | 6.6   |
| 60x30                                    | 5.6                                    | 2.0            | 9.1      | 5.6      | 5.6   |
| 60x40                                    | 2.5                                    | 1.9            | 7.1      | 4.7      | 4.6   |
| Mean                                     |  | 2.2            | 9.1      | 5.5      | 5.6 B |
| 80x20                                    | 6.3                                    | 1.9            | 9.6      | 4.9      | 5.5   |
| 80x30                                    | 5.6                                    | 1.2            | 7.3      | 3.6      | 4.0   |
| 80x40                                    | 3.1                                    | 1.1            | 8.0      | 4.3      | 4.5   |
| Mean                                     |  | 1.4            | 8.3      | 4.3      | 4.7 C |
| Mean 20 (within row)                     |  | 2.6            | 10.3     | 6.0      | 6.3 A |
| Mean 30 (within row)                     |  | 2.0            | 9.0      | 4.9      | 5.3 B |
| Mean 40 (within row)                     |  | 1.8            | 8.5      | 4.7      | 5.0 B |
| Mean                                     |  | 2.1 A          | 9.3 B    | 5.2 C    | 5.5   |

Means with the same letter are not significantly different at  $P = 0.01$ .

### Crop cycle

Under favourable climatic conditions, *Origanum* is a perennial crop. The crop lifespan is about 3-4 years, but many factors can influence longevity (e.g. winter frost, disease, number of cuts). Plant losses are often encountered after the second mowing in autumn, whenever cuts have been made very close to the ground and frost is experienced.

**Table 3. Influence of planting distances in *Origanum*.**

| Space between rows<br>x within rows (cm) | Plant density<br>no./m <sup>2</sup> | Mean weight of plant (g) |          |          |       |
|--|-------------------------------------|--------------------------|----------|----------|-------|
|  |                                     | 1st year                 | 2nd year | 3rd year | Mean  |
| 40x20                                    | 12.5                                | 71                       | 162      | 678      | 304   |
| 40x30                                    | 8.3                                 | 102                      | 346      | 571      | 340   |
| 40x40                                    | 6.3                                 | 131                      | 558      | 532      | 407   |
| Mean                                     |                                     | 101                      | 355      | 594      | 350 A |
| 60x20                                    | 8.3                                 | 99                       | 273      | 522      | 298   |
| 60x30                                    | 5.6                                 | 125                      | 219      | 790      | 378   |
| 60x40                                    | 2.5                                 | 147                      | 500      | 618      | 422   |
| Mean                                     |                                     | 124                      | 331      | 643      | 366 B |
| 80x20                                    | 6.3                                 | 103                      | 288      | 557      | 316   |
| 80x30                                    | 5.6                                 | 134                      | 423      | 546      | 368   |
| 80x40                                    | 3.1                                 | 139                      | 726      | 712      | 526   |
| Mean                                     |                                     | 125                      | 479      | 605      | 403 C |
| Mean 20 (within row)                     |                                     | 91                       | 241      | 586      | 306 A |
| Mean 30 (within row)                     |                                     | 119                      | 329      | 636      | 361 B |
| Mean 40 (within row)                     |                                     | 139                      | 595      | 621      | 452 C |
| Mean                                     |                                     | 116 A                    | 338 B    | 614 C    | 373   |

Means with the same letter are not significantly different at  $P = 0.01$ .

**Table 4. Influence of planting distances in *Origanum*.**

| Space between rows<br>x within rows (cm) | Plant density<br>(no./m <sup>2</sup> ) | Branching (no.) |          |          |       |
|--|--|-----------------|----------|----------|-------|
|  |  | 1st year        | 2nd year | 3rd year | Mean  |
| 40x20                                    | 12.5                                   | –               | 78       | 202      | 140   |
| 40x30                                    | 8.3                                    | –               | 129      | 169      | 149   |
| 40x40                                    | 6.3                                    | –               | 141      | 212      | 177   |
| Mean                                     |  |                 | 116      | 194      | 155 A |
| 60x20                                    | 8.3                                    | –               | 122      | 111      | 117   |
| 60x30                                    | 5.6                                    | –               | 137      | 162      | 150   |
| 60x40                                    | 2.5                                    | –               | 110      | 183      | 147   |
| Mean                                     |  |                 | 123      | 152      | 138 A |
| 80x20                                    | 6.3                                    | –               | 137      | 223      | 180   |
| 80x30                                    | 5.6                                    | –               | 112      | 181      | 147   |
| 80x40                                    | 3.1                                    | –               | 154      | 240      | 197   |
| Mean                                     |  |                 | 134      | 215      | 175 B |
| Mean 20 (within row)                     |  |                 | 112      | 179      | 146 A |
| Mean 30 (within row)                     |  |                 | 126      | 171      | 149 A |
| Mean 40 (within row)                     |  |                 | 135      | 212      | 174 B |
| Mean                                     |  |                 | 124 A    | 187 B    | 156   |

Means with the same letter are not significantly different at  $P = 0.01$ .

### Weed control practices

Weed control in a perennial crop such as *Origanum* presents an important constraint, because it is necessary to keep the crop clean all year round. As many winter or summer species infest *Origanum* fields, tilling is therefore frequent, taking place at least two or three times a year (in autumn, spring and summer). Selective herbicides are also available for weed control in post-transplanting: phenmedipham (application rate 1 kg/ha), alloxym-sodium (0.7 kg/ha), etc. Nevertheless, the use of herbicides on aromatic and medicinal plants is not advisable as it is associated with a number of serious drawbacks.

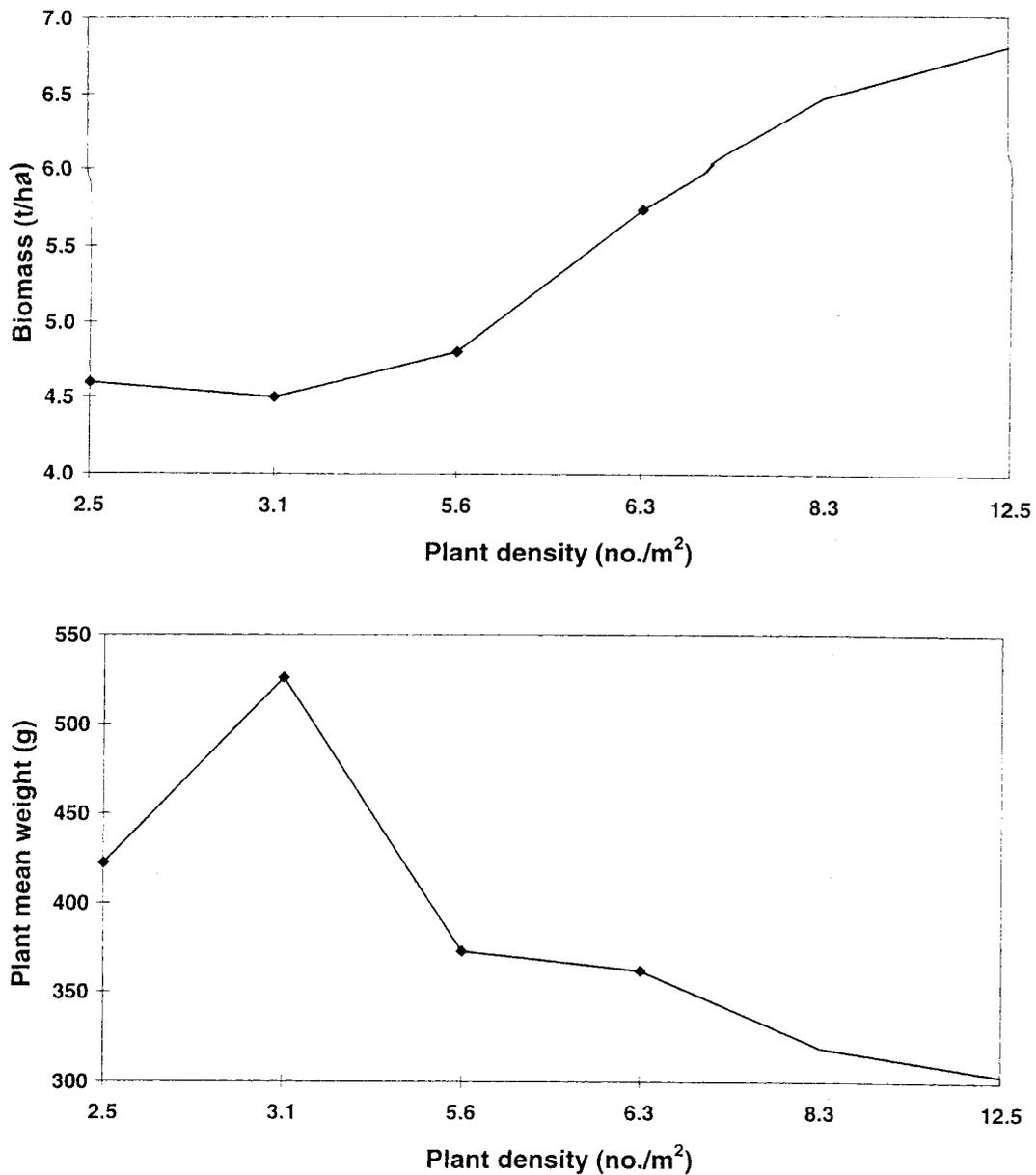


Fig. 3. Influence of plant density on biomass and mean plant weight.

### Irrigation

Normally, *Origanum* is cultivated in dry climatic conditions. Winter rain is usually sufficient for the crop, but to increase the yield and to obtain a second cutting in autumn it is necessary to irrigate during the summer, soon after cutting. It is also advisable to irrigate the crop in spring if there has been no rain over a long period during the winter.

**Table 5. Influence of planting distances in *Origanum*.**

| Space between rows<br>x within rows (cm) | Plant density<br>(no./m <sup>2</sup> ) | Plant height (cm) |          |          |      |
|--|--|-------------------|----------|----------|------|
|  |  | 1st year          | 2nd year | 3rd year | Mean |
| 40x20                                    | 12.5                                   | 32.0              | 62.6     | 60.8     | 51.8 |
| 40x30                                    | 8.3                                    | 30.5              | 51.0     | 58.4     | 46.6 |
| 40x40                                    | 6.3                                    | 28.5              | 42.4     | 59.7     | 43.5 |
| Mean                                     |  | 30.3              | 52.0     | 59.6     | 47.3 |
| 60x20                                    | 8.3                                    | 32.8              | 52.0     | 59.0     | 47.9 |
| 60x30                                    | 5.6                                    | 27.8              | 41.0     | 48.4     | 39.1 |
| 60x40                                    | 2.5                                    | 27.5              | 49.8     | 57.3     | 44.9 |
| Mean                                     |  | 29.4              | 47.6     | 54.9     | 44.0 |
| 80x20                                    | 6.3                                    | 30.8              | 45.4     | 60.1     | 45.4 |
| 80x30                                    | 5.6                                    | 27.5              | 48.2     | 56.0     | 43.9 |
| 80x40                                    | 3.1                                    | 29.3              | 43.8     | 58.2     | 43.8 |
| Mean                                     |  | 29.2              | 45.8     | 58.1     | 44.4 |
| Mean 20 (within row)                     |  | 31.9              | 53.3     | 60.0     | 48.4 |
| Mean 30 (within row)                     |  | 28.6              | 46.7     | 54.3     | 43.2 |
| Mean 40 (within row)                     |  | 28.4              | 45.3     | 58.4     | 44.0 |
| Mean                                     |  | 29.6 A            | 48.4 B   | 57.6 C   | 45.2 |

Means with the same letter are not significantly different at  $P = 0.01$ .

### Harvesting time and yield

*Origanum* is harvested at full blooming for essential oil production or at the beginning of blooming for herb production. Significant differences in yield and oil content have not been observed during the blooming period (Table 6). Oil content in leaves is very low in the October harvest.

Highest yields are obtained in the second year, during the two cuts made in June-July and in October (Table 7). A rough estimate of an average crop production (subject to further verification) indicates a value of 20 t/ha in a 4-year cultivated field.

The harvest index is about 50-55% in the first harvest owing to the high incidence of stems and ca. 60-70% in the second mowing usually done in October.

During the drying process, high temperatures have been found to affect negatively the oil content (Table 8).

**Table 6. Influence of harvesting time on yield and oil content in *Origanum*.**

|                                    | 1st mowing (summer) |        |        | Harvesting time<br>Begin blooming |
|------------------------------------|---------------------|--------|--------|-----------------------------------|
|                                    | Blooming            |        |        |                                   |
|                                    | begin               | full   | end    |                                   |
| Yield                              |                     |        |        |                                   |
| Mean weight of plant (g)           | 237                 | 284    | 253    | 63                                |
| Leaves + inflorescence/plant ratio | 56.2                | 58.7   | 54.1   | 75.2                              |
| Length of flowering branches (cm)  | 20.2                | 18.6   | 18.4   | –                                 |
| Essential oil content              | –                   | –      | –      | –                                 |
| Inflorescences                     | 20.7                | 19.6   | 20.6   | 14.8                              |
| Leaves                             | 16.0                | 13.6   | 13.6   | 10.8                              |
| Stems                              | traces              | traces | traces | traces                            |

**Table 7. Crop duration and yield in *Origanum*.**

|                       | 1st year | 2nd year | 3rd year | Total |
|-----------------------|----------|----------|----------|-------|
| Leaves (t/ha)         | 1.0      | 1.9      | 0.6      | 3.5   |
| Inflorescences (t/ha) | 0.8      | 4.0      | 3.4      | 8.2   |
| Stems (t/ha)          | 0.7      | 4.1      | 2.0      | 6.8   |
| Total plant (t/ha)    | 2.5      | 10.0     | 6.0      | 18.5  |
| Plant height (cm)     | 30       | 48       | 58       |       |
| Mean weight/plant (g) | 120      | 400      | 600      |       |

**Table 8. Influence of drying process temperature on essential oil content in *Origanum* harvests.**

|                  | Control (air temperature, °C) | Temperature (°C) |      |      |     |        |
|------------------|-------------------------------|------------------|------|------|-----|--------|
|                  |                               | 25               | 40   | 60   | 80  | 105    |
| All plants       |                               |                  |      |      |     |        |
| Initial blooming | 10.0                          | 10.0             | 8.0  | 5.5  | 1.5 | traces |
| Final blooming   | 12.5                          | 13.3             | 12.0 | 12.0 | 0.7 | traces |

## Conclusions

*Origanum* subshrubs, spontaneous in many regions of the Mediterranean area, are increasingly becoming a popular cultivated herb for industrial purposes. In the climatic conditions of southern Italy it is a perennial crop (3-4 years), its growing period lasting from March to November. To obtain best harvests, cuts should be carried out twice per year in June and October, but it is necessary to irrigate in summer. In the second cut, oil content of leaves is very low. Plant density influences the yield and the number of branches per plant. The total yield in a 4-year crop has been estimated at about 20 t/ha.

## Suggested reading

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## Bio-agronomical behaviour in Sicilian *Origanum* ecotypes

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### Abstract

The material investigated was collected from 24 sites distributed within the Sicilian Mediterranean maquis and over the western part of the island. The germplasm collection, which is maintained in the field around Villalba (Caltanissetta), includes 214 biotypes belonging to 36 different populations. All sampled accessions have been identified on the basis of their morphological characteristics as *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart (syn. *O. heracleoticum* auct. non L.). The results of these investigations have indicated a large diversity within the examined material. Variability was particularly relevant in regard to several agromorphological traits such as biomass, degree of environmental adaptation and essential oil content.

### Introduction

The most important centre of diversity for the genus *Origanum* is the southeastern Mediterranean basin. In this area about 75% of *Origanum* species are endemic (Ietswaart 1980), *Origanum vulgare* being the most widely distributed taxon.

The etymology of *Origanum* word derives from the Greek *oros* = mountain and *ganos* = ornament, as the species are mostly found in mountainous areas at 400-1800 m asl. A typical characteristic of all *Origanum* species is their high content in essential oil (EO), which is rich in phenolic compounds such as carvacrol and thymol (traces to up to 95%). This trait is often used to characterize oregano 'taxa' into the so-called 'carvacrol-types' or 'thymol-types'.

Other EO compounds are p-cymene and  $\gamma$ -terpinene, considered the precursors of thymol and carvacrol respectively (Granger *et al.* 1964).

Type and quantity of EO compounds are generally subject to remarkable variability depending on the growing area, the growth stage and the part used for oil extraction (Kokkini and Vokou 1989).

### Materials and methods

The trial was carried out to evaluate oregano yield capacity and its qualitative characteristics in local material. Germplasm collecting was carried out for sampling wild populations of *Origanum* species growing in the western part of Sicily.

A germplasm field collection was set up in Villalba, a representative site of the Sicilian hinterland. This collection included 214 biotypes belonging to 36 different populations and originating from 24 sites (Fig. 1). The material was classified, according to morphological traits, as *O. vulgare* subsp. *hirtum* (Link) (syn. *O. heracleoticum* auct. non L.) with the exception of four accessions identified as *O. vulgare* subsp. *vulgare*.



Fig. 1. Collecting sites of *Origanum* species in Sicily.

The vegetatively propagated material was planted in a field using a 100 cm (between rows) x 50 cm (within row) plant density pattern.

A 'starter' fertilization of the fields was carried out supplying 100 kg/ha of  $P_2O_5$  and 100 kg/ha of  $K_2O$ . No irrigation was applied during the trial and weeds were controlled mechanically.

Harvesting was carried out between June and July, according to the earliness of the material, when most plants were at full blooming stage.

Most relevant biometric data related to yield such as dry weight of the inflorescence, leaves, stems and the whole plant, as well as essential oil (EO) content in all plant parts, have been recorded for the most interesting biotypes.

Data obtained have been grouped into size classes. The table below reports the frequencies of biotypes for each of the above-mentioned parameters.

| Character           | Size class | No. of biotypes |
|---------------------|------------|-----------------|
| Inflorescences (%)  | <30        | 25              |
|                     | 30-39.9    | 24              |
|                     | 40-49.9    | 11              |
|                     | >50        | 2               |
| Leaves (%)          | <10        | 25              |
|                     | 10-19.9    | 36              |
|                     | >20        | 1               |
| Oil content (% V/W) | <2         | 17              |
|                     | 2-2.9      | 20              |
|                     | 3-3.9      | 20              |
|                     | >4         | 5               |

Gathered data disclosed the presence in the collection of several interesting biotypes characterized by good qualitative traits, such as high number of

inflorescences/plant and high essential oil content. Among the 62 tested biotypes, 25 showed an inflorescence incidence less than 30% and 13 more than 40%. In almost all biotypes, however, leaf percentage has never been more than 20%. For oil yield, 25 biotypes showed an oil content higher than 3% and 5 biotypes higher than 4%.

The most important phenotypic characteristics of the 62 biotypes are reported in Table 1. Samples have been grouped in the table according to their collecting site, mean height/plant and number of branches/plant. Table 2 shows yield data.

**Table 1. Phenotypic and biometric characteristics of Sicilian *Origanum* biotypes.**

| Collecting site <sup>†</sup> | No. of biotypes | Colour        |             | Height (cm) | No. of branches/plant |
|------------------------------|-----------------|---------------|-------------|-------------|-----------------------|
|                              |                 | Corolla       | Leaves      |             |                       |
| 3                            | 3               | white         | dark green  | 43.8        | 208                   |
|                              | 1               | roseate       | light green | 45.3        | 190                   |
|                              | 2               | white-roseate | light green | 46.7        | 238                   |
| 4                            | 3               | white-violet  | green       | 47.1        | 347                   |
|                              | 3               | white         | green       | 45.1        | 544                   |
|                              | 2               | white         | light green | 45.3        | 456                   |
| 9                            | 2               | white         | light green | 46.6        | 415                   |
|                              | 1               | white         | green       | 46.9        | 595                   |
| 10                           | 1               | white         | light green | 38.0        | 93                    |
|                              | 1               | white-roseate | dark green  | 43.1        | 304                   |
| 11                           | 1               | light rose    | dark green  | 46.5        | 299                   |
| 13                           | 1               | pink          | greenish    | 35.3        | 195                   |
|                              | 1               | white         | light green | 40.9        | 288                   |
| 14                           | 1               | white         | light green | 47.5        | 280                   |
|                              | 1               | roseate       | light green | 47.1        | 498                   |
|                              | 1               | white-roseate | dark green  | 42.2        | 162                   |
| 15                           | 1               | pink          | green       | 42.3        | 365                   |
| 16                           | 1               | white         | dark green  | 47.9        | 370                   |
| 17                           | 1               | roseate       | dark green  | 44.1        | 417                   |
| 18                           | 1               | roseate       | dark green  | 42.4        | 280                   |
|                              | 1               | pink          | dark green  | 41.6        | 87                    |
| 19                           | 1               | white-roseate | dark green  | 51.9        | 305                   |
| 20                           | 1               | white         | green       | 45.0        | 300                   |
| 21                           | 2               | white         | green       | 42.6        | 593                   |
|                              | 6               | white         | dark green  | 41.6        | 322                   |
|                              | 1               | pink          | light green | 41.4        | 105                   |
|                              | 5               | white         | green       | 44.5        | 297                   |
|                              | 1               | pink          | light green | 49.6        | 417                   |
|                              | 7               | white         | green       | 43.7        | 251                   |
|                              | 1               | white         | dark green  | 42.1        | 550                   |
| 22                           | 1               | white         | green       | 37.1        | 325                   |
| 23                           | 2               | white         | green       | 44.3        | 386                   |
|                              | 1               | pink          | green       | 45.4        | 267                   |
|                              | 1               | roseate       | dark green  | 41.6        | 480                   |
| 24                           | 1               | white-roseate | dark green  | 45.3        | 355                   |
|                              | 1               | white         | light green | 47.8        | 420                   |

<sup>†</sup> Numbers refer to Figure 1.

In the field, on average, about 31% of the biotypes showed the highest height and the maximum plant weight (wet and dry weight), about 26% a high number of branches/plant, and about 32% a high essential oil content. Several biotypes showed also an erect growth, which is an important factor for allowing mechanical harvesting.

Table 2. Yield characteristics of Sicilian *Origanum* biotypes.

| Collecting site <sup>†</sup> | No. of biotypes | Weight of whole plant |         | Water content (%) | Inflores.+ leaves plant D.M. | Oil yield (% V/W) |
|------------------------------|-----------------|-----------------------|---------|-------------------|------------------------------|-------------------|
|                              |                 | wet (g)               | dry (g) |                   |                              |                   |
| 3                            | 3               | 1177.1                | 540.0   | 54.1              | 54.2                         | 3.1               |
|                              | 1               | 1086.6                | 510.0   | 53.1              | 32.3                         | 2.9               |
|                              | 2               | 804.5                 | 377.5   | 53.1              | 43.1                         | 1.8               |
| 4                            | 3               | 975.0                 | 396.5   | 57.9              | 55.7                         | 3.2               |
|                              | 3               | 1100.0                | 496.4   | 54.9              | 53.4                         | 3.1               |
|                              | 2               | 1300.0                | 514.3   | 60.4              | 52.3                         | 3.1               |
|                              | 2               | 1160.0                | 483.4   | 58.3              | 51.7                         | 3.4               |
| 9                            | 1               | 1409.6                | 690.0   | 51.0              | 26.5                         | 2.4               |
| 10                           | 1               | 242.6                 | 110.1   | 54.7              | 46.1                         | 3.4               |
|                              | 1               | 1280.4                | 515.0   | 59.8              | 38.3                         | 3.6               |
| 11                           | 1               | 1105.6                | 550.0   | 50.3              | 27.9                         | 1.3               |
| 13                           | 1               | 573.6                 | 240.0   | 50.8              | 32.4                         | 1.7               |
|                              | 1               | 1098.6                | 540.0   | 58.2              | 48.9                         | 3.2               |
| 14                           | 1               | 686.2                 | 330.0   | 51.9              | 31.9                         | 3.4               |
|                              | 1               | 1458.6                | 675.0   | 53.7              | 33.9                         | 2.5               |
|                              | 1               | 325.6                 | 150.0   | 53.9              | 37.5                         | 2.2               |
| 15                           | 1               | 1781.4                | 865.0   | 51.4              | 29.7                         | 1.3               |
| 16                           | 1               | 1204.2                | 540.0   | 55.2              | 33.0                         | 3.9               |
| 17                           | 1               | 1052.6                | 515.0   | 51.1              | 34.2                         | 2.5               |
| 18                           | 1               | 987.5                 | 470.0   | 52.4              | 29.0                         | 1.6               |
|                              | 1               | 475.2                 | 230.0   | 51.6              | 38.7                         | 2.2               |
| 19                           | 1               | 1475.3                | 735.0   | 50.2              | 46.3                         | 1.4               |
| 20                           | 1               | 691.6                 | 325.0   | 53.0              | 47.9                         | 2.8               |
| 21                           | 2               | 1561.6                | 755.0   | 51.6              | 42.3                         | 3.3               |
|                              | 6               | 988.5                 | 433.3   | 56.2              | 50.2                         | 2.8               |
|                              | 1               | 210.2                 | 105.0   | 50.0              | 59.6                         | 4.0               |
|                              | 5               | 823.7                 | 388.0   | 52.9              | 43.5                         | 3.3               |
|                              | 1               | 1752.6                | 740.1   | 57.8              | 52.7                         | 3.3               |
|                              | 7               | 872.4                 | 418.6   | 52.0              | 39.0                         | 2.3               |
|                              | 1               | 737.9                 | 350.0   | 52.6              | 45.8                         | 1.5               |
| 22                           | 1               | 527.7                 | 255.0   | 51.7              | 40.9                         | 1.5               |
| 23                           | 2               | 1295.2                | 612.5   | 52.7              | 33.4                         | 2.5               |
|                              | 1               | 1080.9                | 520.0   | 51.9              | 37.0                         | 1.9               |
|                              | 1               | 956.8                 | 460.0   | 51.9              | 27.2                         | 1.6               |
| 24                           | 1               | 899.6                 | 420.0   | 53.3              | 40.5                         | 1.3               |
|                              | 1               | 2209.5                | 1100.0  | 50.2              | 33.6                         | 1.7               |

<sup>†</sup> Numbers refer to Figure 1.

To evaluate essential oil yields, steam distillation (a very simple method) has been followed in the study. In this method, three types of distillers were used: a standard one (Albrigi) and two 'home made' ones called A-EMI and B-EMI. Results are reported in Table 3.

Table 3. Oil yields (ml/kg DM) of *Origanum* biotypes obtained by steam distillation.

| Compound                  | Distiller type |       |       |
|---------------------------|----------------|-------|-------|
|                           | Albrigi        | A-EMI | B-EMI |
| $\alpha$ -tujene          | 1.84           | 2.6   | 2.8   |
| $\alpha$ -pinene          | 0.18           | 1.3   | 1.5   |
| Other monoterpenes        | 34.5           | 37.5  | 35.8  |
| Thymol + carvacrol (45:1) | 52.3           | 47.1  | 45.0  |
| Sesquiterpenes            | 3.7            | 3.6   | 4.9   |

The separated distillation of inflorescences, leaves and stems of the tested plants showed that EO percentage (ml/kg DM) is higher in the inflorescences (26.3) than

in the leaves (11.3) and that leaves have more carvacrol and thymol precursors than carvacrol and thymol isomers.

The research of biotypes with high inflorescence incidence could therefore be an important aim for future selection work.

*Origanum* oil tests led to the identification of 19 different compounds. Cluster analysis of data obtained identified four clusters (Table 4).

**Table 4. Cluster analysis of *Origanum* biotypes from Sicily.**

| Compound    | Cluster 1 (n=15) |                   |                 | Cluster 2 (n=3) |      |      | Cluster 3 (n=21) |      |      | Cluster 4 (n=11) |      |      |
|-------------|------------------|-------------------|-----------------|-----------------|------|------|------------------|------|------|------------------|------|------|
|             | mean             | med. <sup>†</sup> | CV <sup>†</sup> | mean            | med. | CV   | mean             | med. | CV   | mean             | med. | CV   |
| α-terpinene | 2.4              | 2.5               | 34.6            | 4.0             | 3.9  | 42.2 | 2.1              | 2.1  | 18.9 | 1.9              | 1.8  | 21.9 |
| γ-terpinene | 15.3             | 15.3              | 26.3            | 35.7            | 36.6 | 7.1  | 9.7              | 9.9  | 33.6 | 5.9              | 5.6  | 19.3 |
| p-cymene    | 15.2             | 15.9              | 21.3            | 13.8            | 12.4 | 27.7 | 12.1             | 12.4 | 17.4 | 9.6              | 10.1 | 17.6 |
| thymol      | 44.4             | 44.0              | 9.8             | 28.3            | 29.1 | 25.8 | 56.7             | 56.6 | 4.3  | 63.4             | 63.7 | 3.4  |
| carvacrol   | 0.7              | 0.7               | 52.6            | 0.2             | 0.2  | 46.4 | 0.8              | 0.7  | 72.3 | 1.0              | 1.1  | 44.6 |

<sup>†</sup> CV=coefficient of variation, in %; med.=median.

The first cluster is made of 15 biotypes, the second of 3, the third of 21 and the last of 11. Main differences among clusters depend on concentration of more representative compounds (p-cymene, γ-terpinene and thymol), and ripening period and/or harvesting that in the individual cluster showed the lowest CV. Data disclose the change of thymol content, from 28.3% in the second cluster to 63.4% in the fourth cluster and the opposite course of γ-terpinene, from 5.9% in the fourth cluster to 35.7% in the second; p-cymene, indeed, shows a similar course but less variability.

Having these three components the same carbonic structure, an inter-conversion, depending on a ripening plant stage, could be supposed (Battistutta *et al.* 1995).

To study adaptability behaviour and yield, two fields in two different Sicilian locations were designed and planted with the same material. The fields were in Monreale (site close to Palermo and located at 450 m asl) and Villalba (Caltanissetta, 650 m asl).

Natural rainfall and the applied weed control contributed to the fast growth of the plants. After the first harvest and at the beginning of the autumn rains (mid-September), the plants had their regrowth, reaching the stage of 'stem elongation' in the following year.

In the first year of cultivation, interesting yields were obtained (>2 t/ha), but it was only in the second year that the highest production was eventually achieved in both fields (about 14 t/ha in Villalba and 8.5 t/ha in Monreale).

During the third year of the experiment, a decrease in the production was recorded (31.5% in Monreale and 36.2% in Villalba), followed by an increase in the leaf weight (the inflorescence weight did not increase).

Comparisons of Monreale and Villalba with regard to their EO contents showed no significant differences (Table 5).

**Table 5. Essential oil contents (%) of *Origanum* plants grown at two sites in Sicily.**

| Compound    | Monreale | Villalba |
|-------------|----------|----------|
| thymol      | 54.7     | 58.9     |
| carvacrol   | 1.6      | 1.9      |
| p-cymene    | 11.5     | 11.9     |
| γ-terpinene | 16.4     | 16.9     |
| α-terpinene | 3.8      | 3.9      |
| limonene    | 0.7      | 0.8      |

From the results of this investigation, it appears that Sicilian material is rich in thymol and is characterized by a high percentage of p-cymene and  $\gamma$ -terpinene precursors, although rather poor in carvacrol. It could be said, therefore, that Sicilian oregano could be defined as thymol-rich germplasm.

### Conclusions

The data obtained from this study on oregano Sicilian biotypes are encouraging for the potential application of this material in crop improvement. If we were to pursue this aim, the selection of the best biotypes and the choice of the propagation method would be the first step to take. Cultivation of selected biotypes could be carried out by practical technology. The good essential oil content present in this material could then be used for many purposes, such as antimicrobial additive in food systems, mildew controller in stored foods, antiseptic compound and in cosmetic and hygienic products.

Furthermore, *Origanum* also has the interesting characteristic of being able to grow in marginal environments, thus its erosion-control action due to its rooting apparatus could be used for land preservation in combination with the above-mentioned properties.

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#### **IV. Cultivation and Use in Europe and Northern Africa**

## Some scientific and practical aspects of production and utilization of oregano in central Europe

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### Introduction

The majority of species belonging to the genus *Origanum* L. (Labiatae), and the many others rich in aromatic compounds, have been used as spice for thousands of years. However, new fields of application are being found, on the basis of recent findings, with regard to their antibacterial, antifungicidal, antiviral, nematicidal and antioxidant properties. The term oregano has a very wide meaning commercially: at least 61 species of 17 genera belonging to six botanical families are referred to under this name.

Several efforts (not always successful) have been made to properly classify the genus *Origanum*. According to Flora Europaea, the genus is divided into three sections, i.e. *Origanum*, *Majorana* and *Amaracus*. All three sections were revised by Letswaart (1980), who identified as a whole 38 species and 17 taxa of hybrid origin.

In Hungary, botanists have been carrying out several investigations to gain better knowledge on the intraspecific variation within *Origanum* species. These studies have led to the classification of *Origanum vulgare* into three subspecies: *vulgare*, *prismaticum* and *barcense*. Furthermore, 13 taxa belonging to lower ranks (varietas, forma, lusus) also have been described.

However, in spite of efforts made to better classify *Origanum*, it is felt that further work, possibly better coordinated, is desirable, considering the existence of contradictory data from morphological and chemical evaluations by various workers.

According to the literature, the genus *Origanum* is one of the most studied groups of plants of the Labiatae family with regard to chemical compound contents. Results from these investigations diverge ostensibly from those obtained in botanical studies as far as the species classification is concerned. A clear indication of this situation can be gathered, for instance, in the evaluation of accumulation level of carvacrol, thymol, p-cymene and  $\gamma$ -terpinene compounds.

In central European countries, especially in Hungary, the cultivation of *O. majorana* (syn. *Majorana hortensis* Mönch.) has a long tradition. Commercial material of oregano (*O. vulgare*) is partially collected from wild plants even today. To avoid the disadvantages of exploiting oregano directly from the wild, efforts have been made in the area of its domestication and cultivation. The selection of new cultivars is underway and the material already selected is characterized by 0.5-1.5% oil content containing carvacrol and thymol as the main compounds.

## Importance of oregano

### Utilization as a spice

Records on the use of oregano date back thousands of years: the famous 'hyssop', mentioned in the Bible, is believed to be an *Origanum syriacum* L. plant (Hepper 1987). Today, oregano plant parts and biochemical extracts (herb, leaf, essential oil, etc.) are commonly used in the food industry as a spice. In spite of its long European history, oregano was only introduced into America at the beginning of this century, and afterward to other parts of the world. Today oregano can be considered one of the most important spices both in Mediterranean countries (Carmo *et al.* 1989; Baser *et al.* 1992, 1993; Vokou *et al.* 1988, 1993b) and elsewhere (Anon. 1985; International Organization for Standardization 1985; Bernáth 1993). The overall market of oregano is about 350-500 t in France, 600 t in Germany, 500 t in the UK and 150 t in the Netherlands (Maftai 1992).

The popularity of oregano is increasingly growing as a result of scientific developments achieved in the area of its cultivation and utilization. More and more new interesting varieties are being produced, thus contributing to broadening the horizon of its actual application.

### Antibacterial and fungicide activity

The antibacterial and fungicidal activity of oregano has been reported in many works: its oil has a bactericidal effect against *Erwinia amylovora* (Scortichini and Rossi 1989, 1993) and many other microorganisms such as *Bacillus subtilis*, *Escherichia coli*, *Hafnia alvei*, *Micrococcus luteus*, *Proteus vulgaris*, *Staphylococcus aureus* and *Streptococcus faecalis* (Biondi *et al.* 1993). Research findings also report inhibiting effects on the growth of some plant and animal pathogens, as well as organisms causing food spoilage (Deans and Svoboda 1990; Deans *et al.* 1992; Izzo *et al.* 1995). On honeybee (*Apis mellifera*) pathogens, the bactericidal and fungicide effects of oregano extracts are reported for *Bacillus larvae* (causing American foulbrood), *Ascospaera apis* (causing chalkbrood) and *Bacillus alvei* (a secondary pathogen involved in European foulbrood). These findings, confirmed by Calderone *et al.* (1994), highlight the important role that oregano can also play in the management of honeybee diseases.

Essential oils present in oregano are active against the growth of the fungi *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus ochraceus*, as well as against the bacteria *Campylobacter jejuni* and *Clostridium sporogenes* (Paster *et al.* 1990, 1995; Shaaya *et al.* 1991) and *Clostridium botulinum* (Ismail 1988). The same property was found against *Phytophthora citrophthora*, *Phoma tracheiphila* (syn. *Deuterophoma tracheiphila*) and *Pseudomonas syringae* (Arras 1988). With regard to this latter activity, the high carvacrol content of oregano was found to play a major role (Colin *et al.* 1989). *Origanum syriacum* was found to be effective in controlling a number of pathogens such as *Fusarium oxysporum*, *Macrophomina phaseolina*, *Botrytis cinerea* and *Exserohilum turcicum* (*Setosphaeria turcica*) (Shimoni *et al.* 1993), whereas *O. compactum* is active against spore germination, mycelial elongation and sporulation of *Aspergillus niger*, *Penicillium italicum* and *Zygorrhynchus* sp. (Tantaoui-Elaraki *et al.* 1993).

The antibacterial/fungicidal activity of oregano oil is used today in a number of practical applications. An interesting example is provided by the following case: an aerosol formulation of its extracts (containing 2% propolis and 0.4% mixture of essential oils) was sprayed into three enclosed areas (school hall, analytical

laboratory and library) and a drastic reduction of the microorganisms present in the air was registered (a drop of 74-93%) (Panizzi and Pinzauti 1989).

#### **Antiviral activity**

Ethanollic extracts of *O. vulgare* proved to be active against ECHO9 Hill virus too, in culture of monkey kidney cells, by inducing the formation of a substance with interferon-like activity (Skwarek *et al.* 1994).

#### **Nematicidal activity**

In this area, interesting and promising results have been recorded with leaf extract of *O. vulgare*. The toxicity of the preparation was dependent on concentration and duration of treatment (Ramraj *et al.* 1991). Similar findings were made by Abd-Elgawad and Omer (1995). The main compounds involved in these activities are carvone, p-cymene, terpinen-4-ol and carvone.

#### **Biocide activity**

*Origanum vulgare* biocides (terpenoids, flavonoids, essential oils and tannins) were compared with the activity of synthetic pesticides used at low levels. The LD50 for the diazinon and 2-4 D pesticides was 300-400 and 300-1000 mg/kg, respectively, whereas for the oregano biocide compound (carvacrol) the LD50 was 810 mg/kg (Duke *et al.* 1992). Extracts from *O. vulgare* also have been tested for controlling *Acarapis woodi* pest on bees (*Apis mellifera*).

It is interesting to report here that farmers in southern France traditionally use insecticidal plants to control an important pest, *Acanthoscelides obtectus*, in stored kidney beans (*Phaseolus vulgaris*), and oregano is one of the plants used in this practice. Laboratory trials carried out with a range of dried plants, to confirm these insecticidal properties, have shown significant differences: plants belonging to the Labiatae family provided the best direct and indirect insecticidal effects, *O. serpyllum* being the most effective species (Regnault-Roger and Hamraoui 1993).

#### **Anti-oxidant activity**

The anti-oxidant activity of natural sources, including *Origanum* species, has been recently investigated (Dorofeev *et al.* 1989; Nguyen *et al.* 1991; Sawabe and Okamoto 1994; Takacsova *et al.* 1995). Lagouri *et al.* (1993) have demonstrated the anti-oxidant activity of *O. vulgare* subsp. *hirtum* (Greek oregano), *O. onites* (Turkish oregano), *Coridothymus capitatus* (Spanish oregano) and *Satureja thymbra*. Results from these findings indicate that the anti-oxidant effect may be related to the presence of carvacrol and thymol in the essential oils. Other chemical compounds such as terpenoids may be responsible for the anti-oxidant activity; this is the case of glucosides (Nakatani and Kikuzaki 1987) or flavonoids (Vekiari *et al.* 1993), both extracted from the leaves of oregano. Five phenolic acids with anti-oxidant properties were isolated by Kikuzaki and Nakatani (1989), their activity being greater than the one shown by alpha-tocopherol.

#### **Regulation of growth**

Growth-suppressant properties of Labiatae essential oils, including *O. onites* and *O. vulgare* subsp. *hirtum*, were studied by Vokou *et al.* (1993a). Results indicated that oregano oil had, however, no suppression activity on potato growth, although it showed strong antimicrobial activities against *Erwinia carotovora* strains and other bacteria responsible for crop damage during storage.

### Evidence of plant material

The commercial name of oregano has a very wide meaning and even nowadays refers to at least two large groups of spices, namely European and Mexican oregano (Fleisher and Sneer 1982; Lawrence and Reynolds 1984). The situation is a much more complicated one when we investigate the term 'oregano' from a taxonomic point of view, as in this case the name is used to refer to different spices of diverse botanical origin. The most frequent terms are listed in Table 1. Literature searches have found at least 61 species of 17 genera belonging to six families mentioned under the name oregano. The family Labiatae is considered to be the most important group containing the genus *Origanum* which provides the source of well-known oregano spices – Turkish and Greek types. Two genera of the Verbenaceae family (*Lanata* and *Lippia*) are used for production of oregano herbs, especially in America (Mexican oregano). The other families (Rubiaceae, Scrophulariaceae, Apiaceae and Asteraceae) have a restricted importance. However, we frequently encounter the herbs of the above-mentioned families under the name of oregano in the market (oregano, origanum, oregano de Cartagena, oregano del monte, oregano del campo).

**Table 1. Species used commercially in the world as oregano.**

| Family   | Species   | Commercial name/s found in literature  |
|----------|---|--|
| Labiatae | <i>Calamintha potosina</i> Schaf.   | oregano de la sierra, oregano, origanum  |
|          | <i>Coleus amboinicus</i> Lour. (syn. <i>C. aromaticus</i> Benth)  | oregano, oregano brujo, oregano de Cartagena, oregano de Espana, oregano Frances |
|          | <i>Coleus aromaticus</i> Benth.   | oregano de Espana, oregano, origanum   |
|          | <i>Hedeoma floribunda</i> Standl.   | oregano, origanum  |
|          | <i>Hedeoma incona</i> Torr.   | Oregano  |
|          | <i>Hedeoma patens</i> Jones   | oregano, origanum  |
|          | <i>Hyptis albida</i> H.B.K.   | oregano, origanum  |
|          | <i>Hyptis americana</i> (Aubl.) Urb. ( <i>H. gonocephala</i> Gris.)   | oregano  |
|          | <i>Hyptis capitata</i> Jacq.  | oregano, origanum  |
|          | <i>Hyptis pectinata</i> Poit.   | oregano, origanum  |
|          | <i>Hyptis suaveolens</i> (L.) Poit.   | oregano, oregano cimarron, origanum  |
|          | <i>Monarda austromontana</i> Epling   | oregano, origanum  |
|          | <i>Ocimum basilicum</i> L.  | oregano, origanum  |
|          | <i>Origanum compactum</i> Benth. (syn. <i>O. glandulosum</i> Salzm, ex Benth.)  | oregano, origanum  |
|          | <i>Origanum dictamnus</i> L. ( <i>Majorana dictamnus</i> L.)  | oregano, origanum  |
|          | <i>Origanum elongatum</i> (Bonnet) Emberger et Maire  | oregano, origanum  |
|          | <i>Origanum floribundum</i> Munby ( <i>O. cinereum</i> Noe)   | oregano, origanum  |
|          | <i>Origanum grosii</i> Pau et Font Quer ex letsvaart  | oregano, origanum  |
|          | <i>Origanum majorana</i> L.   | oregano  |
|          | <i>Origanum microphyllum</i> (Benth) Vogel  | oregano, origanum  |
|          | <i>Origanum onites</i> L. (syn. <i>O. smyrneum</i> L.)  | * Turkish oregano, oregano, origanum   |
|          | <i>Origanum scabrum</i> Boiss et Heldr (syn. <i>O. pulchrum</i> Boiss et Heldr.)  | oregano, origanum  |
|          | <i>Origanum syriacum</i> L. var. <i>syriacum</i> (syn. <i>O. maru</i> L.)   | oregano, origanum  |
|          | <i>Origanum vulgare</i> L. subsp. <i>gracile</i> (Koch) letsvaart (syn. <i>O. gracile</i> Koch, <i>O. tyttanthum</i> Gontscharov) | oregano, origanum  |
|          | <i>Origanum vulgare</i> subsp. <i>hirtum</i> (Link) letsvaart (syn. <i>O. hirtum</i> link)  | oregano, origanum  |

| Family           | Species  | Commercial name/s found in literature                              |
|------------------|--|--|
|                  | <i>Origanum vulgare</i> subsp. <i>virens</i><br>(Hoffmanns et Link) letsvaart<br>(syn. <i>O. virens</i> Hoffmanns et Link)           | oregano, origanum, oregano verde                                   |
|                  | <i>Origanum vulgare</i> subsp. <i>viride</i><br>(Boiss.) Hayek (syn. <i>O. viride</i> )<br>Halacsy (syn. <i>O. heracleoticum</i> L.) | * Greek oregano, oregano, origanum                                 |
|                  | <i>Origanum vulgare</i> L. subsp. <i>vulgare</i><br>(syn. <i>Thymus origanum</i> (L.) Kuntze)  | oregano, origanum  |
|                  | <i>Origanum vulgare</i> L.   | oregano, orenga, Oregano de Espana                                 |
|                  | <i>Poliomintha longiflora</i> Gray   | oregano  |
|                  | <i>Salvia</i> sp.  | oregano  |
|                  | <i>Satureja thymbra</i> L.   | oregano cabruno, oregano, origanum                                 |
|                  | <i>Thymus capitatus</i> (L.) Hoffmanns et<br>Link (syn. <i>Coridothymus capitatus</i><br>(L.) Rchb.f.)                               | * Spanish oregano, oregano, origanum                               |
| Verbenaceae      | <i>Lantana citrosa</i> (Small) Modenke   | oregano xiu, oregano, origanum                                     |
|                  | <i>Lantana glandulosissima</i> Hayek   | oregano xiu, oregano silvestre, oregano, origanum                  |
|                  | <i>Lantana hirsuta</i> Mart. et Gall.  | oreganillo del monte, oregano, origanum                            |
|                  | <i>Lantana involucrata</i> L.  | oregano, oregano, origanum   |
|                  | <i>Lantana purpurea</i> (Jacq.)<br>Benth.&Hook. (syn. <i>Lippia</i><br><i>purpurea</i> Jacq.)  | oregano, oregano, origanum   |
|                  | <i>Lantana trifolia</i> L.   | oregano, oregano, origanum   |
|                  | <i>Lantana velutina</i> Mart. & Gal.   | oregano xiu, oregano, origanum                                     |
|                  | <i>Lippia myriocephala</i> Schlecht. & Cham.   | oreganillo   |
|                  | <i>Lippia affinis</i> Schau.   | oregano  |
|                  | <i>Lippia alba</i> (Mill) N.E. Br. (syn.<br><i>L. involucrata</i> L.)  | oregano, origanum  |
|                  | <i>Lippia Berlandieri</i> Schau.   | oregano  |
|                  | <i>Lippia cardiostegia</i> Benth.  | oreganillo, oregano montes, oregano, origanum                      |
|                  | <i>Lippia formosa</i> T.S.Brandeg.   | oregano, oregano, origanum   |
|                  | <i>Lippia geisseana</i> (R.A.Phil.)Soler.  | oregano, oregano, origanum   |
|                  | <i>Lippia graveolens</i> H.B.K.  | * Mexican oregano, oregano, oregano cimarron,<br>oregano, origanum |
|                  | <i>Lippia helleri</i> Britton  | oregano del pais, oregano, origanum                                |
|                  | <i>Lippia micromera</i> Schau.   | oregano, oregano del pais, oregano, origanum                       |
|                  | <i>Lippia micromera</i> var. <i>helleri</i> (Britton)<br>Moldenke  | oregano  |
|                  | <i>Lippia organoides</i> H.B.K.  | oregano, oregano del pais,   |
|                  | <i>Lippia palmeri</i> var. <i>spicata</i> Rose   | oregano  |
|                  | <i>Lippia palmeri</i> Wats.  | oregano, origanum  |
|                  | <i>Lippia umbellata</i> Cav.   | oreganillo, oregano montes, oregano, origanum                      |
|                  | <i>Lippia velutina</i> Mart. et Galeotti   | oregano, origanum  |
| Rubiaceae        | <i>Borreria</i> sp.  | oreganos, oregano, origanum  |
| Scrophulariaceae | <i>Limnophila stolonifera</i> (Blanco) Merr.   | oregano, oregano, origanum   |
| Apiaceae         | <i>Eryngium foetidum</i> L.  | oregano de Cartagena, oregano, origanum                            |
| Asteraceae       | <i>Coleosanthus veronicaefolius</i> H.B.K.   | oregano del cerro, oregano del monte, oregano<br>del campo         |
|                  | <i>Eupatorium macrophyllum</i> L. (syn.<br><i>Hebeclinium macrophyllum</i> DC.)  | oregano, origanum  |

\* Species of main economic importance according to Lawrence and Reynolds (1984).

### Botanical and chemical aspects of *Origanum* genus and its occurrence in European flora

#### Some botanical aspects

There have been many efforts – not all successful – to properly classify the genetic diversity within the genus *Origanum*. The wide distribution of its species and their high morphological variability makes this a rather difficult task. In many cases, the same species are being called by different names, or with wrong synonyms. The classification system for *Origanum* used in Flora Europaea is given in Table 2.

Table 2. Classification of *Origanum* L. according to the Flora Europaea (Tutin 1972).

| Section                           | Species  | Subspecies                                    | Distribution                                  |
|-----------------------------------|--|---|---|
| <i>Origanum</i>                   | <i>Origanum compactum</i> Benth.   |   | SW Spain                                      |
|                                   | <i>Origanum heracleoticum</i> L. ( <i>O. hirtum</i> Link)  |   | SE Europe, from Sardinia to the Aegean region |
|                                   | <i>Origanum vulgare</i> L. [syns. <i>O. dilatatum</i> Klokov, <i>O. vulgare</i> subsp. <i>viride</i> (Boiss.) Hayek] |   | Most of Europe                                |
|                                   | <i>Origanum virens</i> Hoffmanns   |   | SW Europe                                     |
| <i>Majorana</i> (Miller) T. Vogel | <i>Origanum majorana</i> L. (syn. <i>M. hortensis</i> Moench)  |   | S Europe                                      |
|                                   | <i>Origanum dubium</i> Boiss.  |   | E Mediterranean                               |
|                                   | <i>Origanum onites</i> L.  |   | Mediterranean                                 |
|                                   | <i>Origanum microphyllum</i> (Bentham) Boiss.  |   | Crete   |
|                                   | <i>Origanum x minoanum</i> P.H.Davis   |   | Crete   |
| <i>Amaracus</i> Bentham           | <i>Origanum dictamnus</i> L. [syn. <i>Amaracus dictamnus</i> (L.) Bentham]   |   | Crete   |
|                                   | <i>Origanum tournefortii</i> Aiton [syn. <i>Amaracus tournefortii</i> (Aiton) Bentham]                               |   | Kikladhes, Crete                              |
|                                   | <i>Origanum scabrum</i> Boiss et Heldr. [syn. <i>Amaracus scaber</i> (Boiss. et Heldr.) Briq.]                       | <i>scabrum</i>                                | Mountains of S Greece                         |
|                                   |  | <i>pulchrum</i> (Boiss. et Heldr.) P.H. Davis | Mountains of S Greece                         |
|                                   | <i>Origanum liriium</i> Heldr. ex Halácsy [syn. <i>Amaracus liriium</i> (Heldr. ex Halácsy) Hayek]                   |   | Mountains of S Greece                         |
|                                   | <i>Origanum vetteri</i> Briq. et W. Barbey [ <i>Amaracus vetteri</i> (Briq. et W. Barbey) Hayek]                     |   | Karpathos                                     |
|                                   |  |   |   |

According to this system, the genus is divided into three sections: *Origanum*, *Majorana* and *Amaracus*. It appears that the *Origanum* section contains a number of unclear, if not ambiguous, statements. Also in the case of the other two sections, incorrect literature references have been noted. It has been noted, for instance, that *O. majorana* is often mixed up with the other members of the former *Amaracus* genus.

The classification of the *Origanum* genus was revised by Letswaart in 1980. In this work, 38 species and 17 taxa of hybrid origin were proposed. A satisfactory classification of the genus has not been achieved to date and continued efforts are therefore needed to harmonise results obtained by different research groups. Botanists in different countries have always been stimulated by the diversity encountered in *Origanum* and have deployed considerable efforts in trying to better classify it (Soó 1968). Hungarian experts, for instance, have classified *O. vulgare* into three subgroups, namely subsp. *vulgare*, subsp. *prismaticum* and subsp. *barcense* (Table 3). Furthermore, subsp. *vulgare* (var. *vulgare*) is divided into five taxa at the form level, namely f. *vulgare*, *latebracteatum*, *thymiflorum*, *semiglaucum* and *procumbens*. Subspecies *prismaticum* was found to be rather homogeneous, whereas subsp. *barcense* showed a richer diversity, thus requiring further groupings into

three forms (*pilosiusculum*, *grecescui* and *chlorescens*) and four *lusus*, which differ in flower colour (*albiflorum*, *roseum*, *carneum* and *chlorophyllum*). However, the intraspecific classification of *O. vulgare* still does contain many contradictory points and certainly needs further attention. Any future work in this regard should aim at combining results from both chemical and morphological investigations in order to reach a final classification consistent with data originating in these two major research areas, both very relevant for the taxonomy of *Origanum*.

**Table 3. Intraspecific classification of *Origanum* L. according to the Hungarian Flora (Soó 1968).**

| Subspecies   | Variety   | Forma   | Lusus   |
|--|---|---|---|
| <i>vulgare</i>   | <i>vulgare</i>  | – <i>vulgare</i>  |   |
| [syns. <i>O. euvulgare</i><br>Hay., <i>O. genuinum</i><br>Gaud.]                       | [syns. <i>O. bracteosum</i><br>Peters.,<br><i>O. glabrescens</i> Beck.] | – <i>latebracteatum</i> Beck<br>– <i>thymiflorum</i> Rchb.<br>– <i>semiglaucum</i> Boiss.<br>– <i>procumbens</i> Jakucs       |   |
| <i>prismaticum</i> Gaud<br>[syn. <i>O. vulgare</i> var.<br><i>megastachyum</i> Koch]   |   |   |   |
| <i>barcense</i> (Simk.) Jáv.<br>[syn. <i>O. vulgare</i> var.<br><i>puberulum</i> Beck] |   | – <i>pilosiusculum</i> Borhidi<br>– <i>grecescui</i> soó [syn.<br><i>O. macrostachum</i> Grec.]<br>– <i>chlorescens</i> Simk. |   |
|  |   |   | – <i>albiflorum</i> Schur<br>[syn. <i>O. pallidum</i><br>Beckhaus]<br>– <i>roseum</i> Beckhaus<br>– <i>carneum</i><br>Beckhaus<br>– <i>chlorophyllum</i><br>Borb. |

### Chemical diversity of the genus

A review of published data on *Origanum* indicates that this genus has been the object of accurate investigations with regard to its chemical contents. Particularly during the second half of the 1980s, a great amount of scientific work was published in this field. Though it is rather difficult to make a global assessment of these works owing to the botanical inaccuracies of many authors for the material used, such an attempt has been made and the results are given in Table 4. It is obvious that *O. vulgare* and its subspecies are the most frequently analysed material. The majority of plants belonging to this species are characterized by carvacrol, thymol, p-cymene and  $\gamma$ -terpinene as the main compounds, although some exceptions to this have also been reported (Marczal and Vincze-Vermes 1973; Sezik *et al.* 1993). *Origanum onites*, *O. syriacum* and *O. heracleoticum* also have attracted the interest of many scientists. Chemical variability among these species is characterized by a relevant presence of carvacrol, thymol or linalool essential oils, depending on the origin of the analysed material; however, it should be borne in mind that such results are in fact very much dependent on the type of extraction method used for separation of the volatile compounds (Németh *et al.* 1995).

Table 4. Main chemical compounds found in *Origanum* species.

| Species                                 | Compounds   | Reference                    |
|---|---|------------------------------|
| <i>bivani</i>                           | carvacrol, thymol   | Hoppe 1958                   |
| <i>compactum</i>                        | carvacrol, $\gamma$ -terpinene, terpinen-4-ol, $\alpha$ -terpineol, p-cymene, carvacryl methyl ether, thymol, | Benjilali <i>et al.</i> 1986 |
| <i>compactum</i>                        | carvacrol, thymol, p-cymene   | Lawrence and Reynolds 1984   |
| <i>cordifolium</i>                      | carvacrol, $\alpha$ -terpineol, trans- and cis-nerolidol, menthyl acetate,                                    | Valentini <i>et al.</i> 1991 |
| <i>dictamnus</i>                        | carvacrol, $\alpha$ -pinene, $\beta$ -pinene, myrcene, limonene, p-cymene, thymol, linalool, terpin-1-en-4-ol | Lawrence and Reynolds 1984   |
| <i>dictamnus</i>                        | carvacrol, $\gamma$ -terpinene, p-cymene, caryophyllene, borneol, terpin-1-en-4-ol, carvacrol methyl ether    | Harvala <i>et al.</i> 1987   |
| <i>dubium</i>                           | carvacrol, $\gamma$ -terpinene  | Arnold <i>et al.</i> 1993    |
| <i>dubium</i>                           | 1,8-cineole, linalool and camphor   | Souleles 1991                |
| <i>dubium</i>                           | carvacrol, thymol   | Hoppe 1958                   |
| <i>elongatum</i>                        | carvacrol, $\gamma$ -terpinene, terpinen-4-ol, $\alpha$ -terpineol, p-cymene, carvacryl methyl ether, thymol  | Benjilali <i>et al.</i> 1986 |
| <i>floribundum</i>                      | carvacrol, thymol   | Hoppe 1958                   |
| <i>heracleoticum</i>                    | carvacrol, $\gamma$ -terpinene, p-cymene, thymol  | Akguel and Bayrak 1987       |
| <i>heracleoticum</i>                    | carvacrol, $\gamma$ -terpinene, p-cymene  | Fleisher and Sneer 1982      |
| <i>heracleoticum</i>                    | thymol, p-cymene, $\gamma$ -terpinene   | Fleisher and Sneer 1982      |
| <i>heracleoticum</i>                    | thymol, terpinen-4-ol, $\gamma$ -terpinene  | Fleisher and Sneer 1982      |
| <i>hirtum</i>                           | carvacrol, terpene, p-cymole  | Hoppe 1958                   |
| <i>hypericifolium</i>                   | carvacrol, p-cymene, $\gamma$ -terpinene  | Baser <i>et al.</i> 1994a    |
| <i>inutiflorum</i>                      | carvacrol   | Baser <i>et al.</i> 1993     |
| <i>laevigatum</i>                       | bicyclogermacrene, germacrene D, $\beta$ -caryophyllene, myrcene  | Tucker and Maciarello 1992   |
| <i>maioranoides</i>                     | carvacrol, thymol   | Hoppe 1958                   |
| <i>majorana</i>                         | carvacrol   | Baser <i>et al.</i> 1993     |
| <i>majorana</i> var. <i>tenuifolium</i> | cis-sabinene hydrate (cis-thuyanol-4), terpinen-4-ol  | Arnold <i>et al.</i> 1993    |
| <i>maru</i>                             | carvacrol, followed by $\gamma$ -terpinene and p-cymene, thymol   | Akguel and Bayrak 1987       |
| <i>maru</i>                             | carvacrol, thymol   | Hoppe 1958                   |
| <i>minutiflorum</i>                     | carvacrol   | Baser <i>et al.</i> 1991     |
| <i>onites</i>                           | carvacrol, p-cymene (6-12%) and $\gamma$ -terpinene   | Pino <i>et al.</i> 1993      |
| <i>onites</i>                           | carvacrol, p-cymene   | Arnold <i>et al.</i> 1993    |
| <i>onites</i>                           | carvacrol   | Baser <i>et al.</i> 1993     |
| <i>onites</i>                           | carvacrol, $\gamma$ -terpinene, $\beta$ -bisabolene   | Ruberto <i>et al.</i> 1993   |
| <i>onites</i>                           | carvacrol   | Biondi <i>et al.</i> 1993    |
| <i>onites</i>                           | linalool  | Baser <i>et al.</i> 1993     |
| <i>onites</i>                           | carvacrol, thymol   | Lagouri <i>et al.</i> 1993   |
| <i>onites</i>                           | carvacrol   | Kaya 1992                    |
| <i>onites</i>                           | carvacrol, thymol, borneol, p-cymene, $\gamma$ -terpinene   | Vokou <i>et al.</i> 1988     |
| <i>onites</i> [syn. <i>smyrnaceum</i> ] | carvacrol, linalool, cymol, d-camphene, $\alpha$ -pinene, terpene   | Hoppe 1958                   |
| <i>rotundifolium</i>                    | cis-sabinene hydrate  | Baser <i>et al.</i> 1995     |
| <i>saccatum</i>                         | carvacrol, p-cymene,  | Tumen <i>et al.</i> 1995     |
| <i>sipyleum</i>                         | carvacrol, $\gamma$ -terpinene, p-cymene, thymol methyl ether, methyl ether, thymol, carvacrol                | Baser <i>et al.</i> 1992     |
| <i>smyrnaeum</i>                        | carvacrol, $\gamma$ -terpinene and p-cymene, thymol   | Akguel and Bayrak 1987       |
| <i>solymicum</i>                        | p-cymene, thymol, linalool  | Tumen <i>et al.</i> 1994     |
| <i>syriacum</i>                         | p-cymene, phenolic monoterpenes, $\gamma$ -terpinene  | Dudai <i>et al.</i> 1992     |
| <i>syriacum</i>                         | carvacrol, thymol   | Fleisher and Fleisher 1991   |

| Species  | Compounds   | Reference                      |
|--|---|--------------------------------|
| <i>syriacum</i>  | carvacrol, geraniol, geranyl esters, ethyl cinnamate  | Fleisher and Fleisher 1991     |
| <i>syriacum</i> var.<br><i>aegyptiacum</i>                           | carvacrol, p-cymene, $\gamma$ -terpinene, myrcene, $\alpha$ -thujene, carvacrol methyl ether, carvacryl acetate | Halim <i>et al.</i> 1991       |
| <i>syriacum</i> var.<br><i>bevanii</i>                               | carvacrol, thymol, $\gamma$ -terpinene  | Baser <i>et al.</i> 1993       |
| <i>syriacum</i> var.<br><i>bevanii</i>                               | carvacrol, thymol, $\gamma$ -terpinene  | Tumen and Baser 1993           |
| <i>virens</i>  | carvacrol, thymol   | Hoppe 1958                     |
| <i>virens</i>  | carvacrol, thymol   | Hohmann 1968                   |
| <i>vulgare</i>   | carvacrol, linalool, $\gamma$ -terpinene, p-cymene, $\alpha$ -terpinene, terpinen-4-ol                          | Özgülven and Stahl-Biskup 1989 |
| <i>vulgare</i>   | carvacrol, linalool, $\beta$ -caryophyllene, linalyl acetate, terpinen-4-ol                                     | Carmo <i>et al.</i> 1989       |
| <i>vulgare</i>   | carvacrol, thymol, trans- $\beta$ -ocymene,   | Carmo <i>et al.</i> 1989       |
| <i>vulgare</i>   | carvacrol, thymol, Chi-terpinene (1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene), p-cymene,                     | Putievsky <i>et al.</i> 1988   |
| <i>vulgare</i>   | carvacrol, thymol, p-cymene, $\gamma$ -terpinene  | Putievsky <i>et al.</i> 1985   |
| <i>vulgare</i>   | $\beta$ -pinene, $\gamma$ -terpinene, limonene, p-cymene, linalool, linalylacetate                              | Marczal and Vincze-Vemes 1973  |
| <i>vulgare</i> subsp.<br><i>gracile</i>                              | $\beta$ -ocymene, $\beta$ -caryophyllene, germacrene  | Sezik <i>et al.</i> 1993       |
| <i>vulgare</i> subsp.<br><i>virens</i>                               | carvacrol, thymolcamphor, 1,8-cineole   | Lawrence and Reynolds 1984     |
| <i>vulgare</i> subsp.<br><i>viride</i>                               | carvacrol, p-cymene, thymol, $\gamma$ -terpinene  | Lawrence and Reynolds 1984     |
| <i>vulgare</i> subsp.<br><i>viride</i>                               | carvacrol, p-cymene (6-12%) and $\gamma$ -terpinene   | Pino <i>et al.</i> 1993        |
| <i>vulgare</i> subsp.<br><i>viride</i>                               | terpinen-4-ol, germacrene, $\beta$ -bisabolene,   | Sezik <i>et al.</i> 1993       |
| <i>vulgare</i> subsp.<br><i>hirtum</i>                               | carvacrol, thymol, $\gamma$ -terpinene and p-cymene   | Vokou <i>et al.</i> 1993b      |
| <i>vulgare</i> subsp.<br><i>hirtum</i>                               | carvacrol, p-cymene and $\gamma$ -terpinene   | Baser <i>et al.</i> 1994b      |
| <i>vulgare</i> subsp.<br><i>hirtum</i>                               | carvacrol, thymol   | Lagouri <i>et al.</i> 1993     |
| <i>vulgare</i> subsp.<br><i>hirtum</i>                               | carvacrol, p-cymene, $\alpha$ -pinene   | Sezik <i>et al.</i> 1993       |
| <i>vulgare</i> var.<br><i>hirtum</i>                                 | carvacrol, thymol, p-cymene, $\gamma$ -terpinene  | Baser <i>et al.</i> 1993       |
| <i>vulgare</i> subsp.<br><i>vulgare</i>                              | germacrene, terpinen-4-ol, $\beta$ -bisabolene  | Sezik <i>et al.</i> 1993       |
| <i>vulgare</i> subsp.<br><i>vulgare</i>                              | carvacrol, sabinene, cis-ocymene, p-cymene, $\gamma$ -cadinene  | Lawrence and Reynolds 1984     |
| <i>vulgare</i> var.<br><i>creticum</i>                               | carvacrol, linalool, camphene, pinene, p-cymene   | Hoppe 1958                     |
| x <i>applii</i> [syn.<br><i>O. vulgare</i> x<br><i>O. majorana</i> ] | thymol, linalyl acetate, terpinen-4-ol (p-cymene, linalyl acetate, terpinen-4-ol                                | Dellacassa <i>et al.</i> 1994  |

The existing dissimilarities between a classification based upon morphological traits (a classical botanical) and one based upon chemical compounds could be touched upon in the following case regarding two populations of *O. vulgare* subsp. *viride* and three of *O. heracleoticum* of different origin (Table 5, Fig. 1). Both these taxa, *O. vulgare* and *O. heracleoticum*, may have chemotypes of similar characters, in

spite of their morphological diversity. In fact, both species have carvacrol-rich and carvacrol-free chemotypes (carvacrol content is less than 3%) regardless of the origin of the material. Furthermore, by evaluating the accumulation level of carvacrol, thymol, p-cymene and  $\gamma$ -terpinene in these two species, there is no doubt of the presence of a great chemical similarity between them (Fleisher and Sneer 1982; Lawrence and Reynolds 1984).

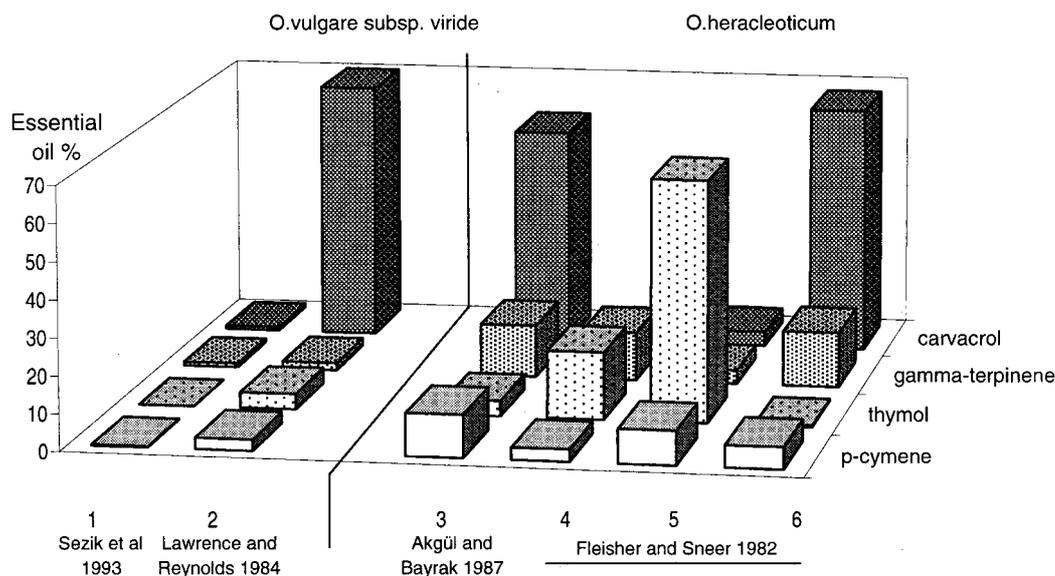


Fig. 1. Classifications of *Origanum* spp. based on chemical compounds.

### Cultivation of oregano in Hungary

In central European countries the cultivation of *O. majorana* has always had a long tradition. In particular, such herbs produced in the south Hungary became famous as 'Hungarian marjoram' all over the world.

The commercial material of *O. vulgare* is still partially collected from wild plants today. The disadvantage of this practice is that the content of chemical compounds extracted from plants gathered in the wild can be non-homogeneous and, moreover, the supply of the material can not be ensured because of yield fluctuations due to changes in the climatic conditions. It is therefore easily explained why the cultivation of oregano has recently become more and more popular as a way to avoid such drawbacks (Bernáth 1993).

Today in Hungary, the type of oregano being cultivated most in the country is characterized by 0.5-1.5% oil content, having carvacrol and thymol as the main compounds. Selection of new cultivars for this crop, however, has been actively pursued over the last few years. Such work is carried out using two taxa of *Origanum*, i.e. *O. vulgare* and *O. creticum* (syn. *O. heracleoticum*). The main characteristics of eight lines belonging to of each of the two taxa are listed in Table 6.

Table 5. Chemical diversity within *Origanum vulgare* subsp. *viride* and *O. heracleoticum* according to literature data (values reported in %).

| Compound   | <i>Origanum vulgare</i><br>subsp. <i>viride</i> |                            | <i>Origanum heracleoticum</i><br>Fleisher and Sneer 1982 |          |          |          |
|--|---|----------------------------|--|----------|----------|----------|
|  | Sezik <i>et al.</i> 1993                        | Lawrence and Reynolds 1984 | Akgül and Bayrak 1987                                    | 'A' type | 'B' type | 'C' type |
|  | (E)-2-hexenal                                   | 0.07                       | —  | —        | —        | —        |
| (E)-2-hexenol  | 0.02  | —                          | —  | —        | —        | —        |
| (E)- $\alpha$ -ocymene   | 3.66  | —                          | —  | —        | —        | —        |
| (E,Z)-2,6-nonadienal   | 0.04  | —                          | —  | —        | —        | —        |
| (Z)-2-nonenal  | 0.05  | —                          | —  | —        | —        | —        |
| (Z)- $\beta$ -ocymene  | 0.38  | —                          | —  | —        | —        | —        |
| (Z)-geranyl acetone  | 0.04  | —                          | —  | —        | —        | —        |
| 1,8-cineole  | 0.88  | trace-0.1                  | 0.30   | 3.4      | 0.1      | 0.2      |
| 1-octen-3-ol   | 0.18  | 0.1-0.4                    | —  | —        | —        | —        |
| 2,5-diethyltetrahydrofuran   | 0.01  | —                          | —  | —        | —        | —        |
| 2-ethyl-1-hexanol  | 0.47  | —                          | —  | —        | —        | —        |
| 3-octanol  | —   | trace-0.1                  | —  | —        | —        | —        |
| 6-methyl-3heptanol   | 0.24  | —                          | —  | —        | —        | —        |
| $\alpha$ -cadinene   | —   | trace-0.1                  | —  | —        | —        | —        |
| $\alpha$ -cadinol  | 3.83  | —                          | —  | —        | —        | —        |
| $\alpha$ -calacorene   | —   | trace                      | —  | —        | —        | —        |
| $\alpha$ -cubebene   | —   | trace                      | —  | —        | —        | —        |
| $\alpha$ -farnesene, geranyl acetate, cadinene   | 5.68  | —                          | —  | —        | —        | —        |
| $\alpha$ -himachalene  | —   | 1.11                       | —  | —        | —        | —        |
| $\alpha$ -humulene   | —   | 3.16                       | —  | —        | —        | —        |
| $\alpha$ -maalinene, terpinen-4-yl acetate, trans-dihydrocarvone, methyl benzoate                | —   | 0.8-1.5                    | —  | —        | —        | —        |
| $\alpha$ -muurolene  | 0.49  | —                          | —  | —        | —        | —        |
| $\alpha$ -muurolene, $\beta$ -bisabolene, delta cadinene   | —   | trace-0.1                  | —  | —        | —        | —        |
| $\alpha$ -p-dimethylstyrene  | —   | trace-0.1                  | —  | 0.6      | 0.2      | 0.3      |
| $\alpha$ -phellandrene   | 0.32  | 0.3-0.7                    | —  | 0.9      | —        | 0.1      |
| $\alpha$ -pinene   | 0.16  | 0.4-0.7                    | 1.05   | —        | —        | —        |
| $\alpha$ -terpinene  | 0.23  | 0.3-0.7                    | 0.40   | 8.5      | 0.5      | 0.8      |
| $\alpha$ -terpineol  | 1.40  | 0.3-0.7                    | 0.15   | 3.8      | 0.2      | 0.3      |
| $\alpha$ -tujone   | —   | trace-0.2                  | —  | —        | —        | —        |
| $\alpha$ -ylangene   | —   | trace                      | —  | —        | —        | —        |
| amyl furan   | 0.02  | —                          | —  | —        | —        | —        |
| $\beta$ -bisabolene  | 6.23  | —                          | —  | —        | —        | —        |
| $\beta$ -bourbonene  | 0.70  | —                          | —  | —        | —        | —        |
| $\beta$ -bourbonene,4,5-epoxy-p-menth-1-ene, linalool, cis-sabinene hydrate, cis-p-menth-2-en-ol | —   | 0.1-0.4                    | —  | —        | —        | —        |
| $\beta$ -caryophyllene   | 0.09  | —                          | —  | 1.5      | 0.8      | 1.0      |
| $\beta$ -pinene  | 0.27  | trace-0.1                  | 0.51   | 0.1      | 0.1      | —        |
| $\beta$ -sesquiphellandrene  | 0.11  | —                          | —  | —        | —        | —        |
| borneol  | —   | —                          | 1.52   | 0.9      | 0.3      | 1.1      |
| bornyl acetate   | 1.01  | —                          | 0.18   | —        | —        | —        |
| bornyl acetate, $\beta$ -ylangene, trans- $\alpha$ -bergamotene                                  | —   | trace-0.2                  | —  | —        | —        | —        |

| Compound   | <i>Origanum vulgare</i><br>subsp. <i>viride</i> |                            | <i>Origanum heracleoticum</i><br>Fleisher and Sneer 1982 |             |             |             |
|--|---|----------------------------|--|-------------|-------------|-------------|
|  | Sezik <i>et al.</i> 1993                        | Lawrence and Reynolds 1984 | Akgül and Bayrak 1987                                    | 'A' type    | 'B' type    | 'C' type    |
| cadina-1(16),4 diene, viridiflorene, $\gamma$ -muurolene | –   | 0.1-0.2                    | –  | –           | –           | –           |
| cadinol  | 0.48  | –                          | –  | –           | –           | –           |
| calamenene   | –   | trace-0.1                  | –  | –           | –           | –           |
| camphene   | 0.09  | trace-0.3                  | 0.30   | 0.2         | 0.3         | 0.2         |
| camphor  | –   | –                          | 1.07   | –           | –           | –           |
| <b>carvacrol</b>   | <b>0.95</b>                                     | <b>62.4-82.6</b>           | <b>58.71</b>   | <b>2.9</b>  | <b>4.0</b>  | <b>67.3</b> |
| carvone  | –   | –                          | 0.29   | –           | –           | –           |
| carvotanacetone  | –   | trace                      | –  | –           | –           | –           |
| caryophyllene oxide                                      | –   | trace-0.1                  | –  | –           | –           | –           |
| caryophyllene, cis-dihydrocarvone                        | –   | 0.4-1.0                    | 0.29   | –           | –           | –           |
| cis-sabinene hydrate                                     | 0.07  | –                          | 0.25   | –           | –           | –           |
| copaene  | –   | trace-0.1                  | –  | –           | –           | –           |
| cuminaldehyde  | –   | trace                      | –  | –           | –           | –           |
| decanol  | 0.07  | –                          | –  | –           | –           | –           |
| $\gamma$ -cadinene                                       | –   | trace-0.2                  | –  | –           | –           | –           |
| <b><math>\gamma</math>-terpinene</b>                     | <b>1.03</b>                                     | <b>1.3-2.3</b>             | <b>14.17</b>   | <b>13.2</b> | <b>8.7</b>  | <b>15.0</b> |
| geraniol   | 0.21  | –                          | –  | –           | –           | –           |
| germacrene   | 15.87   | –                          | –  | –           | –           | –           |
| hexahydrofarnesyl acetone                                | 0.31  | –                          | –  | –           | –           | –           |
| humulene epoxide   | –   | trace-0.1                  | –  | –           | –           | –           |
| isoborneol   | 0.19  | –                          | –  | –           | –           | –           |
| limonene   | 1.32  | 0.1-0.2                    | trace  | 1.4         | 0.2         | 0.3         |
| linalool   | 2.37  | –                          | 0.16   | 1.6         | 0.2         | 0.4         |
| linalyl acetate  | 3.88  | –                          | trace  | –           | –           | –           |
| methyl carvacrol, aromadendrene                          | –   | 0.4-0.8                    | –  | –           | –           | –           |
| methyl chavicol  | –   | trace-0.1                  | –  | –           | –           | –           |
| myrcene  | 0.37  | 0.4-0.8                    | 2.64   | 1.4         | 0.5         | 0.8         |
| nerol  | 0.07  | –                          | –  | –           | –           | –           |
| nerolidol  | 0.24  | –                          | –  | –           | –           | –           |
| nonanal  | 0.12  | –                          | –  | –           | –           | –           |
| nonanoic acid  | 0.06  | –                          | –  | –           | –           | –           |
| octenyl acetate  | 1.20  | –                          | –  | –           | –           | –           |
| p-cymen-8-ol   | –   | trace-0.2                  | –  | –           | –           | –           |
| <b>p-cymene</b>  | <b>0.24</b>                                     | <b>2.7-8.8</b>             | <b>11.51</b>   | <b>3.3</b>  | <b>9.2</b>  | <b>5.9</b>  |
| patchoulane  | 1.62  | –                          | –  | –           | –           | –           |
| piperitenone oxide                                       | 0.45  | –                          | –  | –           | –           | –           |
| piperitone oxide   | 2.81  | –                          | –  | –           | –           | –           |
| sabinene   | 1.59  | trace                      | –  | –           | –           | –           |
| spathulenol  | 1.16  | –                          | –  | –           | –           | –           |
| terpinen-4-ol  | 16.82   | 0.4-0.7                    | 0.16   | 27.2        | 1.4         | 0.7         |
| terpinolene  | –   | trace-0.1                  | trace  | 1.7         | –           | –           |
| terpinolene epoxide                                      | –   | trace-0.2                  | –  | –           | –           | –           |
| <b>thymol</b>  | –   | <b>1.1-7.4</b>             | <b>4.23</b>  | <b>18.2</b> | <b>65.1</b> | <b>0.4</b>  |
| torreyol   | 1.11  | –                          | –  | –           | –           | –           |
| trans-anethole   | –   | trace                      | –  | –           | –           | –           |
| trans-sabinene hydrate                                   | 0.28  | trace-0.1                  | 6.0  | –           | –           | –           |

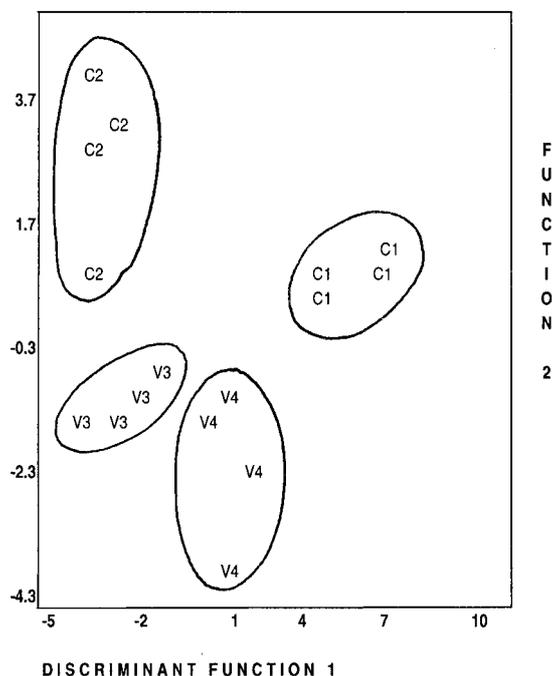
Table 6. Essential oil in *Origanum* lines under selection in Hungary (values reported in %).

| Components          | <i>Origanum creticum</i> (syn. <i>O. heracleoticum</i> ) |      |      |     |      |      |     |      |     |      |      | <i>Origanum vulgare</i> |     |     |     |     |  |  |  |
|---------------------|--|------|------|-----|------|------|-----|------|-----|------|------|-------------------------|-----|-----|-----|-----|--|--|--|
|                     | C11  | C12  | C13  | C14 | C21  | C22  | C23 | C24  | V31 | V32  | V33  | V34                     | V41 | V42 | V43 | V44 |  |  |  |
| $\alpha$ -pinene    | 0.0  | 0.0  | 0.0  | 0.0 | 0.0  | 0.0  | 0.0 | 0.0  | 0.7 | 0.0  | 3.7  | 1.8                     | 2.7 | 0.0 | 0.0 | 1.7 |  |  |  |
| $\beta$ -pinene     | 0.0  | 0.0  | 0.0  | 5.0 | 9.0  | 6.7  | 1.0 | 2.6  | 0.7 | 0.0  | 3.7  | 1.8                     | 2.7 | 0.0 | 0.0 | 1.7 |  |  |  |
| p-cymene            | 3.8  | 5.3  | 5.8  | 1.4 | 5.5  | 14.1 | 7.4 | 24.2 | 7.3 | 21.2 | 14.8 | 12.7                    | 7.0 | 2.9 | 3.1 | 4.7 |  |  |  |
| $\gamma$ -terpinene | 3.2  | 4.4  | 9.1  | 0.0 | 8.6  | 0.0  | 0.0 | 17.6 | 2.5 | 6.4  | 0.0  | 0.0                     | 1.2 | 2.7 | 0.0 | 1.1 |  |  |  |
| linalool            | 0.0  | 0.0  | 0.0  | 1.2 | 2.3  | 23.0 | 7.5 | 0.0  | 1.3 | 2.0  | 3.2  | 3.8                     | 1.6 | 0.0 | 0.0 | 0.0 |  |  |  |
| terpinen-4-ol       | 0.0  | 0.0  | 0.0  | 0.0 | 14.2 | 2.1  | 5.5 | 1.1  | 3.9 | 13.3 | 11.6 | 4.2                     | 2.6 | 3.2 | 2.4 | 0.0 |  |  |  |
| thymol              | 0.0  | 0.0  | 0.0  | 0.0 | 0.0  | 0.0  | 0.0 | 0.0  | 0.0 | 5.3  | 0.0  | 0.0                     | 0.0 | 0.0 | 0.0 | 0.0 |  |  |  |
| carvacrol           | 90.0   | 86.4 | 78.4 | 0.0 | 1.2  | 1.4  | 0.0 | 12.0 | 3.2 | 5.3  | 5.1  | 3.9                     | 4.1 | 7.9 | 7.4 | 4.0 |  |  |  |
| caryophyllene       | 0.0  | 1.3  | 0.9  | 3.3 | 2.1  | 6.1  | 1.5 | 1.8  | 2.3 | 0.0  | 3.1  | 4.6                     | 3.6 | 3.7 | 2.8 | 8.3 |  |  |  |

By using discriminant analysis to compare these lines, it becomes clear that there are large differences between the botanical classification and the chemical characteristics of these plants (Table 7 and Fig. 2). In particular, *O. creticum* lines show a large chemical diversity; for instance, one group (C11-C14) separates well from the others because of the lack of terpinen-4-ol. *Origanum vulgare* lines seem to be closer together: the higher p-cymene ratio ought to be emphasised in the V31-V34 group.

**Table 7. Standardized discriminant function coefficients of essential oil components in *Origanum*.**

| Essential oil components | Function coefficients |          |          |
|--------------------------|-----------------------|----------|----------|
|                          | 1                     | 2        | 3        |
| $\alpha$ -pinene         | 0.39422               | -1.02967 | -0.44935 |
| $\beta$ -pinene          | 1.78103               | 1.26053  | 0.73651  |
| p-cymene                 | -1.04926              | 0.72934  | 1.69165  |
| $\gamma$ -terpinene      | -0.84917              | -0.07430 | -2.11795 |
| linalool                 | -1.23579              | 0.43939  | -1.15759 |
| Terpinen-4-ol            | -2.34818              | -0.15762 | 0.16399  |
| Thymol                   | 1.90060               | -0.90779 | -0.17673 |
| Carvacrol                | 1.35784               | 0.41929  | 0.71663  |
| Charyophyllene           | -0.67612              | -0.84707 | -0.42739 |



C1 (GROUP)= *O. creticum* C11, C12, C13, C14 lines  
 C2 (GROUP)= *O. creticum* C21, C22, C23, C24 lines  
 V3 (GROUP)= *O. vulgare* V31, V32, V33, V34 lines  
 V4 (GROUP)= *O. vulgare* V41, V42, V43, V44 lines

**Fig. 2.** Distribution of *O. vulgare* and *O. creticum* lines in the discriminant field.

In Hungary, oregano is cultivated on light, dry and well-drained soils, which are somewhat alkaline. Propagation can be done vegetatively by separation of roots or by seed. Roots are planted in the field in September-October. The seed should be sown in an open-air nursery with inter-row distances of 25 cm in April. One gram of seed is usually sufficient for sowing 1 m<sup>2</sup> of nursery. The depth of sowing is 5-10 mm. The seedlings can be transplanted to the field in May, when they reach 10-12 cm in height. They are planted with a spacing of 50-60 cm between rows and 20-25 cm within rows. Irrigation is required at the time of planting and a few other times in the first year. In the following years, plants have developed an efficient root system and thus no further irrigation is usually needed. Plants are harvested at blooming stage and dried afterwards in the shade or by artificial means (dryers should not exceed 40°C) to preserve the colour and fragrances of the herb. The lifespan of the crop is about 5-6 years in this country and usually one harvest is done in the first year and two in the following years. On average, the yield ranges from 2.5 to 3.5 t/ha.

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## Selection work on *Origanum vulgare* in France

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### Abstract

The paper summarises the results of research investigations conducted by the Conservation Centre of Milly La Forêt, France from 1991 to 1995. The work aimed at investigating the agronomic performance and chemical compound content of germplasm material of *O. vulgare* ('Greek oregano') gathered from Greece. The material, originating from 32 different locations, was grown out in Milly La Forêt and identified as *O. vulgare* L. subsp. *hirtum* Ietswaart and *O. onites*. Morphological characterization and chemical evaluation work led to some interesting conclusions that will eventually enhance the use of the crop in France. Among the examined material, six main groups of plants were identified on the basis of their essential oils content, the first group being the most common one: (1) thymol rich (>55%) and poor in carvacrol (< 6%); (2) carvacrol rich (>50%) and poor in thymol (< 2.5%); (3) balanced presence of thymol and carvacrol (30-45%) for both components; (4)  $\gamma$ -terpinene rich (>45%), very poor in thymol (<1%) and poor in carvacrol (13-15%); (5) carvacrol rich (45-60%) and very poor in thymol (14-22%).

### Introduction

The main objective of this work is to obtain clones of oregano with good resistance to the cold and having a good productivity in dry matter yield and essential oils. The final goal of this investigation is to have, by 1996, at least 50 clones bearing particularly interesting traits for these characteristics.

The choice to select Greek oregano in this work has been motivated by a number of reasons. First of all, Greece is one of the countries within the Mediterranean region with the highest concentration in oregano diversity. At least 10 of the 13 *Origanum* species that are supposed to occur throughout Europe are recorded there. Secondly, this species, often named *O. vulgare* L. subsp. *hirtum* Ietswaart, is one of the most popular types of oregano on the market.

In fact, the Israeli *Origanum* species, commercialized everywhere in the world, are likely to have originated from Lamia, a Greek locality.

### Reports of activities (1991-95)

#### 1991

In the framework of our activities on *Origanum*, and on the invitation of ITEIPMAI (Institute Technique de Plantes Médicinales, Avignon, France) (which has developed a selection programme on this genus), two plant explorations in Greece were carried out in conjunction with some travels made to that country.

- The first trip was made in the month of July in the mountainous areas of eastern and central Greece and to the Peloponnesus. Thirty-two oregano accessions were gathered during this mission. These plants, in limited amounts, along with some cuttings (with few roots), were sent to Milly La

Forêt. Seeds collected from previous-year dry infructescences of these populations were very disappointing for their very low germination rate.

- The second expedition was carried out in October. Seeds were gathered on the same sites visited previously. Both this mission and the previous one yielded material belonging to either *O. vulgare* subsp. *hirtum* or *O. onites*.

Both species are strongly aromatic and have a high essential oil content whose main components are thymol and carvacrol.

### 1992

Thanks to the seed material gathered in October 1991, 26 populations (20 plants per populations) were planted on soil mounds.

Morphological characterizations allowed the proper distinction of the material from a taxonomic point of view, separating *O. onites* from *O. vulgare* species. On the other hand, it was not possible to distinguish, within *O. vulgare* material, subsp. *hirtum* from subsp. *viride*, as they differ from one another solely on the basis of essential oil gland density (these are more numerous in subsp. *hirtum*). Subspecies *virides* is also a less common taxon than subsp. *hirtum* in the country.

With regard to the growth of this material, all the accessions developed successfully into a vegetative flowering mass thus permitting a first evaluation on their potential with regard to essential oil content. The harvest was thus used to make a distillation, in which 187 samples were analysed as a whole.

### 1993

- During this year (the second year of growth for the material), a smaller study was conducted on those accessions that survived the winter. Flowering parts of these populations were harvested at their full flowering stage. Some 328 individuals were thus sampled and the material weighed and distilled.

Other characters that were measured were the precocity in flowering, the health status and the vigour of each plant.

In September, 36 individuals that were found to have a content in essential oils higher than 7.5 or a yield higher than 8 ml/plant were selected and cloned by dividing up their clumps (two repetitions of 5 plants per clone).

- Along with this trial, some new seeds were obtained by using original mother seeds from Greece corresponding to the best populations recorded in the 1992 investigations. The young plants, not numerous, that were obtained from this planting were then put in open cultivation in summer. They represented the basic material for further investigations made in the following year.

### 1994

- Thirty-one clones out of the 56 multiplied that survived the 1993-94 winter were evaluated for quantitative traits (i.e. dry matter yield of leaves + flowers, essential oil content and yield) (Table 1).

The results obtained with regard to the essential oil content confirm those obtained from material collected in 1993 on the original mother plants. The yields per plant were, however, rather low and were not very consistent with those obtained in 1993.

Table 1. Analysis of accessions, planted in 1993, with high essential oil (EO) content ( $\geq 7.5$ ).

| Number      | DM yield of leaves and flowers (g/plant) |           |           | EO content (ml/100 g DM leaves and flowers) |            |            | EO yield (ml/plant) |            |            |
|-------------|--|-----------|-----------|---|------------|------------|---------------------|------------|------------|
|             | 1994                                     | 1995      | Mean      | 1994  | 1995       | Mean       | 1994                | 1995       | Mean       |
| 94-000-95   | 28                                       | 3         | 16        | 9.6   | 10.3       | 9.9        | 2.7                 | 0.3        | 1.5        |
| 94-262-40   | 36                                       | 100       | 68        | 10.9  | 8.2        | 9.6        | 4.0                 | 8.2        | 6.1        |
| 94-260-27   | 110                                      | 38        | 74        | 9.8   | 9.2        | 9.5        | 10.8                | 3.5        | 7.2        |
| 94-255-23   | 42                                       | 25        | 34        | 5.3   | 13.7       | 9.5        | 2.2                 | 3.4        | 2.8        |
| 94-266-46   | 28                                       | 42        | 35        | 9.1   | 9.8        | 9.4        | 2.5                 | 4.2        | 3.4        |
| 94-255-25   | 35                                       | 24        | 29        | 9.6   | 8.4        | 9.0        | 3.3                 | 2          | 2.7        |
| 94-266-45   | 40                                       | 137       | 88        | 9.2   | 8.8        | 9.0        | 3.7                 | 12         | 7.8        |
| 94-254-07   | 42                                       | 22        | 32        | 9.3   | 8.4        | 8.9        | 3.9                 | 1.9        | 2.9        |
| 94-254-08   | 49                                       | 176       | 113       | 9.2   | 8.4        | 8.7        | 4.5                 | 14.8       | 9.7        |
| 94-260-28   | 12                                       | 4         | 8         | 10.4  | 7          | 8.7        | 1.3                 | 0.3        | 0.8        |
| 94-000-97   | 9  | 75        | 42        | 11.0  | 6.3        | 8.7        | 1.0                 | 4.8        | 2.9        |
| 94-277-89   | 37                                       | 66        | 52        | 8.4   | 8.8        | 8.6        | 3.1                 | 5.8        | 4.5        |
| 94-262-41   | 43                                       | 20        | 32        | 8.8   | 8          | 8.4        | 3.8                 | 1.6        | 2.7        |
| 94-000-92   | 7  | 105       | 56        | 7.3   | 9.4        | 8.3        | 0.5                 | 9.8        | 5.2        |
| 94-000-96   | 68                                       | 3         | 35        | 6.6   | 9.9        | 8.3        | 4.5                 | 0.3        | 2.4        |
| 94-081-102  |  | 63        | 63        |   | 8.2        | 8.2        |                     | 5.2        | 5.2        |
| 94-261-35   | 5  | 25        | 15        | 8.7   | 7.6        | 8.2        | 0.5                 | 1.9        | 1.2        |
| 94-252-02   | 48                                       | 209       | 129       | 8.3   | 7.7        | 8.0        | 4.0                 | 16.1       | 10.1       |
| 94-254-12   | 38                                       | 32        | 35        | 7.3   | 8.6        | 7.9        | 2.8                 | 2.8        | 2.8        |
| 94-262-43   |  | 5         | 5         |   | 7.9        | 7.9        |                     | 0.4        | 0.4        |
| 94-275-85   | 50                                       | 36        | 43        | 7.4   | 7.7        | 7.6        | 3.7                 | 2.8        | 3.3        |
| 94-254-20   | 75                                       | 65        | 70        | 6.8   | 8.2        | 7.5        | 5.1                 | 5.4        | 5.2        |
| <b>Mean</b> | <b>40</b>                                | <b>58</b> | <b>49</b> | <b>8.7</b>                                  | <b>8.7</b> | <b>8.6</b> | <b>3.4</b>          | <b>4.9</b> | <b>4.1</b> |

It should be said at this point that the unusually high results in essential oil content obtained in 1993 on accessions 93-262-05, 93-263-04 and 93-262-14 were in fact not confirmed in 1994. A too-small amount of plant material harvested did not allow us to carry out reliable analyses.

The accession 93-263-04 did not survive the 1993-94 winter. For the other accessions, there was a confirmation of the good results previously recorded, their essential oil content being always, however, closer to more normal values.

- New 1993 seeds (seeds gathered in Greece in 1991)

Seeds which were taken from the best populations found in Greece were put into the nursery very late in 1993; therefore the plants obtained from them had to be considered as being in their first year of cultivation in 1994.

At their 'full flowering stage', 108 plants were collected and distilled. The results obtained from these plants harvested in 1995 are reported in Table 1.

## 1995

- Clones obtained in 1993

The clones which were surveyed in the winter 1994-95, 26 were evaluated once again in 1995. Additional distillations (3 to 6) were carried out on each of them.

We would like to make the following remarks on these data:

1. There has been a good repetitiveness of the results over the 3 years:
  - the mother plant was obtained in 1993 (second year of cultivation);
  - the clone was obtained in 1994 (first year of cultivation);
  - the clone was also made in 1995 (second of cultivation).

2. The highest values in essential oil contents that were obtained in 1993 with clones 93-262-05 and 93-263-04 were not recorded either in 1994 or 1995. These clones still remain, however, very rich in essential oil content.
3. The criteria 'high dry matter yield' could not be applied to isolated plants. The seven mother plants which were selected in 1993 on this basis did produce clones which were just slightly more productive, indeed sometimes less productive than the average value from all the clones together.

The plants studied in 1994 and surveyed during the 1994-95 winter were evaluated once again for quantitative traits in 1995 (on the second year of cultivation).

Table 2 reports the results obtained from those plants which showed, over the 2-year cultivation period, a content in essential oils higher than 7.5%. We would like to highlight once more the good repetitiveness of these values from one year to another, with the exception of accession 94-255-23, whose results will need to be reconfirmed in the following year.

- Evaluation for qualitative traits

The analyses of the essential oils of those clones obtained in 1993 and of the best material obtained in 94-95 tests were carried out in September 1995 at the ITEIPAMI laboratory in Angers. These essential oils were extracted during the summer 1995 by using the leaves and fresh flowers of plants in their second year of cultivation, during their full flowering stage.

The groups of different essential oils can clearly be distinguished:

- thymol rich (>55%) and poor in carvacrol (< 6%): by far the most common type;
- carvacrol rich (>50%) and poor in thymol (< 2.5%);
- balanced presence of thymol and carvacrol (30 to 45%) for each of the two components;
- $\gamma$ -terpinene rich (>45%), very poor in thymol (<1%) and poor in carvacrol (13-15%);
- carvacrol rich (45-60%) and very poor in thymol (14-22%).

### **Multiplication**

As planned, all clones tested in 1992 and 1993 have been multiplied in a standardised manner at the beginning of September 1995. The same was done for the 22 mother plants, found to be the richest in essential oils as found in the 1994-95 tests. Such multiplication was done by:

- splitting of the clump and replanting the parts obtained in the soil under plastic screenhouses;
- stem cuttings: cuttings were then placed in perlite hold in small pot trays located in glasshouses.

As a preliminary assessment, we could say that the results of the cutting establishment vary considerably according to the mother plant or the clone that has been used.

Table 2. Composition of 54 clones and individuals with high essential oil content.

| Name                          | a-thuyene | a-pinene | camphene | b-pinene+sabinene | pic 1 | myrcene | pic 2 | a-terpinene | p-cymen | pic 3 | pic 4 | g-terpinene |
|-------------------------------|-----------|----------|----------|-------------------|-------|---------|-------|-------------|---------|-------|-------|-------------|
| <b>THYMOL group</b>           |           |          |          |                   |       |         |       |             |         |       |       |             |
| A 93-252-03 p8                | 2.14      | 0.63     | 0.16     | 0.42              | 0.45  | 2.71    | 0.43  | 3.19        | 7.17    | 0.00  | 0.06  | 17.80       |
| A 93-252-09 p3                | 2.25      | 0.62     | 0.19     | 0.45              | 0.49  | 2.72    | 0.45  | 3.26        | 6.48    | 0.00  | 0.06  | 20.07       |
| A 93-253-01 p10               | 2.65      | 0.60     | 0.17     | 0.46              | 0.62  | 2.90    | 0.38  | 2.96        | 9.65    | 0.00  | 0.07  | 16.92       |
| A 93-254-12 p4                | 2.20      | 0.65     | 0.13     | 0.73              | 0.24  | 2.64    | 0.34  | 2.02        | 7.52    | 0.03  | 0.56  | 6.89        |
| A 93-255-10 p6                | 2.15      | 0.63     | 0.09     | 0.38              | 0.11  | 2.64    | 0.31  | 1.90        | 6.16    | 0.00  | 0.08  | 7.77        |
| A 93-260-07 p6                | 3.06      | 0.76     | 0.20     | 0.49              | 0.42  | 3.43    | 0.47  | 3.51        | 7.23    | 0.00  | 0.00  | 12.91       |
| A 93-262-05 p6                | 2.82      | 0.58     | 0.11     | 0.37              | 0.15  | 2.87    | 0.38  | 2.15        | 7.71    | 0.00  | 0.05  | 7.06        |
| A 93-262-11 p5                | 2.37      | 0.58     | 0.10     | 0.48              | 0.37  | 2.76    | 0.48  | 1.81        | 7.27    | 0.00  | 0.10  | 6.25        |
| A1 93-262-15 p8               | 2.26      | 0.63     | 0.15     | 0.42              | 0.25  | 2.76    | 0.46  | 2.12        | 5.95    | 0.00  | 0.10  | 7.97        |
| A1 93-263-03 p3               | 2.01      | 0.64     | 0.11     | 0.36              | 0.54  | 2.51    | 0.43  | 2.87        | 6.81    | 0.00  | 0.02  | 14.52       |
| A1 93-264-14 p8               | 3.01      | 0.62     | 0.20     | 0.46              | 0.00  | 3.06    | 0.40  | 2.66        | 8.34    | 0.00  | 2.00  | 9.96        |
| A1 93-266-02 p2               | 2.62      | 0.20     | 0.10     | 0.41              | 0.15  | 2.65    | 0.35  | 2.16        | 7.36    | 0.00  | 0.08  | 8.16        |
| A1 93-266-03 p5               | 2.67      | 0.18     | 0.13     | 0.35              | 0.09  | 2.61    | 0.35  | 1.52        | 6.45    | 0.00  | 0.10  | 4.30        |
| A1 93-266-04 p1               | 2.19      | 0.71     | 0.09     | 0.37              | 0.12  | 2.60    | 0.36  | 1.36        | 6.64    | 0.00  | 0.10  | 3.93        |
| A1 93-266-11 p2               | 2.00      | 0.57     | 0.10     | 0.35              | 0.24  | 2.61    | 0.41  | 3.03        | 6.64    | 0.00  | 0.08  | 15.51       |
| A2 94-254-05                  | 2.44      | 0.73     | 0.19     | 1.11              | 0.39  | 2.68    | 0.34  | 2.17        | 9.40    | 0.06  | 1.10  | 10.09       |
| A2 94-254-07                  | 2.18      | 0.70     | 0.20     | 0.40              | 0.22  | 2.58    | 0.37  | 2.71        | 7.55    | 0.01  | 0.09  | 11.29       |
| A2 94-254-08                  | 2.54      | 0.82     | 0.21     | 0.44              | 0.34  | 2.90    | 0.38  | 2.48        | 8.47    | 0.00  | 0.08  | 10.30       |
| A2 94-254-12                  | 3.54      | 1.09     | 0.14     | 0.68              | 0.13  | 4.11    | 0.57  | 3.15        | 12.69   | 0.00  | 0.16  | 11.81       |
| A2 94-254-20bis               | 2.14      | 0.73     | 0.15     | 0.37              | 0.48  | 2.82    | 0.39  | 1.25        | 6.11    | 0.00  | 0.10  | 3.99        |
| A3 94-255-21                  | 2.23      | 0.63     | 0.10     | 0.39              | 0.04  | 2.59    | 0.31  | 2.17        | 7.99    | 0.00  | 0.09  | 12.26       |
| A3 94-255-25                  | 3.47      | 1.05     | 0.22     | 0.61              | 0.22  | 4.06    | 0.50  | 3.08        | 9.03    | 0.00  | 0.14  | 11.24       |
| A3 94-255-27                  | 2.47      | 0.72     | 0.15     | 0.48              | 0.13  | 2.96    | 0.48  | 3.58        | 6.25    | 0.00  | 0.09  | 20.18       |
| A3 94-261-31                  | 2.04      | 0.72     | 0.27     | 0.39              | 0.30  | 2.43    | 0.33  | 1.95        | 6.95    | 0.00  | 0.08  | 7.98        |
| A3 94-262-41                  | 2.01      | 0.50     | 0.09     | 0.33              | 0.28  | 2.23    | 0.30  | 1.65        | 7.19    | 0.00  | 0.07  | 5.90        |
| A3 94-262-42                  | 2.34      | 0.71     | 0.17     | 0.48              | 0.20  | 2.65    | 0.33  | 1.71        | 7.06    | 0.07  | 0.11  | 6.30        |
| A3 94-266-45                  | 1.93      | 0.76     | 0.15     | 0.40              | 0.64  | 2.67    | 0.50  | 2.06        | 7.61    | 0.00  | 0.09  | 7.53        |
| A3 94-266-46                  | 1.84      | 0.60     | 0.09     | 0.35              | 0.55  | 2.52    | 0.45  | 2.44        | 7.17    | 0.00  | 0.07  | 11.43       |
| A3 94-269-53                  | 4.79      | 1.86     | 0.21     | 0.94              | 0.18  | 5.50    | 0.72  | 2.36        | 14.76   | 0.05  | 0.16  | 7.92        |
| A4 94-270-63                  | 1.98      | 0.67     | 0.09     | 0.41              | 0.25  | 2.57    | 0.34  | 1.57        | 7.76    | 0.00  | 0.08  | 5.86        |
| A4 94-270-69                  | 2.04      | 0.71     | 0.15     | 0.38              | 1.05  | 2.79    | 0.43  | 1.81        | 9.25    | 0.00  | 0.06  | 7.07        |
| A4 94-272-77                  | 2.42      | 0.67     | 0.10     | 0.43              | 0.36  | 2.78    | 0.48  | 2.73        | 8.37    | 0.00  | 0.09  | 13.81       |
| A4 94-277-89                  | 2.34      | 0.61     | 0.11     | 0.43              | 0.28  | 2.73    | 0.00  | 2.87        | 7.78    | 0.00  | 0.09  | 11.94       |
| A4 94-000-92                  | 2.53      | 0.18     | 0.12     | 0.35              | 0.75  | 2.51    | 0.37  | 2.65        | 7.52    | 0.00  | 0.10  | 12.65       |
| A5 94-081-102                 | 2.74      | 0.18     | 0.13     | 0.40              | 0.11  | 2.55    | 0.33  | 2.36        | 8.02    | 0.00  | 0.09  | 11.19       |
| <b>CARVACROL group</b>        |           |          |          |                   |       |         |       |             |         |       |       |             |
| B 93-252-13 p4                | 2.43      | 0.70     | 0.12     | 0.44              | 0.51  | 2.70    | 0.35  | 1.67        | 5.20    | 0.02  | 0.09  | 8.53        |
| B1 93-263-05 p6               | 2.18      | 0.59     | 0.12     | 0.38              | 0.39  | 2.40    | 0.30  | 1.09        | 4.41    | 0.00  | 0.06  | 4.36        |
| B1 93-263-07 p5               | 2.09      | 0.61     | 0.11     | 0.36              | 0.32  | 2.30    | 0.28  | 1.23        | 5.45    | 0.00  | 0.08  | 6.24        |
| B2 94-252-01                  | 4.54      | 1.70     | 0.25     | 0.87              | 0.22  | 4.88    | 0.53  | 3.08        | 12.72   | 0.00  | 0.16  | 16.12       |
| B2 94-252-02                  | 6.49      | 2.15     | 0.29     | 1.09              | 0.49  | 6.56    | 0.75  | 2.59        | 12.21   | 0.02  | 0.15  | 10.33       |
| B4 94-277-88                  | 2.70      | 0.18     | 0.09     | 0.38              | 0.46  | 2.51    | 0.45  | 1.69        | 5.79    | 0.29  | 0.09  | 8.19        |
| B4 94-000-94                  | 1.90      | 0.79     | 0.13     | 0.31              | 0.29  | 2.07    | 0.28  | 0.62        | 4.75    | 0.00  | 0.03  | 2.07        |
| B4 94-000-97                  | 2.37      | 0.28     | 0.13     | 0.33              | 0.20  | 1.97    | 0.26  | 0.70        | 5.64    | 0.08  | 0.05  | 3.13        |
| B5 94-000-98                  | 2.50      | 0.24     | 0.14     | 0.36              | 0.33  | 2.05    | 0.28  | 0.58        | 4.57    | 0.29  | 0.06  | 2.03        |
| B5 94-corse-106               | 0.12      | 0.04     | 0.01     | 0.10              | 0.45  | 1.27    | 0.25  | 1.38        | 6.35    | 0.96  | 0.10  | 23.33       |
| B5 94-corse-107               | 2.74      | 0.25     | 0.08     | 0.53              | 0.49  | 2.62    | 0.33  | 1.71        | 4.99    | 0.18  | 0.11  | 8.93        |
| <b>THYMOL-CARVACROL group</b> |           |          |          |                   |       |         |       |             |         |       |       |             |
| C1 93-264-06 p1               | 3.32      | 0.75     | 0.16     | 0.46              | 0.43  | 2.90    | 0.42  | 2.00        | 5.67    | 0.00  | 0.08  | 7.01        |
| C3 94-262-40                  | 2.46      | 0.20     | 0.10     | 0.34              | 0.66  | 2.53    | 0.29  | 1.28        | 5.38    | 0.00  | 0.07  | 5.44        |
| C4 94-000-96                  | 1.84      | 0.61     | 0.11     | 0.37              | 0.21  | 1.82    | 0.21  | 0.66        | 5.08    | 0.00  | 0.02  | 2.47        |
| <b>γ-TERPINENE group</b>      |           |          |          |                   |       |         |       |             |         |       |       |             |
| D2 93-k3 p1                   | 2.60      | 0.50     | 0.19     | 0.52              | 0.74  | 3.07    | 0.55  | 2.99        | 23.34   | 0.00  | 0.00  | 46.88       |
| D2 93-k5 p6                   | 2.55      | 0.55     | 0.18     | 0.57              | 0.71  | 3.20    | 0.59  | 6.03        | 14.96   | 0.00  | 0.00  | 53.49       |
| <b>CARVACROL-thymol group</b> |           |          |          |                   |       |         |       |             |         |       |       |             |
| E2 94-254-15                  | 3.91      | 1.47     | 0.30     | 0.74              | 0.21  | 4.61    | 0.64  | 2.63        | 9.40    | 0.03  | 0.15  | 12.31       |
| E5 94-081-101                 | 1.99      | 0.63     | 0.13     | 0.36              | 0.44  | 2.79    | 0.17  | 1.75        | 12.73   | 0.74  | 0.06  | 11.58       |
| F4 94-000-95                  | 1.78      | 0.61     | 0.11     | 0.38              | 0.59  | 2.34    | 0.27  | 0.82        | 4.71    | 0.01  | 0.05  | 4.03        |

|  | pic 5 | pic 6 | terpinolene | linalol | pic 7 | borneol | $\alpha$ -terpineol | linalyl acetate | thymol | carvacrol | b-caryophyllene | pic 8 | pic 9 | pic 10 | Total |
|--|-------|-------|-------------|---------|-------|---------|---------------------|-----------------|--------|-----------|-----------------|-------|-------|--------|-------|
| <b>THYMOL group</b>                        |       |       |             |         |       |         |                     |                 |        |           |                 |       |       |        |       |
| 0.69                                       | 0.08  | 0.09  | 0.03        | 0.16    | 0.29  | 0.19    | 0.00                | 58.37           | 2.53   | 1.55      | 0.13            | 0.45  | 0.15  | 99.87  |       |
| 0.54                                       | 0.08  | 0.09  | 0.04        | 0.22    | 0.22  | 0.15    | 0.03                | 54.47           | 5.17   | 1.03      | 0.05            | 0.65  | 0.08  | 99.86  |       |
| 0.55                                       | 0.11  | 0.06  | 0.17        | 0.12    | 0.30  | 0.10    | 0.00                | 57.37           | 1.89   | 1.09      | 0.10            | 0.42  | 0.12  | 99.78  |       |
| 0.62                                       | 0.10  | 0.09  | 0.05        | 0.13    | 0.32  | 0.21    | 0.18                | 69.06           | 3.36   | 0.89      | 0.09            | 0.57  | 0.12  | 99.74  |       |
| 0.47                                       | 0.08  | 0.07  | 0.02        | 0.08    | 0.29  | 0.13    | 0.00                | 71.51           | 3.14   | 1.18      | 0.11            | 0.40  | 0.09  | 99.79  |       |
| 0.53                                       | 0.09  | 0.12  | 0.00        | 0.20    | 0.31  | 0.10    | 0.00                | 61.15           | 2.48   | 0.82      | 0.13            | 0.86  | 0.09  | 99.36  |       |
| 0.66                                       | 0.08  | 0.10  | 0.00        | 0.08    | 0.31  | 0.09    | 0.00                | 70.24           | 2.99   | 0.61      | 0.00            | 0.35  | 0.12  | 99.88  |       |
| 0.66                                       | 0.10  | 0.12  | 0.00        | 0.07    | 0.29  | 0.14    | 0.06                | 70.69           | 3.09   | 1.01      | 0.08            | 1.02  | 0.08  | 99.98  |       |
| 0.57                                       | 0.08  | 0.11  | 0.00        | 0.13    | 0.27  | 0.21    | 0.05                | 69.46           | 3.49   | 1.51      | 0.26            | 0.51  | 0.10  | 99.82  |       |
| 0.60                                       | 0.08  | 0.08  | 0.00        | 0.11    | 0.29  | 0.11    | 0.00                | 63.28           | 2.65   | 0.78      | 0.13            | 0.99  | 0.07  | 99.99  |       |
| 0.68                                       | 0.11  | 0.09  | 0.05        | 0.23    | 0.45  | 0.17    | 0.00                | 63.83           | 4.11   | 0.67      | 0.04            | 0.64  | 0.07  | 101.85 |       |
| 0.52                                       | 0.06  | 0.06  | 0.18        | 0.07    | 0.21  | 0.15    | 0.05                | 69.83           | 2.82   | 0.90      | 0.14            | 0.56  | 0.07  | 99.86  |       |
| 0.44                                       | 0.09  | 0.07  | 0.22        | 0.11    | 0.30  | 0.21    | 0.00                | 74.06           | 3.74   | 0.56      | 0.00            | 0.94  | 0.07  | 99.56  |       |
| 0.56                                       | 0.07  | 0.07  | 0.17        | 0.06    | 0.20  | 0.13    | 0.00                | 74.75           | 4.33   | 0.29      | 0.05            | 0.57  | 0.00  | 99.72  |       |
| 0.66                                       | 0.07  | 0.06  | 0.17        | 0.09    | 0.19  | 0.13    | 0.00                | 60.76           | 4.08   | 0.60      | 0.08            | 1.15  | 0.13  | 99.71  |       |
| 0.51                                       | 0.10  | 0.15  | 0.00        | 0.25    | 0.39  | 0.25    | 0.24                | 62.29           | 2.44   | 0.96      | 0.18            | 0.93  | 0.24  | 99.63  |       |
| 0.55                                       | 0.07  | 0.00  | 0.04        | 0.31    | 0.32  | 0.15    | 0.12                | 64.68           | 2.33   | 1.06      | 0.20            | 1.28  | 0.20  | 99.61  |       |
| 0.51                                       | 0.10  | 0.10  | 0.00        | 0.26    | 0.42  | 0.16    | 0.00                | 64.83           | 2.87   | 0.69      | 0.12            | 0.75  | 0.08  | 99.85  |       |
| 0.78                                       | 0.10  | 0.14  | 0.00        | 0.10    | 0.30  | 0.08    | 0.00                | 56.54           | 2.45   | 0.88      | 0.12            | 0.14  | 0.00  | 99.70  |       |
| 0.64                                       | 0.08  | 0.11  | 0.12        | 0.20    | 0.35  | 0.15    | 0.00                | 74.89           | 3.65   | 0.53      | 0.00            | 0.60  | 0.00  | 99.85  |       |
| 0.70                                       | 0.07  | 0.07  | 0.00        | 0.11    | 0.31  | 0.13    | 0.00                | 65.03           | 3.01   | 0.90      | 0.15            | 0.39  | 0.20  | 99.87  |       |
| 0.69                                       | 0.11  | 0.17  | 0.00        | 0.22    | 0.47  | 0.18    | 0.00                | 60.34           | 2.10   | 0.95      | 0.09            | 0.81  | 0.17  | 99.92  |       |
| 0.49                                       | 0.08  | 0.12  | 0.00        | 0.16    | 0.32  | 0.14    | 0.00                | 56.66           | 2.44   | 1.06      | 0.19            | 0.49  | 0.10  | 99.74  |       |
| 0.63                                       | 0.08  | 0.18  | 0.00        | 0.40    | 0.29  | 0.13    | 0.00                | 68.85           | 3.64   | 0.64      | 0.00            | 0.97  | 0.43  | 99.68  |       |
| 0.78                                       | 0.06  | 0.14  | 0.04        | 0.11    | 0.27  | 0.13    | 0.06                | 68.81           | 7.41   | 0.88      | 0.08            | 0.36  | 0.21  | 99.89  |       |
| 0.76                                       | 0.08  | 0.12  | 0.04        | 0.19    | 0.29  | 0.13    | 0.00                | 71.04           | 3.68   | 0.64      | 0.10            | 0.19  | 0.22  | 99.61  |       |
| 0.62                                       | 0.07  | 0.09  | 0.03        | 0.15    | 0.13  | 0.16    | 0.00                | 69.42           | 2.84   | 0.87      | 0.06            | 0.51  | 0.13  | 99.42  |       |
| 0.72                                       | 0.09  | 0.09  | 0.03        | 0.08    | 0.27  | 0.19    | 0.00                | 65.55           | 2.96   | 1.20      | 0.00            | 0.92  | 0.09  | 99.70  |       |
| 0.41                                       | 0.09  | 0.09  | 0.11        | 0.09    | 0.20  | 0.08    | 0.00                | 55.96           | 2.49   | 0.47      | 0.00            | 0.33  | 0.00  | 99.77  |       |
| 0.75                                       | 0.09  | 0.10  | 0.02        | 0.09    | 0.27  | 0.13    | 0.00                | 68.76           | 5.61   | 0.80      | 0.07            | 1.32  | 0.21  | 99.80  |       |
| 0.70                                       | 0.10  | 0.07  | 0.00        | 0.12    | 0.29  | 0.17    | 0.00                | 67.40           | 3.42   | 0.58      | 0.05            | 0.89  | 0.20  | 99.73  |       |
| 0.54                                       | 0.07  | 0.15  | 0.00        | 0.10    | 0.32  | 0.16    | 0.00                | 59.56           | 5.17   | 0.79      | 0.12            | 0.59  | 0.10  | 99.91  |       |
| 0.66                                       | 0.09  | 0.09  | 0.02        | 0.13    | 0.29  | 0.16    | 0.00                | 63.72           | 3.23   | 0.88      | 0.15            | 0.74  | 0.07  | 99.41  |       |
| 0.66                                       | 0.07  | 0.14  | 0.00        | 0.16    | 0.29  | 0.16    | 0.00                | 63.17           | 4.46   | 0.54      | 0.06            | 0.38  | 0.12  | 99.94  |       |
| 0.71                                       | 0.07  | 0.10  | 0.03        | 0.15    | 0.30  | 0.14    | 0.00                | 63.69           | 5.58   | 0.28      | 0.09            | 0.65  | 0.05  | 99.94  |       |
| <b>CARVACROL group</b>                     |       |       |             |         |       |         |                     |                 |        |           |                 |       |       |        |       |
| 0.59                                       | 0.09  | 0.09  | 0.03        | 0.14    | 0.36  | 0.13    | 0.04                | 0.47            | 72.83  | 1.63      | 0.07            | 0.59  | 0.06  | 99.88  |       |
| 0.62                                       | 0.09  | 0.11  | 0.04        | 0.44    | 0.50  | 0.10    | 0.07                | 0.15            | 78.60  | 2.00      | 0.15            | 0.07  | 0.15  | 99.37  |       |
| 0.65                                       | 0.07  | 0.15  | 0.00        | 0.13    | 0.34  | 0.08    | 0.05                | 0.26            | 76.68  | 1.44      | 0.22            | 0.20  | 0.29  | 99.63  |       |
| 0.86                                       | 0.14  | 0.17  | 0.00        | 0.15    | 0.33  | 0.00    | 0.00                | 0.57            | 51.52  | 0.76      | 0.00            | 0.29  | 0.00  | 99.86  |       |
| 0.59                                       | 0.17  | 0.12  | 0.14        | 0.13    | 0.30  | 0.13    | 0.00                | 0.18            | 52.94  | 1.59      | 0.00            | 0.35  | 0.00  | 99.76  |       |
| 0.52                                       | 0.07  | 0.12  | 0.02        | 0.11    | 0.33  | 0.09    | 0.00                | 2.01            | 72.32  | 0.66      | 0.10            | 0.61  | 0.14  | 99.92  |       |
| 0.48                                       | 0.06  | 0.14  | 0.02        | 0.16    | 0.28  | 0.09    | 0.00                | 2.31            | 81.09  | 1.05      | 0.19            | 0.24  | 0.18  | 99.53  |       |
| 0.41                                       | 0.06  | 0.13  | 0.08        | 0.19    | 0.33  | 0.09    | 0.00                | 0.49            | 81.49  | 0.66      | 0.00            | 0.42  | 0.18  | 99.67  |       |
| 0.36                                       | 0.06  | 0.11  | 0.24        | 0.18    | 0.31  | 0.06    | 0.00                | 0.48            | 82.14  | 1.27      | 0.12            | 0.60  | 0.22  | 99.58  |       |
| 0.00                                       | 0.05  | 0.15  | 0.32        | 0.25    | 0.42  | 0.13    | 2.94                | 0.27            | 51.26  | 2.67      | 0.14            | 0.96  | 2.19  | 96.11  |       |
| 0.52                                       | 0.10  | 0.08  | 0.17        | 0.11    | 0.47  | 0.13    | 4.46                | 0.46            | 64.96  | 2.35      | 0.37            | 1.47  | 0.13  | 98.74  |       |
| <b>THYMOL-CARVACROL group</b>              |       |       |             |         |       |         |                     |                 |        |           |                 |       |       |        |       |
| 0.72                                       | 0.09  | 0.11  | 0.00        | 0.19    | 0.39  | 0.12    | 0.00                | 30.58           | 43.61  | 0.55      | 0.00            | 0.34  | 0.09  | 99.99  |       |
| 0.39                                       | 0.07  | 0.10  | 0.10        | 0.12    | 0.28  | 0.11    | 0.00                | 36.30           | 42.12  | 0.67      | 0.10            | 0.32  | 0.17  | 99.60  |       |
| 0.59                                       | 0.04  | 0.16  | 0.04        | 0.15    | 0.22  | 0.09    | 0.00                | 46.54           | 36.83  | 0.26      | 0.00            | 0.96  | 0.09  | 99.37  |       |
| <b><math>\gamma</math>-TERPINENE group</b> |       |       |             |         |       |         |                     |                 |        |           |                 |       |       |        |       |
| 0.00                                       | 0.14  | 0.20  | 0.00        | 0.35    | 0.35  | 0.27    | 0.04                | 0.24            | 14.42  | 0.94      | 0.16            | 0.89  | 0.34  | 99.72  |       |
| 0.00                                       | 0.11  | 0.13  | 0.05        | 0.29    | 0.33  | 0.18    | 0.00                | 0.75            | 13.50  | 0.77      | 0.13            | 0.60  | 0.20  | 99.87  |       |
| <b>CARVACROL-thymol group</b>              |       |       |             |         |       |         |                     |                 |        |           |                 |       |       |        |       |
| 0.52                                       | 0.12  | 0.13  | 0.04        | 0.26    | 0.36  | 0.09    | 0.00                | 14.20           | 46.15  | 0.88      | 0.12            | 0.58  | 0.00  | 99.85  |       |
| 0.65                                       | 0.09  | 0.11  | 0.42        | 0.23    | 0.38  | 0.17    | 0.00                | 17.30           | 45.98  | 0.32      | 0.06            | 0.45  | 0.24  | 99.77  |       |
| 0.80                                       | 0.06  | 0.16  | 0.04        | 0.15    | 0.25  | 0.05    | 0.00                | 22.34           | 58.51  | 0.52      | 0.00            | 0.81  | 0.29  | 99.68  |       |

## *Origanum majorana* L. – some experiences from Eastern Germany

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### **Abstract**

The collaborative activity related to the *Origanum* germplasm collection of the Gatersleben genebank is presented, along with details regarding its management. Gatersleben is situated in the German State of Saxon-Anhalt. In the same State there is also a large *Origanum majorana* growing area, the largest in the country. About 500 ha are being cultivated with the help of contract-growing arrangements between growers and processing factories. Information on the large production programme, organized by the specialised factory MAWEA, is presented.

### **Introduction**

The cultivation of marjoram (*Origanum majorana* L.) has a long historical tradition in the eastern parts of Germany. For more than 100 years marjoram has been cultivated in the area around the town of Aschersleben (Heeger 1956). Based on this tradition, marjoram is still grown today on 550 ha in Germany. More than 95% of this cultivation is practised in the small area near Aschersleben in Saxony-Anhalt. This area produces about 8% of the whole medicinal and aromatic plant cultivation in Germany.

In the same area, the genebank of IPK (Institute of Plant Genetics and Crop Plant Research) has a large collection of medicinal and aromatic plants (Hammer 1993). Joint programmes have been developed between the genebank and MAWEA, several of which deal with aromatic plants such as *Ocimum* spp. (Junghanns and Hammer 1994), marjoram and other species of the genus *Origanum*, recently included in the joint investigations.

### **Cultivation**

The basic cultivation requirements for growing these aromatic species are good climatic conditions and a highly fertile soil. The fields have to be without stones. Marjoram is mainly cultivated after legumes or potatoes and it comes in the crop rotation before wheat or barley. In autumn the field has to be well-ploughed and levelled, and in spring needs to be ready for sowing. Mid-April is the best time to sow marjoram. This is sown directly in the field at 0.5 to 1.0 cm depth (Fig. 1). Sowing equipment, which should be able to guarantee the even laying of the seed, is very important to ensure the success of this operation. To answer this specific need, special sowing machines have therefore been built in cooperation with both farmers and agrotechnical and processing industries.

The most delicate time during the crop cultivation is the seed-germination stage. Most damage during the whole growing period occurs at this time, fungal diseases and bad weather conditions (heavy rainfall, hot and dry periods) being some of the hazards for the crop. After this stage, and during the growing up of the plantlet

(Fig. 2), another very important factor is represented by weed control. Herbicide use is possible but it is nevertheless also necessary to have mechanical weed control (3 to 5 times) and a final manual weed control on all marjoram fields. The costs of the final manual control alone is about 1000 DM/ha (equivalent to ca. US\$650).

In August, marjoram commences flowering and reaches the right stage for harvesting (Fig. 3). Harvesting machines and seed equipment are both adapted from machines used for other agricultural operations (Fig. 4). The yield is about 2.5 t/ha of dried total plant weight with a basic moisture content of 14%. The harvested material is directly dried using specialised drying equipment available on the farms.



**Fig. 1.** Sowing of marjoram in the second half of April.



**Fig. 2.** Established field of marjoram, 7 weeks old.



**Fig. 3.** Marjoram at harvesting stage (beginning of flowering) in the second half of August (field of ca. 40 ha).



**Fig. 4.** Marjoram harvester (developed from a sugar beet harvester).

## Processing

All herb crops are being cultivated under contract growing systems. This type of contract takes into account aspects such as quality parameters and time of delivery, as well as a free-of-charge supervision from sowing to harvest by the contracting firm. Once dried, the material is delivered by each farm to the processing partner. This processing factory assesses both the quantity and the quality of the material received and stores it throughout the processing period.

Processing starts first with a mechanical division and passes through more than 20 steps until cleaned pure leaves are produced. Among the most important quality

parameters in the final product are (1) leaf content higher than 99.5%, (2) total ash content max. 12%, (3) essential oil content more than 1.2% and (4) moisture content max. 14%. The processing steps are controlled by a quality-security system and all necessary parameters are standardised. The final product obtained at the end of the processing cycle is marketed along with an informative note containing all quality parameters relevant to its proper use in the food industry and at home.

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## Cultivation, selection and conservation of oregano species in Israel

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### Abstract

Two species of oregano, *Origanum vulgare* L. and *O. syriacum* L. (syn. *Majorana syriaca* L. = 'za'atar' in Arabic), are grown commercially in Israel for use as fresh and dry herbs. The two species have been selected from wild populations originating from Israel and Greece. Their combined export value is estimated at US\$ 3 million per year. Another species of oregano, *O. dayi*, is reported to grow spontaneously in the northern part of the Negev; this species is a rather rare taxon and has never been cultivated. The selection of high-quality cultivated varieties has been a result of the availability of large genetic diversity gathered during extensive germplasm-collecting missions targeting wild *Origanum* populations. Because of the very small size of oregano seeds, the species' perennial habit and the fact that the plant is harvested more than once in a year, the crop is propagated by stem cuttings planted directly in the field. In Israel, oregano germplasm collections are conserved both as living plants and as seed.

### Introduction

Oregano (Labiatae family) is a perennial species which grows spontaneously in areas across the Mediterranean region, particularly in hilly locations. In these areas oregano is harvested mainly from wild populations, once or twice a year, at flowering stage.

About 20 years ago, when we started research and development projects on aromatic plants, we decided to focus on Mediterranean herbs such as oregano, sage, satureja and thyme, which thrive in the wild, under similar climatic conditions (Ravid and Putievsky 1985, 1986).

Our search for high-quality oregano varieties ('Greek type', i.e. *Origanum vulgare*) among the material of seed companies and botanical gardens ended without any positive results. Therefore we organized a germplasm-collecting trip to Greece where we sampled more than 70 different oregano populations from all over the country.

The same approach was followed for *Origanum syriacum*, for which more than 25 populations were found in Israel, shortly after this species became a protected plant in the country.

Plants of both species, produced from seeds or cuttings, were grown for 3 years under intensive cultivation systems: three harvests per year were made, yield data were gathered and selected plants were then propagated (from cuttings) to establish a uniform plot of 12 m<sup>2</sup> each. For each species, 20 to 25 plots were eventually established. Part of the results that are being presented in this paper originate from this trial.

### Agrotechnique and physiology

The very small size of oregano and za'atar seeds (1000 seeds weigh  $\pm 0.1-0.2$  g), and the low germination rate of the material originating from the wild (Cohen *et al.* 1980; Putievsky 1983), persuaded us to learn more about stem-cutting propagation in those species (Kuris *et al.* 1980, 1981; Putievsky *et al.* 1983b; Raviv *et al.* 1985) to enable establishment of commercial fields from selected material. Thanks to these studies, today we are able to propagate oregano (as well as other spices) by means of unrooted stem cuttings. This specific agrotechnique allows us to propagate the plants directly in the same field from which the cutting was taken (Putievsky 1978a; Ravid and Putievsky 1987).

Data on yield components were collected during several years of commercial growth (Putievsky and Basker 1977; Putievsky and Kuris 1979) during which herbicides (Putievsky *et al.* 1977) and drying processes were used (Putievsky *et al.* 1982; Segal *et al.* 1982). Agronomic aspects that were studied in this work included the effect of fertilization (Putievsky 1978b; Putievsky *et al.* 1990), irrigation (Sanderovich 1983; Sanderovich *et al.* 1984; Putievsky *et al.* 1991), date of harvest (Putievsky and Chizer 1978; Putievsky and Ravid 1984; Dudai *et al.* 1986, 1988a) and plant height (Putievsky *et al.* 1989) on yield components during at least 2 years of growth. Some experiments were carried out with pure clones (e.g. harvest), while others (fertilization, herbicides, etc.) with different clones in order to find the best treatments for each of those clones expected to be used for commercial purposes.

In addition to these studies dealing with agrotechnique systems, it was decided to focus attention also on physiological aspects, in view of the relevant role that these play in controlling – or at least influencing to some extent – both the quality and quantity of the final product. Studies were conducted on environmental factors (Dudai *et al.* 1994), including the effects of temperature and daylength (Cohen *et al.* 1980; Putievsky 1983; Dudai 1988; Putievsky *et al.* 1988; Dudai *et al.* 1989). Among the results obtained in these investigations is the finding that oregano plants, like many other species, grown under different temperatures or different light intensity flower earlier as temperature and light increase (Table 1).

**Table 1.** Effect of temperatures and shade on date of flowering and full bloom of *O. syriacum* plants.

| Day/night<br>temp. (°C) | No. days from starting LD <sup>†</sup> to |            | Shade<br>(%) <sup>‡</sup> | Date of            |            |
|-------------------------|---|------------|---------------------------|--------------------|------------|
|                         | onset of flowering                        | full bloom |                           | onset of flowering | full bloom |
| 17/12                   | 82  | 105        | 0                         | 5.5                | 18.5       |
| 22/17                   | 51  | 63         | 30                        | 15.5               | 25.5       |
| 27/22                   | 44  | 52         | 50                        | 19.5               | 29.5       |
| 32/27                   | 40  | 52         | 70                        | 22.5               | 18.6       |

<sup>†</sup> LD= long day – 16-h photoperiods.

<sup>‡</sup> Plants grown outdoors under screens giving various percentages of shade, according to Dudai *et al.* (1989).

As the day became longer – due to natural or artificial light – essential oil content increased and its composition changed (Table 2). On the basis of these results it is apparent that the origin of this variation is linked to changes in the photosynthesis of the plant.

The relationship between number of leaves per plant and plant parts and the essential oil content and composition, as well as number and type of glandular hairs (Werker *et al.* 1985a, 1985b; Dudai *et al.* 1988c) is presented in Tables 3 and 4 respectively.

**Table 2.** Effects of daylength and light on the essential oil (EO) content and composition in leaves of the thymol chemotype of *Origanum syriacum*.

| Daylength <sup>†</sup> | EO content (%) | Oil composition (% of total oil) |          |        |           |
|------------------------|----------------|----------------------------------|----------|--------|-----------|
|                        | in fresh leaf  | $\gamma$ -terpinene              | p-cymene | thymol | carvacrol |
| Short day (8+0)        | 0.38b          | 3b                               | 44a      | 38c    | 1b        |
| Long day (8+8)         | 0.82a          | 9a                               | 20b      | 57b    | 2a        |
| Long day (12+4)        | 0.88a          | 11a                              | 14c      | 65a    | 3a        |

<sup>†</sup> Hours of natural light + incandescent light.

Within columns, figures followed by the same letter do not differ significantly at  $P < 0.05$  (according to Dudai *et al.* 1992).

**Table 3.** Percentages of main constituents of essential oil in *Origanum syriacum* leaves at different ages.

| Leaf no. (from top) | Leaf length (mm) | $\gamma$ -terpinene | p-cymene | thymol | carvacrol | Total |
|---------------------|------------------|---------------------|----------|--------|-----------|-------|
| 1 <sup>†</sup>      | 4                | 4.0                 | 3.8      | 55.8   | 4.9       | 68.5  |
| 2                   | 8                | 26.5                | 16.5     | 45.0   | 0.7       | 88.7  |
| 3                   | 11               | 10.2                | 8.5      | 66.7   | 0.4       | 85.8  |
| 4                   | 14               | 5.3                 | 1.2      | 81.6   | 0.2       | 88.3  |
| 5                   | 16               | 4.8                 | 6.6      | 79.2   | 0.4       | 90.0  |
| 6                   | 18               | tr <sup>‡</sup>     | tr       | 79.8   | 3.5       | 83.3  |
| 7                   | 18               | tr                  | tr       | 74.6   | tr        | 74.6  |
| 8                   | 18               | tr                  | tr       | 75.5   | tr        | 75.6  |

<sup>†</sup> Also includes all the younger leaves to the shoot apex.

<sup>‡</sup> Traces. According to Werker *et al.* (1985b).

**Table 4.** Essential oil (EO) content and composition in two chemotypes of *Origanum syriacum*.

| Plant part            | Chemo-type <sup>†</sup> | Fresh matter   |                | Relative percentages of main essential oil components |                      |                      |           |                |         |            |
|-----------------------|-------------------------|----------------|----------------|---|----------------------|----------------------|-----------|----------------|---------|------------|
|                       |                         | % <sup>‡</sup> | EO content (%) | $\alpha$ + $\beta$ -pinene                            | $\alpha$ -terp-inene | $\gamma$ -terp-inene | p-cy-mene | caryo-phyllene | thy-mol | carv-acrol |
| Leaves                | A                       | 44             | 1.3            | 1.4   | 1.6                  | 7.6                  | 11.7      | 0.7            | 0.7     | 70.0       |
|                       | B                       | 29             | 1.8            | 2.4   | 2.8                  | 17.4                 | 14.6      | 0.6            | 52.8    | 2.8        |
| Calyces               | A                       | 19             | 2.4            | 2.4   | 1.6                  | 7.5                  | 3.3       | 0.4            | 0.5     | 78.6       |
|                       | B                       | 31             | 2.6            | 2.6   | 2.9                  | 12.8                 | 6.3       | 0.8            | 64.9    | 3.9        |
| Corollas <sup>§</sup> | A                       | 4              | 1.3            | 1.3   | 0.4                  | 2.2                  | 3.9       | 1.1            | 1.3     | 84.3       |
|                       | B                       | 3              | 1.7            | 1.6   | 2.0                  | 10.2                 | 9.0       | 1.3            | 64.4    | 5.3        |

<sup>†</sup> Chemotype A: carvacrol var. 28/x; chemotype B: thymol var. 27/241.

<sup>‡</sup> Including stems.

<sup>§</sup> Including stamens. According to Dudai *et al.* (1988c).

In Tables 5 and 6 the maximum essential oil content in the two species appears in different seasons, i.e. in summer (June) for oregano, in early autumn (September) for za'atar. In oregano, a phenol-rich plant, the most common essential oil compounds are the two monoterpene hydrocarbons  $\gamma$ -terpinene and p-cymene, which are the biogenetic precursors (via enzymic hydroxylation) of the two phenolic terpenes, thymol and carvacrol. Therefore the content of  $\gamma$ -terpinene decreased as flowering progressed, and in non-flowering plants in early summer the thymol content in the essential oil increased. On the other hand, no such correlation was found in za'atar material (Table 5): in this species the flowering stage was accompanied by a reduction in essential oil and phenolic monoterpene content (Table 6) along with other plant morphological changes (Putievsky and Ravid 1984; Dudai 1988; Dudai *et al.* 1986, 1988a, 1988c, 1989, 1992, 1994; Putievsky *et al.* 1988).

Table 5. Essential oil content and its two main components in *Origanum vulgare* and *O. syriacum* during the growing season.

| Month | Essential oil content<br>in fresh matter (%) |             | Content of two main components (%) in essential oil |           |                     |           |
|-------|--|-------------|---|-----------|---------------------|-----------|
|       | Oregano                                      | Za'atar     | Oregano   |           | Za'atar             |           |
|       |  |             | $\gamma$ -terpinene                                 | thymol    | $\gamma$ -terpinene | thymol    |
| 3     | 0.51   | 0.31        | 15  | 48        | 27                  | 52        |
| 4     | 0.85   | 0.39        | 18  | 57        | <b>34</b>           | 50        |
| 5     | 0.96   | 0.51        | 19  | <b>68</b> | 31                  | 43        |
| 6     | <b>1.07</b>                                  | 0.85        | 20  | 54        | 16                  | <b>57</b> |
| 7     | 0.92   | 1.01        | <b>28</b>   | 38        | 19                  | 50        |
| 8     | 0.90   | 1.10        | 24  | 30        | 20                  | 48        |
| 9     | 0.73   | <b>1.21</b> | 21  | 28        | 21                  | 46        |
| 10    | 0.60   | 1.20        | 20  | 43        | 18                  | 46        |
| 11    | 0.52   | 1.00        | 16  | 46        | 16                  | 41        |
| 12    | 0.50   | 0.60        | 15  | 48        | 15                  | 37        |

Table 6. The influence of flowering on essential oil content and its main components in oregano (*Origanum vulgare*) and za'atar (*O. syriacum*).

| Flowering<br>stage | Essential oil content<br>in fresh matter (%) |         | Content of two main components (%) in essential oil |        |                     |        |
|--------------------|--|---------|---|--------|---------------------|--------|
|                    | Oregano                                      | Za'atar | Oregano   |        | Za'atar             |        |
|                    |  |         | $\gamma$ -terpinene                                 | thymol | $\gamma$ -terpinene | thymol |
| Vegetation         | 1.0  | 1.2     | 21  | 50     | 8                   | 58     |
| Flowering start    | 1.2  | 1.0     | 20  | 51     | 5                   | 50     |
| Full bloom         | 1.5  | 0.6     | 18  | 53     | 3                   | 46     |
| Seed formation     | 1.8  | 0.5     | 14  | 68     | 2                   | 44     |

### Selection

A high level of variation was found for each character in the material originating from the wild. Such expected variability, once exposed to intensive selection pressure (such as artificial irrigation, frequent harvests, herbicide treatments, etc.), did reduce the population size very rapidly, due to the death of all those plants not fitted to cultivation. This first selection step, combined with single plant selection, is of great economic importance. Furthermore, the method of vegetative propagation – set up for members of the Labiatae family, including oregano – enabled us to preserve and multiply superior selected plants for commercial cultivation purposes in a relatively short time.

An additional hybridisation (intra- and interspecific) programme was also initiated at the institute using open- and hand-pollination systems, involving mainly basil and salvia and, at a later stage, also oregano, satureja, thyme and other species.

With regard to oregano (*O. vulgare*), this work started by sowing in greenhouses seeds collected from wild populations and transplanting the seedlings into experimental plots. Za'atar plants were transferred directly from the wild to the experimental field. After 3 years of growth and observation, plants that represented the range of variation in the field were chosen for further experiments. These plants (lines), forming a separate plot at least 12 m<sup>2</sup>, were propagated vegetatively by stem cuttings, and yield components were collected for a few years before quality clones were eventually recommended to growers.

Our selection work with za'atar included 44 plants (lines=clones) which had been selected from more than 800 plants taken from the wild. Yield components were collected for each plant (=clone) during 6 years of growth. The data on average yield per population/year (from the second year up to the sixth year of

growth) are summarised in Table 7. Some populations (like No. 4) seemed to be superior to others, at least in Newe Ya'ar conditions, as far as the total yield is concerned. However, this was not the case for all examined components (e.g. percentage of essential oil in dry leaves). The main component in the essential oil of each population was thymol or carvacrol (Table 7), but some variation was found in each population (Fleisher *et al.* 1980; Putievsky *et al.* 1983a; Dudai *et al.* 1988b).

The need to combine interesting characteristics in one line can be met by artificial crosses, or by use of male sterile plants, to obtain natural hybridisation (Putievsky 1992, 1993).

**Table 7. Yield components of *Origanum syriacum* clones under cultivation conditions, recorded on plants collected from wild populations (yields represent average values of the second up to the sixth year of growth production).**

| Accession number and origin of the clone | Fresh yields (kg/m <sup>2</sup> ) | Dry leaf yield (kg/m <sup>2</sup> ) | % from fresh matter | Essential oil             |                              |                |
|--|-----------------------------------|-------------------------------------|---------------------|---------------------------|------------------------------|----------------|
|  |                                   |                                     |                     | Content (% in dry leaves) | Yields (cc/ m <sup>2</sup> ) | Main component |
| 4 - Carmel mountain                      | 3.0 - 3.8                         | 540 - 704                           | 18.0 - 18.5         | 4.1 - 4.7                 | 26-29                        | thymol         |
| 12 - West Bank (Uria)                    | 1.9 - 3.0                         | 257 - 523                           | 13.5 - 17.4         | 3.2 - 5.0                 | 11-25                        | thymol         |
| 18 - West Bank (Chalchol)                | 2.6 - 3.2                         | 378 - 488                           | 14.5 - 15.7         | 3.6 - 4.2                 | 15-17                        | carvacrol      |
| 27 - Yoqneam (Yizreel valley)            | 3.0 - 3.3                         | 488 - 546                           | 16.3 - 17.0         | 4.0 - 4.8                 | 20-26                        | thymol         |
| 28 - Yoqneam (Yizreel valley)            | 2.6 - 3.7                         | 496 - 594                           | 16.0 - 19.1         | 3.6 - 5.1                 | 21-26                        | carvacrol      |

Over the last few years, papers dealing with this selection programme have been published, with regard to thymol chemotypes (Putievsky and Ravid 1982; Putievsky *et al.* 1987) and carvacrol types (Putievsky and Ravid 1982, 1984; Ravid and Putievsky 1985; Putievsky *et al.* 1987).

Today, commercial fields of clones of both thymol and carvacrol types from both species are grown in Israel. Different species and clones are grown for different purposes: for fresh market, for the dried leaves industry and for essential oil production. In some cases the same field is harvested for both dried leaves (spring and autumn) and essential oil production (mainly the summer harvest).

Both species of oregano are conserved in the country as living plants in the field (all clones, selected from the first collections) and as seed (obtained in open-pollination situations), collected from the same living plants and stored in dry cold conditions (6-8°C).

With regard to the priority actions needed at the international level, to ensure a proper conservation of oregano genetic resources we would like to recommend the following initiatives:

- genetic diversity: building of a Herbarium collection as a reference material;
- seed-conservation management: research study on germination procedures and standards, and all major aspects related to seed storage;
- better use: setting up a living collection for proper classification and seed production;
- species conservation: establishment of a seedbank collection.

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## Experiences with oregano (*Origanum* spp.) in Slovenia

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### Abstract

The paper focuses on different aspects of the present knowledge on oregano (*Origanum* spp.) in Slovenia. In this country wild oregano (*Origanum vulgare* L.) is recorded from 50 localities, mainly on limestone, brown and rendzinas soils and altitudes ranging from 100 to 1500 m asl. Its geographic distribution is not only limited to the sub-Mediterranean region. Although, at present, wild oregano populations are not endangered in their natural habitats, the survey of its distribution across the country and sampling of its diversity have been initiated to ensure the proper preservation of its genepool. Evaluation and comparison trials of wild (*O. vulgare*) and introduced (*O. heracleoticum* L.) populations of oregano are also being carried out to investigate the potential of oregano as a spice and a medicinal plant. The most convenient micropropagation procedure for clonal propagation of oregano is presented here. Experience in the country with regard to the cultivation of introduced 'southern' oregano (*O. heracleoticum* L.) is also recorded. The first cultivation trial of this species began in 1984 in the sub-Mediterranean region of Istria, but thereafter several additional studies aimed at estimating the influence of pedoclimatic conditions on growth, development, yield and quality, as well as herbicide selectivity of introduced southern oregano were undertaken. In the Istrian area, maximum yield (9600 kg/ha dry weight per year, two harvests/year) of oregano was achieved in the third year of cultivation, with a plant density of 63 500 plants/ha, carried out on a terra rossa type of soil. In this area, the first harvest takes place between May and June and the second in mid-August. Essential oil content ranged between 3.1 and 4.1%, with high levels of carvacrol. Presently, southern oregano is cultivated on a relatively small area (1-2 ha). Interest in its cultivation among farmers is on the increase.

### Introduction

Many discrepancies among taxonomists are found with regard to the number of species, subspecies and botanical varieties in the genus *Origanum* (Melegari *et al.* 1995), which often leads to some confusion. Nevertheless, if we were to follow Ietswaart's taxonomic revision (Ietswaart 1980; Tucker 1986) *Origanum vulgare* L. with its six subspecies seems to be the largest species in the genus. Because of their large variability in chemical and aroma characteristics, *Origanum* ecotypes (biotypes) offer, besides their common use as a culinary herb or as raw material for the extraction of flavouring substances for foods, a broad variety of possibilities for alternative use in agriculture and the pharmaceutical and cosmetic industries. The essential oils of *Origanum onites* (Turkish oregano), *O. vulgare* subsp. *hirtum* (Greek oregano) and *O. dictamnus* (Cretan dittany) possess antibacterial (Aureli *et al.* 1992; Biondi *et al.* 1993; Vokou *et al.* 1993) and/or antifungal properties (Arras and Picci 1984; Guérin and Réveillère 1985; Colin *et al.* 1989; Paster *et al.* 1993). Also, antimicrobial action is reported for *O. vulgare* extracts (Mirovich *et al.* 1989; Izzo *et al.* 1995) which contained phenolcarboxylic acids (identified as cinamic, caffeic, p-hydroxybenzoic, syringic, protocatechuic, vanillic acid) as presumably active

substances (Mirovich *et al.* 1989). Fumigant toxicity of oregano essential oils for store-room insects also has been confirmed (Shaaya *et al.* 1991).

Traditionally, '*Origanum vulgare herba*' is used in respiratory tract disorders – like cough or bronchial catarrh – as an expectorant and spasmolytic agent, in gastrointestinal disorders as a choleric, digestive, eupeptic and spasmolytic agent, and in urinary tract disorders as a diuretic and antiseptic agent. With regard to these properties, we should say that extensive documentation on the supposed drug effects of *O. vulgare* (Repertorio fitoterapico 1994) was in fact submitted to the attention of the department dealing with phytotherapeutic issues of the German Ministry of Health in 1988 for official endorsement. The Commission in charge of this matter did not actually approve the report (Banz. No. 122 of 6/7/88), owing to the lack of scientific evidence to support the above-mentioned properties. Nevertheless, in spite of the negative outcome, we feel fairly comfortable in believing the reports of the spasmolytic effects for *Origanum* spp. as these species do contain flavonoids (flavanone group – naringin, flavone group – apigenin and luteolin, flavonol group – quercetin) and flavonoid-glycosides (luteoline-7-glucoside, apigenin-7-glucoside) (Harvala and Skaltsa 1986; Skaltsa and Harvala 1987; Bohm 1988; Soulèlès 1990), some of which are known to be actively involved in this property.

The anti-oxidative effect of *Origanum* (Lamaison *et al.* 1993; Sawabe and Okamoto 1994; Takácsová *et al.* 1995) is probably the consequence of the content of phenolic compounds such as hydroxycinnamic derivatives (terpenoid substances – diterpenes or sesquiterpenes, like rosmarinic acid).

Also, very interesting results have been obtained in Poland, where Skwarek *et al.* (1994) discovered that *O. vulgare* extracts, when applied to ECHO9 Hill virus and cultured in monkey kidney cells, induced the formation of a substance with an interferon-like activity.

The above-mentioned findings on *Origanum*'s alternative uses represent the basis for further pharmacological investigations. However, to successfully pursue their official approval, release and successful commercialization, these curative uses also have to meet quality requirements and standards such as the ISO, NF, DIN standards and EU recommendations. These requirements need to be clearly defined if we are to be successful in promoting the utilization of these species in this area and achieve the marketing of active compounds in EU countries and elsewhere. As in phytotherapy, the basic approach, i.e. in respect of safety, effectiveness and quality, should also be applied to other marketed oregano products to make them competitive with others.

The aim of this contribution is to review the research work done on *Origanum* in Slovenia and present future research activities on conservation and evaluation of oregano natural ecotypes which are intended to be used for future commercial uses.

### ***Origanum* species in Slovenia: present knowledge**

#### **Geographical distribution**

To draw a complete distribution map of wild oregano in Slovenia, the Herbarium of the University of Ljubljana, literature data and floristic/vegetation inventories were consulted. *Origanum vulgare* subsp. *vulgare* is a common species, present in hilly and mountainous areas, particularly on carbonate aggregates, (limestone, brown soils and rendzinas). Generally, wild oregano grows in forest clearings, on forest edges, in shrubberies by the roadside and dry meadows. It occurs from 100 to

1500 m asl but can probably also be found at higher altitudes. It is often found thriving with the following plant associations: *Origanetalia*, *Prunetalia*, *Agropyretalia*, *Quercetalia pubescentis*, *Erico-Pinetalia* and in *Mesobromion* meadow association. Wild oregano populations were found in over 50 localities, most frequently in pre-alpine and in predinaric subsectors (Fig. 1). Although in herbarium or past literature data there was no information on wild oregano distribution in the southwestern and northeastern regions of Slovenia, a floristic inventory carried out in 1994 and 1995 showed 11 localities in the Mediterranean region/north-coastal sector where wild oregano populations were to be found. On the basis of such findings, wild oregano should be considered among those species which most frequently occur in Slovenia. Today wild oregano populations are not under threat of genetic erosion. Further surveys (to be made especially in the Mediterranean region of Slovenia) are likely to increase the number of populations reported to occur in the country. Figure 2 shows the geographic distribution of wild oregano in Slovenia.

The Slovenian national *ex situ* genebank for medicinal and aromatic plants holds several foreign and/or domestic genotypes of *O. vulgare* subsp. *vulgare*. Considering the recent progress made on the mapping of oregano populations in Slovenia, it is likely that in the next 5 years the number of oregano accessions conserved at the Slovenian genebank will steadily increase, owing to the new samples expected to be gathered from these newly discovered populations.



**Fig. 1.** Phytogeographic regions in Slovenia: 1 = Southeast Alpine sector/Alpine region; 2 = Subalpine subsector; 3 = Prepannonian subsector; 4 = Pre-alpine subsector; 5 = Northwestern border of the Predinaric subsector; 6 = Predinaric subsector; 7 = North-coastal sector/ Mediterranean region.

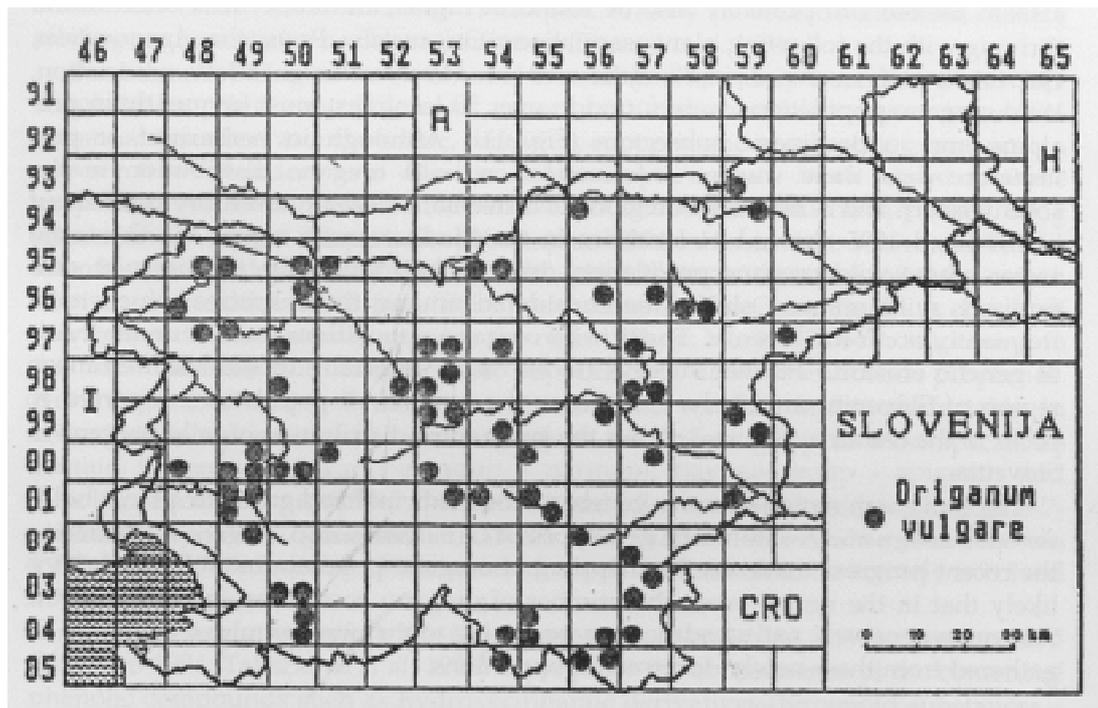


Fig. 2. Geographic distribution of wild oregano (*Origanum vulgare* subsp. *vulgare*) in Slovenia.

#### Tissue culture

Because of the high morphological and chemical variability present in *O. vulgare*, current techniques such as tissue culture have been studied to obtain rapid and economical propagation of the plant, which would ensure homogeneity of traits within each accession. Furthermore, vegetative propagation tends to shorten the juvenile period and induce precocity, a factor that has been observed in *Origanum* and *Lavandula* species (Raviv and Putievsky 1987).

There is not much information about *in vitro* culture of oregano in the current literature with the exception of the Kumari and Saradhi (1992) report on plant regeneration from calli cultures. The Slovenian experience is thus based upon previous micropropagation work carried out in the country (Erzen-Vodenik 1987, 1990; Baricevic 1990) and studies carried out elsewhere in other Labiatae species (Frett 1987; Furmanova and Olszowska 1987; Olszowska and Furmanova 1990).

Slovenian genebank material of *O. vulgare* subsp. *heracleoticum* has been used for setting up the micropropagation procedure. In this investigation, sterilisation of plant material, culture media and rooting capacity were studied. Screening for optimal *in vitro* conditions was also carried out taking into account velocity, morphological uniformity and low cost input in this procedure. The statistical evaluation of the results obtained in the study is underway.

**Methodology.** Stems were collected from adult plants grown in the field. Segments 3-4 mm long were sampled from each node (axillary bud). Concurrently, apical segments were also sampled and introduced into the culture to follow eventual differences in the development of *in vitro* shoots with the other cuttings.

The sterilisation procedure was investigated by using different times (10, 15 or 20 min) of sterilisation of plant cuttings with calcium hypochlorite solutions ( $\text{Ca}(\text{OCl})_2$ ; 5% Cl) after surface-disinfection of plant material with 70% ethanol.

For screening the most convenient basic medium for shoot growth and proliferation, Murashige and Skoog or Nitsche and Nitsche macro- and micro-elements, supplemented with thiamine HCl (0.4 mg/L), myo-inositol (100 mg/L), adenine-sulphate (10 mg/L), sodium hydrogen phosphate ( $\text{NaH}_2\text{PO}_4 \cdot 12 \text{H}_2\text{O}$ ; 0.174 g/L), sucrose (25 g/L) and agar (0.6 g/L) were used. The influence of cytokinins (kinetin, 0.1 or 0.5 mg/L), auxins (IAA or NAA, 0.1 mg/L) or gibberelins ( $\text{GA}_3$ ; 0.005 mg/L) addition to the growth medium was also investigated.

Rooting medium was composed of Murashige and Skoog macro- and micro-elements, sucrose (25 g/L), agar (0.6 g/L) and of synthetic growth regulator  $\text{B}_9$  (Alar 85<sup>R</sup>, active compound Daminozid (85%), Uniroyal Chemical UK; 0.010 g/L). The media, adjusted to pH 5.7, were placed in test tubes (diameter 25 mm, length 150 mm) and autoclaved at a pressure of 49kPa for 30 min.

Cultures were maintained in a growth chamber, where artificial light was provided by parallel fluorescent tubes, installed above and below the cultures (luminous intensity 2000 luxes, i.e.  $31.4 \mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetically active radiation; chamber temperature  $20 \pm 2^\circ\text{C}$ , photoperiod of 12 hours light).

The hairy surface of oregano makes it sensitive to primary-born *in vitro* infections. Infections were found when a 10-min sterilisation with  $\text{Ca}(\text{OCl})_2$  was applied. However, after surface-disinfection with 70% ethanol (30 seconds), sterilisation procedure using 5%  $\text{Ca}(\text{OCl})_2$  containing a few drops of surfactant (Tween 20) for 15 minutes and thereafter rinsing of explants three times with sterile distilled water, a successful healthy culture was obtained with no infections. Longer exposure of plant tissue to  $\text{Ca}(\text{OCl})_2$  solution (20 min) caused leaf damage and low survival of plantlets.

**Results.** Tissue culture has to be set up with axillary buds of oregano because apical cuttings frequently enter the fructification stage *in vitro*, thus weakening the vigour of vegetative shoots. In this case the material cannot be used for further *in vitro* propagation.

The optimal micropropagation medium consisted of Murashige and Skoog (MS) macro- and micro-elements, supplemented with thiamine HCl (0.4 mg/L), myo-inositol (100 mg/L), sodium hydrogen phosphate ( $\text{NaH}_2\text{PO}_4 \cdot 12 \text{H}_2\text{O}$ ; 0.174 g/L); sucrose (25 g/L) and agar (0.6 g/L). Under the above-mentioned conditions, addition of kinetin or other hormones did not improve the proliferation rate (7 to 9 plantlets per 8 weeks) or development of oregano. In the first stage of the culture, gibberelins ( $\text{GA}_3$ ; 0.005 mg/L) induced slight elongation of the internode stem distance. Because of the possible – although presently unknown – side effects of hormones on malformation of clone material and the associated cost, the use of plant hormones in the plant-proliferation stage of oregano is not recommended.

Rooting capacity on simple medium, containing MS macro- and micronutrients, sucrose (25 g/L), agar (6 g/L) and  $\text{B}_9$  (0.010 g/L) root stimulant (which also tends to shorten internode stem distance) was relatively high (95%) and *in vitro* rooting of plantlets was fast enough (10-14 days on average).

In greenhouses oregano plants acclimatised rapidly (3 weeks on average) to *in vivo* growth conditions and the percentage of plantlets that survived in field conditions was surprisingly high (96%). *In vitro* descendants were much more vigorous than seed-propagated plants. This phenomenon also was observed in our experiments on thyme (*Thymus vulgaris* L.) micropropagation procedure.

### Cultivation

Scientists from Slovenia and the Federal Republic of Yugoslavia reported on experiences with oregano cultivation in different regions of former Yugoslavia (Cok and Kota 1989; Kota and Cok 1989; Macko and Cok 1989).

In the Istrian area Greek oregano (*Origanum heracleoticum* L.) was introduced into cultivation in 1984, after preliminary ecological studies on species acclimation and on herb quality were made. Cultivations have been set up in three sub-Mediterranean areas (Dvori-Isola, 260 m asl, limestone brown soils on calcareous flish, plant density 63 500 plants/ha; Smarje-Capodistria, 200 m asl, limestone brown soils on calcareous flish, plant density 57 000 plants/ha; Savudrija, sea level, terra rossa soil type, plant density 63 500 plants/ha). These areas border with the Adriatic sea (45°31' lat.; 300 days of growth period; average temperature in the growth period 15.2°C; average winter temperature 5.5°C; average rainfall in the growth period 912 mm and during winter time 139 mm; 2346 hours of insolation) (Baricevic *et al.* 1995).

In all the localities oregano plants were manually planted in May (Dvori-Isola on 4 May; Smarje-Capodistria on 22 May; Savudrija on 27 May) (Cok and Kota 1989). Plants were harvested at the beginning of the flowering period and naturally dried (dry, airy and shady place). The yield per hectare and the quality (content of essential oil and its composition measured by a gas chromatograph) of dry herb were measured.

Table 1 shows the yield of oregano herb along with location, planting date, plant density and age of the culture. In the case of earlier planting (beginning of May) or of applied irrigation (both practices done in Vojvodina region, Federal Republic of Yugoslavia) harvest of oregano can be achieved in September the first year of cultivation. At Dvori-Isola, planting was completed 3 weeks earlier than at Smarje-Capodistria and Savudrija where, owing to the late planting date, there was no harvest in the first year of cultivation (Table 1).

With regard to the yield of the first cut, the highest herb yield (6600 kg/ha) was obtained at Savudrija (Table 2). According to Cok and Kota (1989) such a high yield in this locality is related to the precocity of oregano springtime growth due to higher warming capacity of terra rossa soil type compared with limestone brown soils. Furthermore, results show the importance of the earliness of the first harvest date for securing second cuttings in the following years of cultivation. For example, the second harvest in the second and subsequent years failed at Dvori, where oregano was harvested for the first time 3 weeks later than the other two localities (Table 1). Yield of the second harvest is usually lower than the first, and takes place in mid-August. The yield of the first harvest done in the third year of cultivation is usually higher than that of the second year. In the Istrian area, maximum yield was achieved in the third year (Savudrija, terra rossa soil, 9600 kg/ha dry weight per year, harvest twice a year) or in the fourth year of cultivation (Dvori-Isola, 9800 kg dry weight/ha, one harvest per year) of oregano cultivation with plant density of 63 500 plants/ha.

The essential oil percentage of Greek oregano grown in Istria ranged between 3.13 and 4.15%. According to gas chromatographic analysis, essential oil of Greek oregano cultivated in this area is of high quality, due to high carvacrol and relatively low tymol content (85.16 and 0.84% respectively).

**Table 1.** Yield (kg/ha) of dry oregano plants (*Origanum heracleoticum* L.) cultivated at different localities in the Istrian region, with respect to plant density, planting date and age of culture (Cok and Kota 1989).

| Locality  | Surface area (m <sup>2</sup> ) | Planting date | Plant density/ha | Year of cultivation |       |       |       |
|-----------|--------------------------------|---------------|------------------|---------------------|-------|-------|-------|
|           |                                |               |                  | 1st                 | 2nd   | 3rd   | 4th   |
| Dvori     | 500                            | May 4         | 63 500           | 600                 | 4.700 | 7.500 | 9.800 |
| Smarje    | 600                            | May 22        | 57 000           | –                   | 6.450 | 9.325 | –     |
| Savudrija | 300                            | May 27        | 63 500           | –                   | 9.200 | 9.600 | –     |

**Table 2.** Yield (kg/ ha) of dry oregano plants (*Origanum heracleoticum* L.) in the first and second cutting period with respect to cultivation sites (Istrian region) and age of culture (from Cok and Kota 1989).

|         |             | Locality |       |         |       |           |       |
|---------|-------------|----------|-------|---------|-------|-----------|-------|
|         |             | Dvori    |       | Smarje  |       | Savudrija |       |
|         |             | Date     | Yield | Date    | Yield | Date      | Yield |
| Year 1: | 1st cutting | Sept 30  | 600   | –       | –     | –         | –     |
|         | 2nd cutting | –        | –     | –       | –     | –         | –     |
| Year 2: | 1st cutting | June 30  | 4.70  | June 10 | 4.215 | May 15    | 6.600 |
|         | 2nd cutting | –        | –     | Aug 15  | 2.235 | Aug 15    | 2.600 |
| Year 3: | 1st cutting | June 8   | 7.500 | May 15  | 6.000 | June 16   | 7.400 |
|         | 2nd cutting | –        | –     | Aug 15  | 3.325 | Aug 15    | 2.200 |
| Year 4: | 1st cutting | June 20  | 9.800 | –       | –     | –         | –     |
|         | 2nd cutting | –        | –     | –       | –     | –         | –     |

In the same Istrian localities, the 2-year investigations were carried out with the aim of determining the quantity of N, P, K, Ca and Mg in dry plants of Greek oregano (Kota and Cok 1989). It is well known that quantities of these macro-elements in the plant depend on location, soil type, age of plantation, number of harvests, plant density and cultivation method. Our investigation found the following contents in the dry herb: nitrogen 0.9425-2.484%, phosphorus 0.181-1.275%, potassium 1.120-1.770%, calcium 0.775-1.891% and magnesium 0.129-0.410%. Nitrogen was the most abundant element, whereas potassium and calcium were 30% lower; phosphorus and magnesium contents were similar to K and Ca and 10 times lower than N.

These data could be considered useful parameters to investigate oregano crop needs for macronutrients. As to this latter aspect, maximum nutrient uptake of Greek oregano in Istrian pedoclimatic conditions ranges as follows: nitrogen 92.4-125.3 kg/ha, phosphorus 13.7-17.8 kg/ha, potassium 112.7-172.5 kg/ha, calcium 77.7-106.2 kg/ha and magnesium 12.7-16.0 kg/ha.

On the basis of 2-year results obtained by studying weed control in Greek oregano, the application of Sinbar herbicide (2 kg/ha), early in spring, at the beginning of the vegetation period was recommended (Macko and Cok 1989). Application of this product in later years resulted in the occurrence of resistant weed species (especially *Cirsium* spp. and *Convolvulus* spp.). Another possibility of chemical weed control, especially in those years when residual action of Sinbar is to be avoided, is represented by the application of the following combination of products: Sinbar (1.5 kg/ha) + Venzar (1.5 kg/ha) or Sinbar (1 kg/ha) + Prometrin (1.5 kg/ha). It is important to point out that application of herbicides at the beginning of the vegetation period of oregano results neither in a decrease of yield nor in a decrease of essential oil content.

### Pharmacology

In 1966 an international project started aiming at the screening of native plants of former Yugoslavia for their potential use in USA and Yugoslavian agriculture (Mayer *et al.* 1971). In the framework of this project 1466 samples belonging to 754 species were sent to Washington DC, USA, for chemical and pharmacological assays. Very interesting results were obtained from these investigations: for instance a very high carvacrol content (60-85%) was found in a biotype of *O. heracleoticum* (= *O. vulgare* spp. *hirtum* (Link) Ietswaart). Such a finding was particularly relevant, in view of the fact that this species is known – according to the USA Cancer Chemotherapy National Service Center – to have high antitumoral activity.

### Further research activities on *Origanum* spp. in Slovenia

#### Strategy on conservation of natural habitats

The awareness of the role that medicinal and aromatic plants play in the continuous search for new active principles and possible development of new products in the pharmaceutical, food and cosmetic industry, along with the 'back to nature' attitude of the public, are among those factors that have stimulated many workers, including us to widen the chemical prospecting of natural resources. At the same time, greater attention to the preservation of the genetic diversity of raw material – medicinal and aromatic plants – and of their ecosystems also became an important issue. It is for this reason that leading world agricultural institutions and pharmaceutical industries have introduced programmes for the inventory of medicinal plants at the national level and for assessing the potentials of natural resources through collecting of wild accessions and their *in situ* and *ex situ* conservation (Wagner and Farnsworth 1990; Widrlechner and Foster 1991; Marwick 1995). The estimation of the endangered status of these species, as well as the study on the sustainable use of wild medicinal and aromatic plants for selection and breeding purposes, also represent an important task for many national research programmes.

An important aspect related to the preservation and sustainable use of plant genetic resources is the standardisation of the characteristics of raw material. This issue has become increasingly important with respect to the medicinal products marketed in the European Community. To abide by these new rules and to contribute effectively to the safeguarding of these species, directives for the national programme dealing with medicinal and aromatic plants have been proposed to the Slovenian Ministry of Agriculture, Food and Forestry. The preservation strategy of natural resources of medicinal and aromatic plants (among which *O. vulgare* and *Salvia officinalis* serve as a model) is an important part of this national programme. The natural resources preservation strategy consists of the four following steps: inventory, active preservation (*in situ* conservation), sustainable use (access only to germplasm collection for *ex situ* conservation purposes) and prevention of mass exploitation of natural resources through successive introduction of cultivation of known medicinal plant genotypes in suitable climatic conditions. Among the many benefits foreseen as a result of this programme focusing on medicinal and aromatic plants, are the following: maintenance of natural equilibrium; improvement of the landscape; contribute to the preservation of natural heritage for future generations; contribute to creating new job opportunities in the area of crop production and processing; contribute to greater safety in the area of natural remedy exploitation;

contribute to the production of more effective and better quality herb remedies, and eventually contribute to enhancing Slovenian competitiveness on the European market.

#### Evaluation of oregano biotypes/genotypes

In consideration of the known geographic variation of *O. vulgare*, morphological characteristics (total plant pubescence, glandular and non-glandular trichomes, plant height, leaf size, leaf/stem ratio) and essential oil contents (Kokkini *et al.* 1994), there is a need to evaluate natural populations of wild oregano (*O. vulgare* subsp. *vulgare*) recorded in Slovenia. Even though Slovenian material is supposed to contain less essential oil than introduced genotypes of Greek oregano (*O. vulgare* subsp. *hirtum*), a comparison with populations from other countries is essential. Reports on the presence of high-quality essential oils also in material from northern latitudes (Deans and Svoboda 1990) suggest that a final assessment of the potentials of Slovenian medicinal and aromatic species can only be made after screening such material and comparing results with exotic germplasm.

#### Conclusions

This paper has tried to review pharmacological properties of oregano and provide general information on research activities conducted on this crop in Slovenia. Oregano species offer many opportunities in the development of new active preparations to be used for applications in the field of animal and human pathology as well as crop protection. To secure official approval of these alternative uses, a better definition of plant material from a chemical, taxonomic, morphological and pharmacological point of view is essential. On the basis of results from these investigations, recommendations on how best to use this material should be made, thus providing crop selection and breeding programmes with precious advice on the direction to take in the improvement of these valuable plant genetic resources.

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## Status of cultivation and use of oregano in Turkey

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### Abstract

Oregano has been used in the Anatolian region of Turkey since ancient times. Records here date its use back to the 7th century BC. In this region the crop has mainly been used as a spice and as a medicine to treat various health disorders. The natural occurrence of the 23 species of oregano, reported to be indigenous to Turkey, is recorded in the following floristic regions: the Euro-Siberian, the Mediterranean (including the Aegean part) and the Irano-Turanian. The cultivation of oregano is very popular in Turkey and a marked increase in the area devoted to the cultivation of this crop has been noticed in the last few years. Within the framework of the initiatives conducted by the Medicinal and Aromatic Plants National Research Programme and the National Plant Genetic Resources Programme, oregano is being investigated at various levels, by different Institutes throughout the country. These activities are being carried out in cooperation with, and under the coordination of, the Aegean Agricultural Research Institute (AARI). The research areas covered by these investigations include breeding, agronomy, seed physiology, technology, taxonomy, collecting, ecogeography, conservation and evaluation of oregano genetic diversity.

### Introduction

In Anatolia, utilization of plants dates back to the Palaeolithic era (50 000-7000 BC). First records were taken from Hittite tablets (1600-1200 BC) in which plants used were described by illustrations. Oregano (as a condiment herb used for flavouring fish, meat, vegetables and wine) has been used in Anatolia since approximately the 7th century BC. In Turkey, it has been used as a traditional remedy to treat various ailments since the classical period. Today, it is still used as a spasmodic, antimicrobial, expectorant carminative and aromatic for whooping and convulsive coughs, digestive disorders and menstrual problems. It is used topically as an antiseptic and astringent, and for gargling. Of course, oregano herbs and their volatile oils are also widely used in the spice industry. With regard to the above-mentioned properties, leaves and inflorescence (spicules) are used as herbal tea in many locations across Turkey (Nakiboglu *et al.* 1994; Zeybek 1995). Its wide use in Turkey and the high demand for exportation have encouraged crop cultivation, rather than relying completely on harvests from wild populations.

### Ecogeography, distribution and endemism

Species of the genus *Origanum* are mainly found in the Mediterranean region. Most species (about 75% of them) occur exclusively in the East Mediterranean subregion and some in its Euro-Siberian part. The distribution of *Origanum* in Turkey is mostly confined to its Mediterranean (including the Aegean Region), Euro-Siberian and Irano-Turanian Regions (Table 1). These floristic regions are given in Figure 1. Of the 40 oregano species officially recognised, 22 of them have so far been found in

Table 1. *Origanum* species found in Turkey.

| Section                     | Species   | Area                                | Occurrence | Endemism | Phytogeographic region† |
|-----------------------------|---|-------------------------------------|------------|----------|-------------------------|
| Amaracus (Gleditsch) Vogel  | <i>O. boissieri</i> letsvaart                                 | S Anatolia                          | Rare       | Endemic  | E. Med                  |
|                             | <i>O. saccatum</i> P.H.Davis                                  | S Anatolia                          | Rare       | Endemic  | E. Med.                 |
| Anatolicon Bentham          | <i>O. solymicum</i> P.H.Davis                                 | SW Anatolia                         | Rare       | Endemic  | E. Med.                 |
|                             | <i>O. hypericifolium</i> O.Schwarz&P.H.Davis                  | SW Anatolia                         | Rare       | Endemic  | E. Med.                 |
|                             | <i>O. sipyleum</i> L.   | W, C and S Anatolia                 | -          | Endemic  | E. Med.                 |
|                             | <i>O. rotundifolium</i> Boiss.                                | NW Anatolia                         | -          | -        | Eur.-Sib.               |
|                             | <i>O. acutidens</i> (Hand.-Mazz.) letsvaart                   | E Anatolia                          | -          | Endemic  | Ir.-Tur.                |
| Brevifilamentum letsvaart   | <i>O. haussknechtii</i> Boiss.                                | E Anatolia                          | Rare       | Endemic  | Ir.-Tur.                |
|                             | <i>O. bargyli</i> Mouterde                                    | S Anatolia                          | Very rare  | -        | E. Med.                 |
|                             | <i>O. brevidens</i> (Bornm) Dinsm.                            | S Anatolia                          | -          | Endemic  | E. Med.                 |
|                             | <i>O. leptocladum</i> Boiss.                                  | S Anatolia                          | Rare       | Endemic  | E. Med.                 |
|                             | <i>O. munzurense</i> Kit Tan & Sorger                         | E Anatolia                          | Rare       | Endemic  | Ir.-Tur.                |
|                             | <i>O. amanum</i> Post   | S Anatolia                          | -          | Endemic  | E. Med.                 |
|                             | <i>O. bilgeri</i> P.H.Davis                                   | S Anatolia                          | -          | Endemic  | E. Med.                 |
|                             | <i>O. micranthum</i> Vogel                                    | S Anatolia                          | Rare       | Endemic  | E. Med.                 |
|                             | <i>O. minutiflorum</i> O.Schwarz&P.H.Davis                    | SW Anatolia                         | Rare       | Endemic  | E. Med.                 |
|                             | <i>O. majorana</i> L.   | S Anatolia (native)                 | -          | -        | E. Med.                 |
| Majorana (Miller) letsvaart | <i>O. onites</i> L.   | W and S Anatolia                    | -          | -        | E. Med.                 |
|                             | <i>O. syriacum</i> L.   | S Anatolia                          | -          | -        | E. Med.                 |
|                             | <i>O. vulgare</i> L. subsp. <i>hirtum</i> (Link) letsvaart    | S and W Anatolia                    | -          | -        | E. Med.                 |
| Origanum                    | <i>O. vulgare</i> L. subsp. <i>gracile</i> (C.Koch) letsvaart | E Anatolia                          | -          | -        | Ir.-Tur.                |
|                             | <i>O. vulgare</i> L. subsp. <i>viride</i> (Boiss.) Hayec      | Mainly N. Rare in C and SW Anatolia | -          | -        | -                       |
|                             | <i>O. vulgare</i> L. subsp. <i>vulgare</i>                    | N Anatolia                          | -          | -        | Eur.-Sib.               |
| Protaticorolla letsvaart    | <i>O. leavigatum</i> Boiss.                                   | S Anatolia                          | -          | -        | E. Med.                 |
|                             | <b>Doubtful records</b>                                       |                                     |            |          |                         |
|                             | <i>Origanum dictamnus</i> L.                                  | W Anatolia                          |            |          |                         |
|                             | <i>O. calcaratum</i> Juss.                                    | S Aegean                            |            |          |                         |
|                             | <i>O. symes</i> Gorgion Disolona                              | W Anatolia                          |            |          |                         |

† See Figure 1 for explanation of regions.

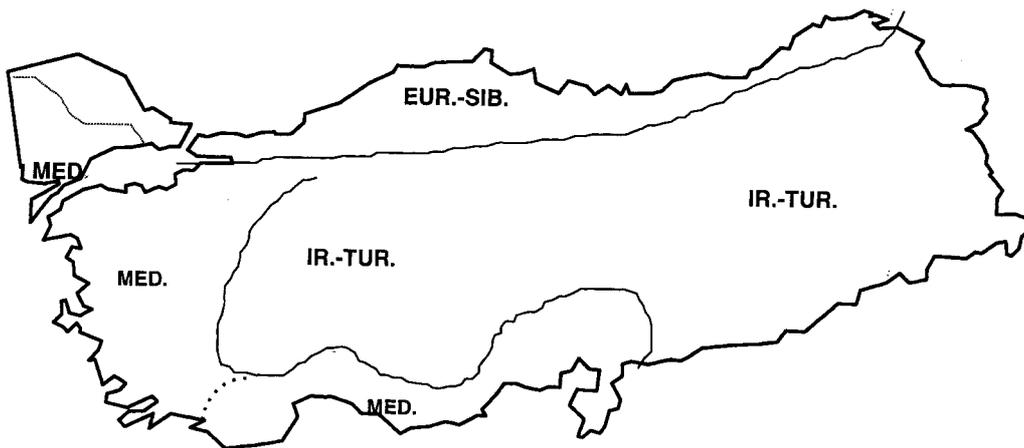


Fig. 1. Phytogeographic regions of Turkey. Med.=Mediterranean; Eur.-Sib.=Euro-Siberian; Ir.-Tur.=Irano-Turanian.

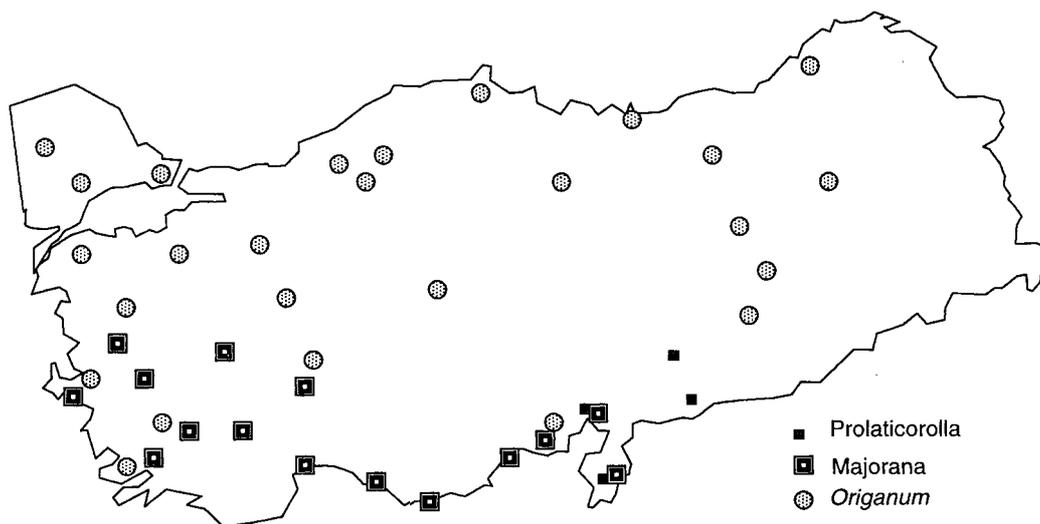


Fig. 2. Distribution of *Origanum* sections *Majorana*, *Origanum* and *Prolaticorolla* in Turkey.

the Turkish flora alone, such taxa being grouped into eight sections (Ietswaart 1982; Davis 1988). The most recently discovered new species of *Origanum* is soon to be published by Duman *et al.* (1996), which will bring the number of oregano species occurring in the country to 23. The distribution of *Origanum* genus in Turkey is shown in Figures 2 and 3.

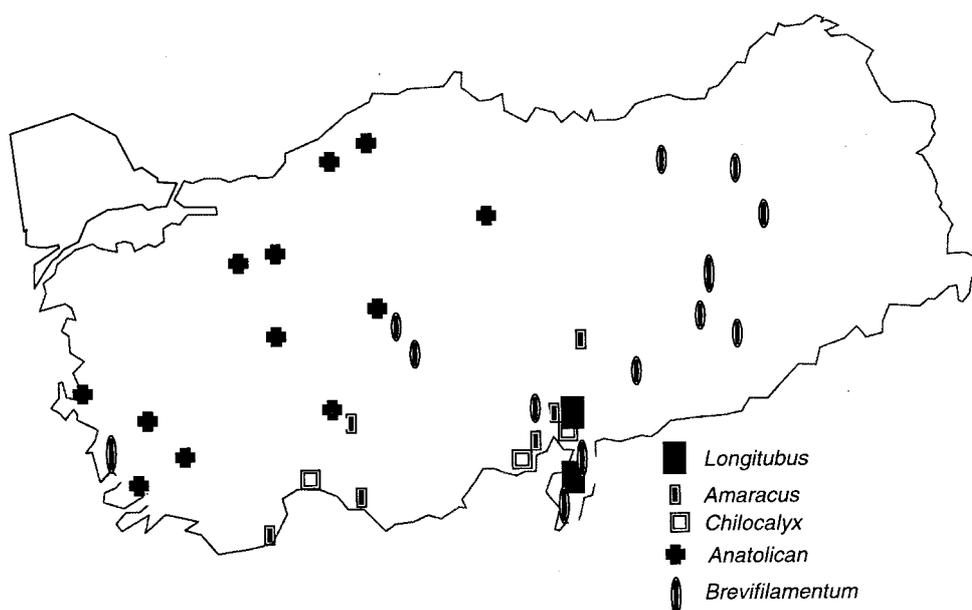


Fig. 3. Distribution of *Origanum* sections *Amaracus*, *Anatolicon*, *Brevifilamentum*, *Longitubus* and *Chilocalyx* in Turkey.

### Hybrids in Turkey

Some intersection hybrids from the Turkish Flora (Table 2) were determined (Ietswaart 1982; Anon. 1996c); additional putative hybrids were not confirmed, although their presence is reported in literature (Ietswaart 1982).

Table 2. Intersection and putative hybrids within *Origanum*.

| Intersection hybrids  | Distribution | Peculiar traits   |
|---|--------------|---|
| Sect. <i>Longitubus</i> Ietswaart   | S Anatolia   | Hybrids are nearer first parent but more branched and smaller flowered                |
| Sect. <i>Prolaticorolla</i> Ietswaart<br><i>O. amanum</i> Post x <i>O. laevigatum</i> Boiss.                                      |              |   |
| Sect. <i>Anatolicon</i> Bentham   | SW Anatolia  | Nearer second parent but differing by untoothed calyx with lip = ½ length of parent's |
| Sect. <i>Majorana</i> (Miller) Ietswaart<br><i>O. sipyleum</i> L. x <i>O. onites</i> L.   |              |   |
| Sect. <i>Anatolicon</i> Bentham   | SW Anatolia  |   |
| Sect. <i>Origanum</i><br><i>O. sipyleum</i> L. x <i>O. vulgare</i> subsp. <i>hirtum</i> (Link) Ietswaart                          |              |   |
| Sect. <i>Majorana</i> (Miller) Ietswaart  | SW Anatolia  | Habitus same as <i>onites</i> , slightly dentate calyx                                |
| Sect. <i>Origanum</i><br><i>O. vulgare</i> subsp. <i>hirtum</i> (Link) Ietswaart x <i>O. onites</i> L.                            |              |   |
| Sect. <i>Majorana</i> (Miller) Ietswaart  | S Anatolia   | Close to first parent from which it differs by 2-lobed dentate calyx                  |
| Sect. <i>Prolaticorolla</i> Ietswaart<br><i>O. syriacum</i> L. var. <i>bevanii</i> x <i>O. laevigatum</i> Boiss                   |              |   |
| <b>Putative hybrids</b>   |              |   |
| Sect. <i>Origanum</i>   | S Anatolia   | Resembling the latter parent but with a regularly 5-toothed calyx                     |
| Sect. <i>Chilocalyx</i> (Briq.) Ietswaart<br><i>O. vulgare</i> subsp. <i>hirtum</i> (Link) Ietswaart x <i>O. micranthum</i> Vogel |              |   |

In the following scheme the geneflow across sections is summarised:

| Section           | <i>Prolaticorolla</i> | <i>Majorana</i> | <i>Chilocalyx</i> | <i>Origanum</i> |
|-------------------|-----------------------|-----------------|-------------------|-----------------|
| <i>Longitubus</i> | X (I)                 | –               | –                 | –               |
| <i>Anaolicon</i>  | –                     | X (I)           | –                 | X (I)           |
| <i>Majorana</i>   | X (I)                 | –               | –                 | X (I)           |
| <i>Origanum</i>   | –                     | X (I)           | X (P)             | –               |

(I=natural hybrids, P=putative hybrids)

A crop-centred genepool for *Origanum vulgare* and *O. onites* (Fig. 4) has been proposed (Anon. 1996c), following the genepool concept of Harlan and De Wet (1971).

### Genetic resources activities

Genetic resources activities on oregano are being carried out in Turkey within the framework of the Medicinal and Aromatic Plants Group of the National Plant Genetic Resources Research Programme (NPGRPP). The Medicinal and Aromatic Plants Genetic Resources Programme (MAPRP) is involved in various aspects related to plant genetic resources, namely germplasm survey and collecting, taxonomy, conservation (both *ex situ* and *in situ*) and characterization and evaluation. Taking into account these areas of interest, oregano species present in the country have been successfully collected, identified and evaluated.

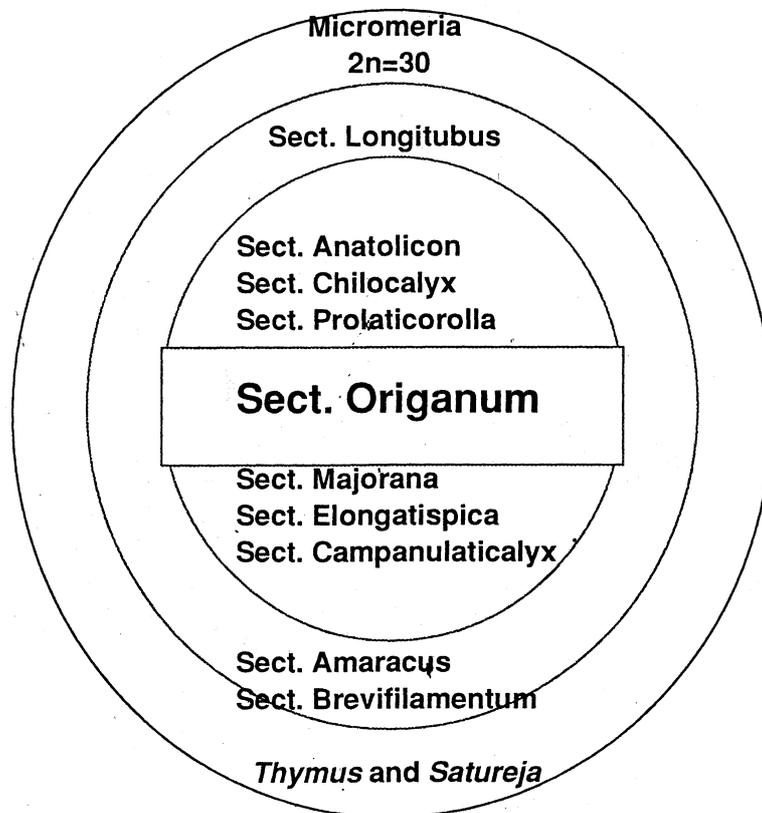


Fig. 4. *Origanum* genepool.

### Conservation

AARI attempts to adopt complementary conservation strategies wherever this practice is possible.

#### *Ex situ* conservation

*Ex situ* conservation activities have been carried out at the AARI genebank since 1964. Seed conservation and vegetative conservation in field conditions are two methods being applied at this genebank. International rules are being applied for conservation in each of the genebanks of those institutes which are part of the conservation network coordinated by AARI.

The AARI seed genebank has been designed for long-term (base collection) and medium-term (active collection) conservation. Cold rooms are at  $-18^{\circ}\text{C}$  to  $-20^{\circ}\text{C}$  for the long term and at  $0^{\circ}\text{C}$  for medium-term storage. Additional storage facilities at  $4^{\circ}\text{C}$  also exist for working samples. For the safekeeping of base collection material (i.e. storage of duplicates of the base collection), another storage facility, to be located in Ankara, is foreseen. International rules are also respected with regard to the viability tests of the material as well as the monitoring of its moisture content.

Seed morphology and germination methods of *Origanum* species have been studied since 1995. These studies have indicated that these seeds are non-endospermic, with axile foliar embryos and thin mucilaginous seed coats. Germination aspects of this investigation are still under study.

The conservation programme involves collaboration with partners within the country and outside, under the terms of the Turkish National Conservation Code of Conduct for plant germplasm exchange and transfer.

As of 1995, 123 seed samples and 442 herbarium specimens of *Origanum* species are being maintained at the AARI Genebank and Herbarium.

The genetic resources material of oregano has been utilized by breeding and agronomy programmes throughout Turkey.

### Characterization

Some 52 samples of *Origanum* material gathered from west and southwest Anatolian regions have been evaluated using 40 morphological characters (Anon. 1996c). Such analysis was carried out using the Principal Components Analysis (PCA) through the TARIST programme (Acikgoz 1993). Table 3 lists the material studied in this work, whereas results of the PCA are shown in Figure 5.

**Table 3. *Origanum* material from Anatolia used in the evaluation study carried out at AARI.**

| Section                             | Species  |
|-------------------------------------|--|
| <i>Anatolicon</i> Bentham           | <i>O. spyleum</i> L.   |
| <i>Majorana</i> (Miller) letswaart  | <i>O. onites</i> L.  |
| <i>Majorana</i> (Miller) letswaart  | <i>O. majorana</i> L.  |
| <i>Origanum</i>                     | <i>O. vulgare</i> L.   |
| <i>Amaracus</i> (Gleditsch) Vogel   | <i>O. saccatum</i> P.H.Davis   |
| <i>Amaracus</i> (Gleditsch) Vogel   | <i>O. solymicum</i> P.H.Davis  |
| <i>Majorana</i> (Miller) letswaart  | <i>O. syriacum</i> L.  |
| <i>Anatolicon</i> Bentham           | <i>O. hypercifolium</i> O.Schwarz&P.H.Davis                                    |
| <i>Prolaticorolla</i> letswaart     | <i>O. leavigatum</i> Boiss   |
| <i>Chilocalyx</i> (Brik.) letswaart | <i>O. bilgeri</i> P.H.Davis  |
| Intersection hybrids (Natural)      | <i>O. onites</i> L. x <i>O. vulgare</i> subsp. <i>hirtum</i> (Link) letswaart  |
|                                     | <i>O. spyleum</i> L. x <i>O. vulgare</i> subsp. <i>hirtum</i> (Link) letswaart |

The PCA diagram shows two main groups: (1) sections *Chilocalyx*, *Majorana*, *Prolaticorolla* and *Origanum* including hybrids were formed in the first large group on the left side of the diagram; (2) section *Anatolicon* with the two subgroups belonging to *O. sipyleum* and *O. hypericifolium* are included in the second group on the right; whereas one sample belonging to *O. saccatum* (section *Amaracus*) singled out from the second group (right uppermost circle).

Cytogenetic and palynological studies were also undertaken within the framework of this project. Results on the pollen morphology were published by Nakiboglu *et al.* (1994). Further research in these fields is expected to be carried out soon on additional material from South Anatolia and other parts of Turkey.

### *In situ* conservation

Conservation of *Origanum* is being pursued within the framework of the project 'In Situ Conservation of Genetic Diversity in Turkey' (started in 1993), in which such activity is considered a complementary initiative. In this large project, the initial step has been the identification of pilot conservation areas for wild ancestors of major crops. After the survey and inventorying of those sites for target species, the gene management zones will be determined and thus *in situ* conservation will take place. This work is part of the national plan on conservation of plant diversity of Turkey, which has now been completed.

In the selection process of these *in situ* conservation sites, the ecological heterogeneity, the ability to control the site and easiness of access to the site for monitoring and management are important elements that are taken into account. Even though oregano species will not be targeted in the pilot project (which will focus on *Prunus* spp. and various species of the Leguminosae family), medicinal and aromatic plant species – and possibly *Origanum* spp. – are expected to be targeted in the second stage of the project.

### Documentation

A documentation system for plant genetic resources is operative in AARI and covers all species preserved at the genebank. In addition, provisions have been made for merging data from other crop databases connected with the main documentation system, to facilitate data comparisons with related fields of investigation.

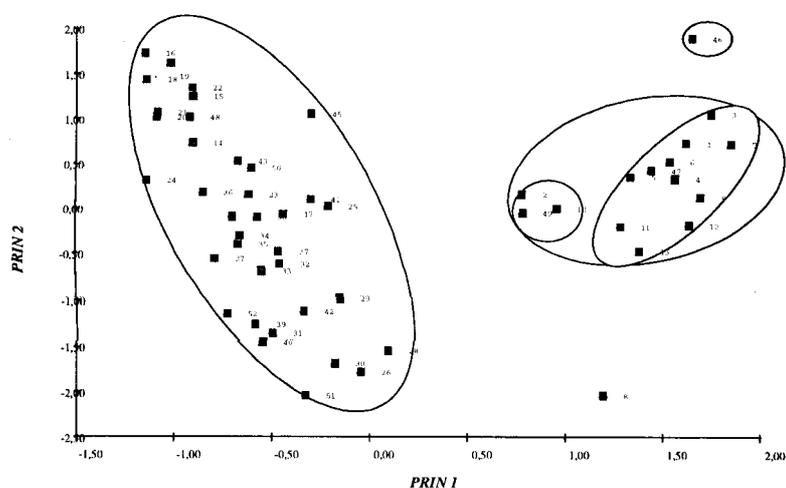


Fig. 5. Scatter diagram of *Origanum* species in Prin. 1 and Prin. 2.

## Agronomy, breeding and cultivation

### Agronomy

Although Turkey is one of the main oregano exporters in the world, a small amount of this crop is also being imported into the country. Export and import figures of for oregano are shown in Table 4 (data taken from Anon. 1995). The largest importing country of oregano from Turkey is the USA, whose import corresponds to approximately 50% of the total Turkish export.

**Table 4. Oregano exports and imports in Turkey.**

| Year | Oregano amount (t) |          |
|------|--------------------|----------|
|      | Exported           | Imported |
| 1989 | 3668.4             | 0        |
| 1990 | 3818.5             | 0.045    |
| 1991 | 3976.4             | 0.500    |
| 1992 | 4744.1             | 122.1    |
| 1993 | 4854.8             | 340.5    |
| 1994 | 6435.4             | 464.2    |
| 1995 | 4449.3             | –        |

Since harvests from the wild are the primary source for local use and exportation of oregano (amounting to 4000 t, mainly exported to the USA), genetic erosion is unfortunately recorded for various species including *O. onites*. More studies on how to promote better exploitation of *O. onites* were therefore commenced by AARI.

In fact, within the framework of NMARP, agronomic studies on this species – first initiated in the country in the early 1970s – have been undertaken in three different locations in the Aegean Region in the framework of a project entitled 'Agro-technique Research on *O. onites* L.'. On-farm trials and demonstrations were conducted to transfer results from this project to the growers. The following is a summary of major results from this work:

"planting patterns differ according to inter row distances and number of rows in the plot. Yield of air-dried plants and number of harvests varied by locations depending on the amount of nitrogen fertilization applications and planting patterns. However, planting patterns and nitrogen fertilization did not affect the etheric oil content and the chemical compound ratio of etheric oil components such as carvacrol" (Ceylan *et al.* 1994).

Yields of green herb, air-dried plants ('drog herba') and both air-dried inflorescences and leaves ('drog folia') in different planting patterns are given in Figure 6 (Ceylan *et al.* 1994).

### Propagation

The effect of the time of harvest and different levels of growth regulator on the rooting of *O. onites* L. is currently being investigated by AARI. Preliminary results from these studies suggest that 1000 ppm of IBA should be the recommended dosage of growth regulator to obtain the highest number and weight of rooting in oregano (Anon. 1996d).

The ageing of *O. onites* L. cultivation also has been studied. The establishment period of such a plantation is one year, whereas its economic lifespan is usually 6 years. The economic lifespan of this cultivation is calculated as the period from which crop production starts until the time when variable costs are equal to the

gross income. Gross expenditures (from sowing to threshing) and gross income are almost equivalent to each other in the first (foundation) year (Anon. 1996a).

In the AARI investigation, the economic lifespan of *O. onites* cultivations has not yet been exhausted (Anon. 1996a).

### Harvesting and handling

With regard to the planting pattern, a distance of 45 cm between rows was found the best and also the most suitable distance for mechanization. The best harvest time to capture the highest amount of etheric oil in *O. onites* is when 50% of the plants in the field have commenced flowering. In relatively small fields, harvest is usually done by hand, mechanical harvesting (by means of a mower) being recommended only for large fields.

After harvesting, plants should then be dried in the shade. A 25-cm stack height is preferred during drying operations in order to facilitate the accumulation of etheric oil content. Although drying under natural conditions is a common procedure, drying ovens operating at 30-35°C can also be used in commercial scale production. A moisture content of 7% (minimum) to 12% (maximum) is required.

### Threshing

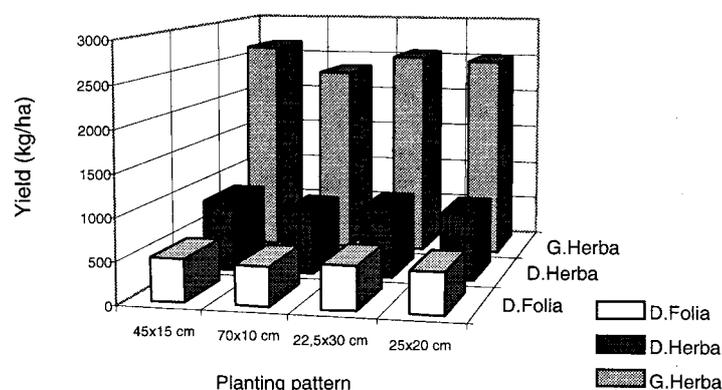
The separation of dried leaves and spike-like inflorescences from stems is done by hand when the same amount of material is involved (in large production, threshing machines are used). Combined threshing machines are usually preferred.

Since volatile oil percentage gradually decreases after 4-5 months of storage, 'drog folias' should be kept in conditions of cool and relatively low humidity.

In Turkey, commercial companies dealing with oregano are usually well equipped with regard to proper crop processing.

### Breeding

Research activities in this area started in Turkey in 1972 (Bayram 1995). No cultivation and improvement of *O. onites* was available before that time in the country. More than 100 *O. onites* populations originating from various parts of the country were studied according to their morphology, 'drog herba yield', 'drog folia yield' and etheric oil contents (Otan *et al.* 1994). More than 5000 individual clones also were evaluated for their earliness, high yield, morphology and etheric oil content (greater than 2.5%). Further selection study is now underway on the 200 most promising clones (Anon. 1996b).



**Fig. 6.** Yields of green herb, 'drog herba' (air-dried plants) and 'drog folia' (air-dried inflorescences and leaves) in different planting patterns.

Chemistry aspects, especially assessment of the etheric oil composition in oregano, are being studied in various Turkish Laboratories, such as the Department of Field Crops Agricultural Faculty Aegean University in Izmir, the Anadolu University Medicinal and Aromatic Plant and Drug Research Centre in Eskisehir, the Cukurova University in Adana and the Istanbul University in Istanbul.

AARI works in close collaboration with the Department of Field Crops of the Agricultural Faculty, Aegean University, as far as agronomy, breeding and chemical analysis investigations on oregano are concerned, and with the Department of Biology of the Education Faculty, Eylul University for genetic resources studies.

### Research needs and future activities

By reviewing past accomplishments and looking at present and future needs in the area of oregano, a number of problems still need to be properly addressed. The following are therefore research topics dealing with agronomy and genetic resources that we feel should be considered as high priority.

#### Agronomy

- Determination of post-harvest storage conditions (storage environment and chemical compound interactions);
- Drying conditions and their effect on chemical composition;
- Sustainable farming.

#### Genetic resources

- Evaluation of existing collections
- Collecting for filling existing gaps in germplasm collections;
- Assessing genetic diversity by use of advanced techniques (i.e. isoenzyme analyses);
- Reproductive biology of related taxa;
- *In situ* conservation.

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## Oregano (*Origanum vulgare* L.) in Albania

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### Abstract

The ecogeographical characteristics of Albania, particularly its soil and climate, represent ideal conditions for the spontaneous growth of oregano in many places throughout the country, especially in *Lauretum-Castanetum* plant association areas. Oregano is also being cultivated in the country, but on a limited area. During recent IPGRI-supported plant germplasm collecting expeditions carried out in Albania over the last 3 years, samples of *O. vulgare* diversity were gathered and then deposited for safe conservation at the Germplasm Institute (CNR) of Bari, Italy. These missions have allowed observation of great genetic diversity within the species but, at the same time, worrying signals of its genetic erosion were detected. So far, no specific studies have been carried out on oregano in Albania. In fact, in most of the botanical literature produced in the country, its taxonomy is still very unclear. For instance, three species are mentioned in the Excursionist Flora of Albania, i.e. *Origanum vulgare* L. or 'red type' (local name, related to the flower colour), which is widely dispersed in the northern part of Albania, *Origanum heracleoticum* L. or 'white type', which is widely dispersed in the southern part of Albania, and *Origanum majorana*. In any other bibliographic sources the red type is classified as *O. vulgare* subsp. *viridis*. Both types grow as perennial plants. Normally, flowering takes place in July–September for the 'red type' and in May–July for the 'white type'. Oregano is widely used as a spice in Albanian cuisine: to make tea, as a medicinal and pharmaceutical plant, for dyeing, as well as a good melliferous plant. Because of its known ability to establish itself in poor soils and dry areas, oregano is a symbol of poverty and stoicism for Albanian people, representing a very popular element in their culture. Oregano annual production in Albania (dried leaves with 13% moisture content) ranges from 550 to 600 t, of which about 500 t are exported (mainly to Greece, Germany and Italy). The rest of the production is used for national consumption. Harvest, processing and trading of oregano follow official standards set out by National Authorities.

### Introduction

Oregano (= 'rigoni' in the Albanian language) is widely used by Albanians, but surprisingly, few of them know where and how this crop is grown. Oregano is considered as a gift of nature which does not need any care to grow. With the exception of short notes found in some Albanian books dealing with the country's flora, no specific study or publication has been made so far on Albanian oregano. Only some analyses on its chemical content have been made to assess the quality of exported oregano. For the preparation of this paper various sources have been consulted, including Albanian literature and reports of germplasm-collecting missions; personal communications with different Albanian specialists as well as their own knowledge and experience also have been taken into account.

### Ecological conditions

Albania belongs to the Mediterranean climatic belt, with a hot dry summer and rainy winter. The southwestern part of the country is strongly influenced by the Mediterranean Sea. Owing to its geographical position (the country lies between 42°39' and 39°38' N and 21°04' and 19°16' E), the sunlight lasts from 2406 to 2731 hours per year and the total radiation in the southwestern part ranges from 153 to 197 kcal cm<sup>-1</sup> year<sup>-1</sup>. Rainfall is very variable throughout the country, averaging 1430 mm (Isidorov 1955; Mandili 1983; Anon. 1991). The country is divided into four main agro-ecological zones based on altitude and temperature (Anon. 1994).

About 70% of the Albanian territory is hilly and mountainous, average altitude being 704 m asl. Soils are usually of the red type, poor in main macronutrients, especially potassium. Heavy soil erosion is present in many areas of the country.

### Geographic distribution and taxonomy

Oregano in Albania is mainly spontaneous, common in *Lauretum-Castanetum* vegetation, though it may also be found growing up to the *Fagetum* area. Recent germplasm-collecting missions (Hammer *et al.* 1994; Gladis *et al.* 1995) have pointed out that oregano is more widespread in the southern part of the country, in poor red soil, through rocks, particularly in the mountainous districts of Tepelene, Gjirokaster, Fier, Vlore, Berat, Kolonje, Skrapar, Permet and Gramsh.

Oregano can be found as small isolated populations, but more frequently it is recorded in association with other shrubs within the *Lauretum* vegetation where *Quercus coccineus*, *Paliurus spinachristi* and *Phlomis fruticosa* are the most common plants. Better growing conditions for oregano have been recorded, however, in the *Castanetum* area, where its plants are developed up to 70-90 cm high, and also have a better aroma.

From a taxonomic point of view, Albanian literature sources provide a rather confused introduction for *Origanum* species. This might be related to the fact that oregano is an underutilized crop which has been neglected by scientists who have not given adequate attention to it so far. Three species are mentioned in the Excursionist Flora of Albania (Demiri 1983): *Origanum vulgare* L., *Origanum heracleoticum* L. and *O. majorana*. In another publication from the same author (Demiri 1979), only *O. vulgare* is mentioned, whereas in the State Standards (Anon. 1988) *O. vulgare* subsp. *viridis* is the species reported to occur in the country. Referring to the oregano flower's colour, Albanians have distinguished two species as follows: *O. heracleoticum* is the 'white oregano' or simply oregano and *O. vulgare* is the 'red oregano'. The 'white type' is widely distributed in the southern part of Albania, the 'red type' in the northern part.

### Oregano as part of ethnocultural components in Albania

Plants have always played a special role in the diverse and rich culture of the Albanian people, very close relationships existing between them and folk songs, poems, proverbs, pictures and sculptures throughout the country (Xhuveli 1996). Oregano is used by Albanians as a symbol of poverty associated with bravery and stoicism; here are some common Albanian expressions:

- "This soil can produce only oregano" (when referring to very poor soils).
- "The boys of oregano" (when referring to very poor people).
- "He stands like the oregano at the top of rock" (when referring to the stoicism and bravery of a person).

### Biomorphological characteristics

Both types of oregano (red and white) are perennial plants. The plant of *O. vulgare* or 'red oregano' grows up to 90 cm in height. Flowers are characterized by purple bracts, 4-5 mm long and bearing some glands in the upper part. Normally, flowering time is July-September. Flower calices are 4-7 mm long and their colour varies from white to purple.

The plant of *O. heracleoticum* or 'white oregano' grows up to 60 cm in height. It usually has green bracts (2-3 mm long) with dense glands. The calyx is 4-5 mm long and its colour is white (very rarely purple). Flowering takes place in May-July.

Albanian oregano contains 0.7-1.1% of essential oil and 310 mg in 100 g of fresh leaves of Vitamin C. More than 29% of the seed content is essential oil. The essential oil contains about 32 main components such as the  $\alpha$ - and  $\beta$ -pinene, the  $\alpha$ -terpinene, dipenten, linalol, acetate, linalol, borneol, p-cymen, thymol and carvacrol. Carvacrol, dipenten, p-cymene and the esters are present in greater quantity than the other components (Demiri 1979).

### Collecting of oregano genetic resources

Four multicrop germplasm-collecting missions have been organized from 1993 to 1995 in Albania thanks to IPGRI support. The international team of experts (from the IPK Genebank of Gatersleben, Germany, the Germplasm Institute of Bari, Italy and the Agricultural University of Tirana, Albania) launched these missions with the aim of rescuing local plant germplasm under threat of genetic erosion, which seems to have increased dramatically after the latest socioeconomic changes faced by the country. The genetic erosion of oregano is occurring in two ways: first of all, through harvesting from the wild (especially when done for export purpose), which is today much more intensive than before. More and more people, (often not familiar with this work) are undertaking the harvesting of its spontaneous populations, attracted by the good market price of oregano. It is sad to say that unfortunately, great is the damage caused by these commercial harvests, which sometimes lead to the complete spoilage of the plants. The second cause of erosion is represented by the heavy presence of grazing animals: in fact even though animals do not particularly feed on oregano (they dislike this plant for the presence of essential oils), they do, however, cause serious damage during their grazing in the fields.

Five oregano samples were collected during these missions, two samples from the northwestern part (Torrovica Mountain in the Shkodra District), one from the south (Dukati village, in the Vlora District) and two samples (one under partial cultivation) in the southernmost part of Albania, close to the Greek border (Vrisera village, in the Gjirokastra District). Collected material is now preserved at the Germplasm Institute of Bari, Italy (Anon. 1993).

### Annual production and use of oregano

No official statistics have been produced yet on oregano production in Albania, regardless of their market destination (domestic use or exportation). However, according to approximate estimates, about 550-600 t/year (dried leaves with 13% moisture content) of oregano are produced in the country, of which some 450-500 t are exported, the remainder being utilized for domestic consumption. Main importers of the Albanian oregano are Greece, Germany and Italy.

Apart from being used as a spice for flavouring meals – it is indeed the most popular spice in Albania – oregano is also used as tea, as a medicinal plant, for dyeing cloth and also as a melliferous species for good-quality honey. Its strong characteristic flavour give roasted meat dishes a very particular taste. Irreplaceable also is its aroma for preparing special Albanian dishes such as stuffed peppers, tomatoes and eggplants, baked potatoes, etc. As a spice, oregano is sometimes mixed with winter savory (*Satureja montana*) in the proportion of 60% oregano and 40% savory. Oregano tea is more widely used in the northern part of the country.

With regard to its curative properties, ground oregano mixed with salt and added to boiled eggs is at the same time a tasty food and a good medicament to stop stomach troubles caused by diarrhoea. It is also used as a sedative for the nervous system, against cough and to treat many more health disorders (Kokalari *et al.* 1980). Oregano is an excellent melliferous plant: its little flowers, rich in nectar, are easily visited by bees during the long flowering period of the plant (May-September). Another interesting use of oregano is related to its insect-repellent properties: dried and ground oregano is traditionally used to keep flies away from the house or from places where food and dairy products are being processed.

### The cultivation of oregano

Two types of oregano cultivation are practised in Albania: in home gardens and in open fields. The traditional home-garden cultivation is practised more in the southern part of the country. In this case, the plants are grown to meet family needs only. The cultivation of oregano in open fields is not done following any particular modern agronomic criteria. As for home gardens, seeds taken from spontaneous populations are also being used for field cultivation. Over the last few years, a small oregano experimental field has been set up by the Forest and Pasture Institute of Tirana. Some preliminary data from this investigation concerning plant fresh weight, dried weight, yield of dried leaves and stalk/leaf ratio are provided below (from Sahatciu, pers. comm.).

|                               | 1st year | 2nd year | 5th-6th years |
|-------------------------------|----------|----------|---------------|
| Fresh weight (g/plant)        | 115      | 481      | –             |
| Dry weight (g/plant)          | –        | 155      | –             |
| Yield of dried leaves (kg/ha) | 225      | 300-400  | 1500          |
| Stalk/leaf ratio              | –        | –        | 3.1           |

Some of the main agrotechniques used in this study include the following:

- preparation of nursery (seed plots). For this purpose 200-300 g seed/ha are used (2-3 g/m seed-plot);
- transfer of seedlings (2-month-old plants). The seedlings should be 4-5 cm long. Usually 40 000 seedlings/ha are used;

- time of harvest: the best period is when the upper branches of the plant are half flowered;
- drying: to keep the green colour, drying under shade is practised;
- commercialization: according to official standards (Anon. 1988), the processed material is considered ready when it has (1) less than 3% of abnormal coloured leaves, (2) less than 3% of remains of stems (up to 2 cm long), and (3) less than 0.5% of soil matter;
- packing: the dried herb is packed in bales of 50 kg, approximately 55 x 60 x 70 cm in size.

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## Short communications

Following are reported the abstracts of three papers which were expected to be delivered during the Workshop. Although the contributions from Logozzo and Benjilali were not delivered owing to the absence of their authors, their abstracts are in these proceedings because they contain some relevant information on the conservation and use of oregano in Italy and Morocco. The contribution from Switzerland is also included as a short communication.

### **MEDEA: Conservation on-farm for safeguarding Mediterranean germplasm**

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#### **Abstract**

The Association MEDEA was born in 1994, with the main goal of safeguarding Mediterranean neglected plant genetic resources, particularly aromatic and medicinal species. The 20 MEDEA members are involved in the management of as many farms, located in diverse environments of Basilicata, Campania and Apulia regions. Sites of Basilicata region are those most suitable for the growing of MAP species. MEDEA members operate by collecting and preserving indigenous germplasm material of MAP species with 'in situ specific' conservation actions. Other main objectives of MEDEA are: (1) to promote and exchange germplasm accessions between different institutions; (2) to keep at least one duplicate collection in any farm or site of any other member; (3) to encourage the promotion, evaluation, and exchange of germplasm collections, and (4) to maintain a database of genetic resources maintained by the association. MEDEA takes care of a field catalogue of MAP species collected in the Basilicata region during several missions and grows out some collections of *Salvia officinalis*, *Thymus serpyllum*, *Origanum vulgare* and *Matricaria chamomilla*.

## Origanum: what does this mean? The case of Morocco

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### Abstract

The term 'oregano', 'origan' or 'origanum' has more a commercial meaning than a botanical one. Many crop species that do not belong to the genus *Origanum* are known on the international market as 'oregano'. In fact some of these species do not even belong to the Labiatae family. Conversely, in trading, some true species of *Origanum* are called by different commercial names. In Morocco, there are five species belonging to the genus *Origanum*. Three of them are endemic to the country: *O. grosi* Pau and F.Q., *O. frontqueri* Pau and *O. elongatum* Emb. and Maire. The two other species, i.e. *O. virens* Hoffing and Link and *O. compactum* Benth., are common in the Iberian peninsula and Morocco. Among the five species, two are widely exploited for marketing and industrial purposes: dried leaves are used for drugs and flavoring (*O. compactum*). This exploitation is very important for some local economies across the country. The estimated Moroccan yearly production of 1500 t of oregano (vegetable material) corresponds to more than 2000 working days paid at minimum wage for agricultural employees in the country. And this is only for harvesting. Indeed, for many local communities, this is more than a small contribution to the welfare of the population. The exploitation of oregano directly from the wild could be dangerous, particularly for some species which are not so abundant. This is for instance the case of *O. elongatum*. But what can we do? The prohibition of exploitation of this natural resource is not the solution. Greater attention placed on the exploitation of these species would be beneficial to those same people who now exploit them, and thus provide a more sustainable system.

## Oregano in Switzerland: Market, production and research

**Charly Rey**

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### Abstract

#### Market

In Switzerland, it is not possible, because of lack of detailed statistics, to know the actual market of oregano. However, this condiment crop is well known and appreciated particularly in the part of the country with a prevalence of Italian-speaking people (Canton Ticino) and other immigrants from Mediterranean countries, who use this plant a lot to flavour popular dishes like pizza. Furthermore, oregano is becoming increasingly popular among Swiss people of German culture, in the traditional cuisine. Some firms like McCormick, Dixa, Migros and Coop between them divide the importation of this spice from Chile, Turkey, Morocco and Egypt. First-quality oregano is sold in Switzerland at the rate of 4-5 Swiss Francs/kg. Because of the high cost of living in Switzerland, and therefore high cost of labour, it is not possible for local farmers to be competitive with the imported material. However, a growing biological market demand supports some cultivation close to large cities or in mid-altitude areas up to 1000 m asl. This indigenous production is estimated to be about 2 t of dry material per year. On the other hand, a year-round large market of fresh plants, sold in bouquets or pots, has been developed for production originating in the Canary Islands, North Africa and Switzerland.

#### Research

Three years ago, to answer the demand from industries and from farmers, we started a modest programme of study on oregano in Arbaz (920 m asl), aiming to assess variety performances. Some species –*O. vulgare* subsp. *vulgare*, *O. vulgare* subsp. *virens* and *O. heracleoticum* – have been investigated for agronomic and chemical characters during the last 2 years. The large phenotypic heterogeneity observed in the material analyzed has induced us to work for the amelioration of these varieties to achieve higher uniformity. Flower biology has been studied on both indigenous and introduced material. We have found some male sterile plants and we have been using them as female parents to better control the hybridization and the genetic homogeneity in relation to the base populations used. In addition, as a result of this selection work, there has been an improvement in the dry matter yield and essential oils content. If today, to ensure homogeneity in their cultivation, the big European producers carry out expensive clonal multiplication with all the material they grow, by cutting the stems of the plants, the situation might be different in the near future, when the availability of hybrid seed of oregano clones will make these operations much easier and cheaper.

## **V. Marketing and Commercial Production**

## The world market of oregano

**Gilbert W. Olivier**

Ludwig Mueller Co. Inc., New York, USA

### Introduction

I am indebted to the International Plant Genetic Resources Institute and especially to Dr Stefano Padulosi for having invited me to talk to you today on the 'Importance of Oregano in the World'. But first let me tell you that it is a personal pleasure for me to stand before you and address such a distinguished gathering.

As you may know, I come from the United States and am the Vice President of the Ludwig Mueller Co. in New York. Our firm is the leading American company selling Mediterranean Oregano (and other spices, of course) in America. I was also the Vice President of the American Spice Trade Association, commonly known as ASTA, and have been serving on its Board of Directors for the past 6 years. Just as an aside, ASTA is quite international in nature and we count members from 37 different countries.

I think it will be useful today to begin our discussion with an overview of spice consumption in the United States inasmuch as we may be one of the higher per capita users of spices in the world.

From figures obtained from the US Department of Agriculture, it appears that our total domestic consumption stands at about 379 000 metric tons per year. This represents an import volume of 240 000 metric tons and a domestic production of about 140 000 metric tons per year. (These domestic numbers include dehydrated vegetables, primarily garlic and onions.) On a per capita basis, we Americans consume about 0.25 kg of spice per year and it is interesting to note that we now consume about 0.25 kg more per capita than 10 years ago. We were once known to be a 'meat and potatoes' country, so the annual increase in consumption bears witness that America has a fascination with foods from other lands.

### Oregano: what is it?

And, this brings us to oregano - the one spice that brings us here today. I am told that the name 'oregano' derives from the Greek language for the 'joy of the mountains'. It is a wild herb and indeed, it favours hilly terrain. Oregano's story is perhaps one of the most unusual in the herb world. It has been known and used for centuries but its mass popularity only occurred in recent years and its rise has been meteoric.

For many centuries oregano played a significant role in peoples' daily lives inasmuch as it was used for food, household products and antiseptics. It also provided a source for medical treatment.

A publication of the American Spice Trade Association tells us that oregano is a herb of the mint family (Labiatae), but it is there that the simplicity ends and we encounter what botanists have been debating for centuries: its proper classification. Tradition in commerce recognises different plants as 'oregano' but the public frequently gets it confused with marjoram; in fact, in some commercial circles oregano is still called wild marjoram. ASTA goes on to say that to understand the situation it is important to note that 'oregano' is a commercial or popular term. According to the "L. H. Bailey *Hortorium*" of the Cornell University, the best - and simplest - accepted

botanical classification for oregano today breaks down this way: "oregano, which comes from the Mediterranean area is *Origanum vulgare*", meaning it is a wild species of the genus *Origanum*, and marjoram, *Origanum marjorana*, is a member of the same genus, usually cultivated.

Long-established trade practices also recognise the leaves of certain plants in Mexico to be oregano since their flavour and aroma bear a family resemblance to Mediterranean oregano but it has been determined that Mexican oregano is from the genus *Lippia*, again, quite distinct from *Origanum*. But let's leave this topic to those experts that will address this issue later in the day in their papers.

So, the two main designations for oregano in commerce, at least in the United States today, are Mediterranean and Mexican. In Italy, Turkey, Greece and other Mediterranean countries, and especially in Mexico, it became a tradition to pick the wild oregano and use it in many different dishes. Its affinity for tomato-based sauces, lamb, seafood and almost any garlic-flavoured dish was recognised and enjoyed. This escaped the attention of much of the world and certainly America until World War II.

Cookbooks before that time hardly ever mentioned oregano and it was not until American soldiers returned from Europe that pizza and, of course, oregano were discovered in the United States. The interesting part is that oregano not only provided a distinctive flavour, but it was placed on top of the pizza so that people noticed it as well, and suddenly, there was an explosion of interest in oregano.

### Sources of oregano

As mentioned above, in commerce we divide oregano into two distinctive categories – Mediterranean Oregano and Mexican Oregano – because these have distinct characteristics.

#### Mexico

For example, Mexican Oregano, compared with the Mediterranean type, is much stronger and more robustly flavoured. The leaves are larger and somewhat darker and it has a higher content of essential oils, about 3-4%. In Mexico, major producing states are Chihuahua, Durango, Zacatecas, Jalisco and San Luis Potosi. A normal crop production of oregano in Mexico would be about 3000 tons, of which about 2000 tons come to the United States. The best estimate we can get is that between 600 and 800 tons are consumed locally in Mexico and that the remainder is used for small export markets. Transport from Mexico to the USA is rather easy because trucks can be loaded and delivered within 1-2 days to dealers and manufacturers. In recent years Mexico has experienced several droughts and during those times the crops were substantially reduced because of reduced plant development. The harvest of this crop commences in mid-September as the plants exhibit maximum foliage and continue until the first frosts, which are usually in November in the higher altitudes. Harvesting is all done on a small scale and competes with farmers' work in chillies, corn and their own garden crops. The leaves are picked and generally placed on the ground near the oregano bushes and simply sun-dried for 4-5 days. Wholesalers travel through the 'Ejidos' or peasant-owned lands and buy their holdings.

#### Mediterranean

On the other hand, the oregano coming from Greece, Turkey and Albania is milder and typically has about 2-2.5 volatile oil (s.v.o). Harvesting is done much the same way as in Mexico, with whole families going up into the hills and mountains to cut

the bushes. Unlike in Mexico where oregano is fairly uniform in flavour and taste, the oregano obtained from the major Mediterranean exporting countries, namely Turkey and Greece, can vary substantially. There is an old adage in the trade that oregano from either side of the same mountain may be different, and we found out that this is essentially true.

At the moment Turkey seems to have captured a dominant position in the worldwide trade of oregano. Italy, on the other hand, harvests a great deal of the product, but we believe that it is in such heavy demand internally that little finds its way into international markets. The Moroccan and Israeli types are somewhere in between the Mediterranean and Mexican, and as such do not have major applications for industrial purposes.

The exporters in Turkey have become quite expert in cleaning the leaves and removing the heavy concentration of dirt, stems, excreta, etc. that arrives from the hills. Several new modern factories have sprung up and use the newest technology available. Also the exporters in Turkey have become quite good in blending the various kinds of oregano to suit the various usages and tastes of buyers in various countries. In fact, few exporters now ship Turkish oregano as collected because specifications by major users require that they guarantee the level of essential oil, bulk index, stems and insect fragments. This is quite distinct from the mesh size of the finished product and the cleanliness specifications which are regulated by the US Food & Drug Administration and checked by ASTA-approved laboratories upon arrival.

Because no one species of oregano can meet all the criteria, the exporters have invested heavily in sophisticated laboratories which enable them to mix various varieties and come up with the product required. This is certainly true for the majority of exports to the United States, Europe and it has just begun for Japan as well. For example, some manufacturers now require Turkish oregano with a minimum 3% volatile oil and this requires the exporters to blend a heavier percentage of white oregano in the product. On the other hand, because this oregano has so much volatile oil it attracts more insects and the level of insect fragments makes it very difficult for the product to pass Food and Drug Administration rules in the United States. So blending is becoming an art and some exporters are finding ways around that by controlling the sourcing of the raw material including sourcing from controlled agriculture, where available, so that the level of insects and their fragments can be kept to a minimum. This a major reason why Turkish oregano has advanced so much in the USA and other world markets.

Greece, on the other hand, also has an excellent quality of raw material but unfortunately, it seems they have not kept up with similar technological progress. As a result we have seen their volume of shipments, at least to the USA, drop sharply.

Table 1 shows the quantity in tonnes imported by the USA from 1991 through 1995. On a 5-year average, annual consumption is about 6000 t (equivalent to about 13 million pounds). You will observe that Mexico and Turkey are the major suppliers.

**Table 1. Quantity of oregano imported by the USA in the 1991-95 period.**

| Supplier      | USA oregano imports (t) |      |      |      |      |
|---------------|-------------------------|------|------|------|------|
|               | 1991                    | 1992 | 1993 | 1994 | 1995 |
| Mexico        | 2186                    | 1558 | 2080 | 2009 | 2100 |
| Turkey        | 2731                    | 2411 | 2717 | 3588 | 3392 |
| Greece        | 440                     | 272  | 321  | 401  | 139  |
| Israel        | 267                     | 192  | 144  | 247  | 168  |
| Morocco       | 171                     | 32   | 72   | 100  | 140  |
| Miscellaneous | 340                     | 204  | 595  | 350  | 156  |
| Total         | 6135                    | 4669 | 5929 | 6695 | 6095 |

### Consumption: trends and patterns

Japan and to a lesser extent countries in the Far East, such as Korea, Thailand, Singapore, Malaysia and the Philippines, are beginning to exhibit interest in oregano. It seems that the major American chains, such as McDonald's, Kentucky Fried Chicken, Pizza Hut, Taco Bell, etc. are making inroads with those countries' younger generation and are gradually changing their taste toward Western-style foods and even in their eating habits; by this I mean the acceptability of fast food.

In the United States oregano consumption boomed from one and a half million pounds in the 1960s to well over 13 million pounds today. What is behind this huge volume increase as regards oregano usage? There are many reasons related to the way all spices are used, but the most important is America's current fascination with foods from all over the world, particularly France, Italy and Mexico. Each of these brings oregano into a more prominent position, but the biggest news is that our huge fast food industry has entered the ethnic marketplace at full speed. The industry is producing and introducing Americans to the specialties of other countries. Americans in the Eastern United States from New York, Boston and all the way south to Florida have learned about Mexican foods, although quite far from Mexico, and rural areas in the Mid-West and South have also found pizza. There are now giant restaurant chains involved in the ethnic market; for example, Pizza Hut for pizzas of course, and Taco Bell, which specialises in Mexican foods. This explosion has reached the point where even McDonald's serves Italian pizza and Mexican fajitas - a sandwich-like snack - both heavy users of oregano.

Food manufacturers have joined the movement with thousands of ethnically based products, from frozen dinners to sauces, to soups and snacks. Just a few years ago you could have found only two or three Italian-style spaghetti sauces on the shelves. Today it is not unusual for just a single supermarket to carry a dozen or more brands, and for small local markets to carry at least four or five where just a short time ago they might not have carried more than one. Oregano, of course, is an important ingredient in the seasoning used in spaghetti sauce.

An additional factor in the increased use of oregano is that, as a nation, we have been advised to cut back on salt and fat. It is estimated that at any given time over half the American population is on some type of diet. Of the countless others who are not on a particular diet, there is a move to use less red meat and to eat a great deal more chicken and fish. All of this is a bonanza for oregano, for as you reduce sodium, fat or calories the biggest loss is in 'flavour'. Oregano has been able to take advantage of this trend since it adds a new element and choice to otherwise lost flavour.

From Figure 1, which was prepared by an organoleptic panel of a major spice company in the USA you will observe the correlation between the flavour and aroma profiles for Mexican, Turkish and Israeli Oregano. This panel has broken down the aroma into earthy/musty, hay, mint and medicinal, which you can observe on the top portion. The flavour has been evaluated as a combination of medicinal, musty, hay, bitter, mint and green, on the bottom. It is clear that as regards aroma, the Mexican and Turkish are very similar, with the Israeli close behind, while there are distinct differences between them as far as flavour is concerned.

Oregano usage has soared on the wings of pizza and other Italian specialties. Although Mexican-style foods do not put quite the same spotlight on oregano that pizza does, oregano still plays an important role in the ever-increasing popularity of chilli-flavoured dishes and hot foods. As a result, demand is brisk in that area as well.



**Fig. 2.** Aroma and flavour profiles for Mexican, Turkish and Israeli oregano evaluated at 0.10% in 70° spring water. The counterpoint = 0; each integral radiating outward = 1.0 cm on the 15 cm Line Scale, where 1.25 cm is Threshold, 5 cm is Slight, 10 cm is Moderate and 15 cm is Strong.

However, and regrettably, some exporters of Mediterranean oregano tried to take advantage of this bonanza. For the past 3-4 years a dark cloud appeared as some exporters have adulterated shipments for purely economic reasons by adding several herbs (some identifiable, some not) including, and especially adding, sumac (*Rhus coriaria*). Of course, these adulterants were of far lesser value, and they thought they had discovered a 'get rich quick' opportunity. The situation got so serious that now the ASTA mandates that all shipments arriving in the United States must be tested and warranted to be free of sumac. Also, other adulterants are currently being isolated and shippers put on notice. We are now happy to report that we have seen good progress in that area and that sumac has not been found in any lots imported from the 1995 crop from any origin, and the level of other adulterants is also decreasing.

I will conclude by saying that oregano has become our leading herb and that its consumption is still increasing. As mentioned, in 1995 it reached about 14 million pounds in the USA alone. That tonnage of something as bulky and lightweight as oregano has to be taken seriously - after all 1 lb, about 454 grams, of oregano, is enough to season pizza for a family of four every day for one year. Multiplied to cover the entire US, that's six billion slices. You must agree that this is very, very impressive.

## Recent initiatives in the development of medicinal and aromatic plant (MAP) cultivation in Italy

**Alessandro Bezzi**

ISAFSA, Forest and Range Management Research Institute, Villazzano di Trento, Italy

The information I will be providing might not be related directly to oregano, but I feel that it will be useful to provide you with a picture of what is happening in the Italian field of medicinal and aromatic plant cultivation (MAP), not only from a technical and scientific point of view, but also from a promotional one.

### Italian Association of MAP Producers

For many years the MAP situation has been a difficult and precarious one and can largely be summarized as follows:

- the fragmentation of producers, who are few in number and scattered across the Italian territory;
- an extreme lack of technical information, particularly regarding market trends;
- few contracts between farmers and processing industries, the majority of the production remaining almost totally in the hands of wholesalers.

For this reason the Forest and Range Management Research Institute of Trento (Istituto Sperimentale per l'Assessmento Forestale e per l'Alpicoltura di Trento, ISAFSA), which was established by the Ministry of Agricultural, Food and Forest Resources of Italy (MIRAAF) and whose activities concern experimental work in particular, has decided to concentrate its efforts on solving some of the problems mentioned above, stimulated also by the recommendations provided by the Ministry of Agriculture.

At a meeting in Nyons, France, in December 1994, which was attended by experts from many EU countries, the need to bring together the European farmers into a single association of producers, to enable them to collect the requests of European producers and present them at the correct moment to the various European Community commissions, was widely recognised.

As Italy did not yet have an association to represent this category, my Institute (ISAFSA) took the necessary steps to establish an association to bring together producers, cooperatives and also the institutions involved in experiments or in some way involved in the promotion of MAP production. Therefore, the Italian Association of Medicinal and Aromatic Plants (Federazione Italiana dei Produttori di Piante Officinali, FIPPO) was eventually founded in Verona, at the 'HERBORA '95' Conference, on 27 March 1995, by 32 members. On that occasion, the founding members approved a statute, the major points of which are mentioned below.

#### Article 1. Establishment

The Italian Association of Medicinal and Aromatic Plants (omissis) has been founded to represent, protect and assist the MAP producer members affiliated to international, national and regional public administration bodies competent in the field, as well as with planning.

### Article 3. Scope and Purpose

The Association's aim is primarily to protect and safeguard the moral and economic interests of the producers of medicinal and aromatic plants (MAPs) with regard to technical, legal and financial/fiscal matters. In order to carry out its aims, the Association agrees to:

1. contribute, under its own initiatives, to the planning and the development of MAP production through an information network, in order to provide a better knowledge of the MAP market and of the industrial products derived from MAPs;
2. co-ordinate the various stages of production, the first processing and marketing of MAPs (and of all MAP derivatives) cultivated directly by its members, in accordance with European Community policies and with regional and national agricultural planning policies;
3. carry out technical training workshops for all those working in the production, first processing and marketing of MAPs, provide professional training courses and promote the use of information on MAPs, also at consumer level;
4. promote technical assistance and consultancy both for the development of activities that companies plan to carry out in the MAP field, as well as for access to possible public funding by local, regional, governmental and European Community bodies for the development of single or joint projects;
5. promote the diffusion of conventional and biological agricultural techniques and methods according to EEC regulation (U.E.) 2092/9J, also by creating laboratories to certify/guarantee the products obtained from present crops and biological methods;
6. promote the setting up of companies to produce facilities for first processing and the marketing of MAP products;
7. promote producer associations for plants used primarily in the perfume industry, medical field etc. according to EEC regulation 1360/78 and 2083/80;
8. participate in applied research programmes concerned with mechanization, genetic improvements and methods for increasing production and quality of drugs, and the reduction of production costs, by making use of the collaboration of experts and of public and private institutions operating in the experimental field;
9. promote and sustain European Community initiatives in order to fulfil the objectives of a common policy in the field.

### Article 4. Membership

The number of members that may join is unlimited. Those who qualify for membership are as follows: MAP producers (whether individual or cooperatives, consortiums or other associated companies), experts in the field of cultivation, processing and marketing of MAPs; bodies that promote the production of MAP crops, even by means of research and experiments (this contingency must not total more than 20% of the total number of members).

In 1996 the members of the Association grew to 46. A second annual general meeting of the association took place in Verona on 24 March 1996, during the 'HERBORA '96' Conference. On that occasion the Federation's activities for 1996 were reviewed. A summary of these initiatives is as follows:

- registration of the Federation at the Registry office in Trento (Italy);
- nomination of ISAFSA as the temporary seat of the Federation's secretariat;
- subscription/registration of the FIPPO and EUROPAM (European Federation of MAP Producers);
- participation of the President at two meetings of EUROPAM, in Brussels and Strasbourg;
- two meetings of the Management Group in Milan;
- production of two FIPPO bulletins/newsletters.

All the activities of the President, the Management Group and the secretariat were carried out on a voluntary basis, without remuneration.

In particular, the two FIPPO Newsletters have represented an important tool for spreading information on the Association's activities and on the sector in general, including the MAP market. However, owing to a lack of financing, the Newsletter

was placed in a monthly magazine "Erboristeria Domani" which is very kindly sent by the editors to all FIPPO members free of charge.

The Association has so far financed itself depending solely on the annual subscription fees of its members, to cover its costs.

The work of the Association is contributing to the sharing of knowledge and experience among its members so as to have a positive influence on farmers' cultivation choices and, in the long term, the planning of MAP cultivation in Italy.

Unfortunately, not all the initiatives in the area of MAP species, presently operational in Italy are moving in the same direction as those of institutions like ISAFA. We would like to promote MAP cultivation as an element of diversification of agricultural products, as a source of income for farmers and as an example of activities that can be considered among those leading to ecologically compatible or sustainable agriculture.

In fact, there are obvious or latent initiatives with a tendency towards the contrary. This is the case of the law proposals regarding the MAP sector that, on the grounds of regulating the herbal sector as a whole, would require MAP farmers to possess a diploma in phytopreparation or herbalism. The same applies to Italian laws presently governing the registration of phytoiatric products, which make it compulsory for those of vegetable origin to undergo the same tests and requirements necessary for the registration of phytoiatric products of synthesis. This process is extremely complicated and makes little sense (e.g. neem extract which is commonly used in biological agriculture and has not yet been registered in Italy). Thus, a process which is favoured by everyone, and which would contribute to extending the use of a sustainable agriculture even in Italy, is further slowed by the lack of adaptability or by a simplification of current legislation.

### **New project of MIRAAF on MAP production**

As coordinator of the ministerial project on the cultivation of MAPs, named 'Incremento della Produzione di Piante Officinali - IPPO' (= increase of the production of MAPs), I would like to share another piece of information with you. It concerns the intention of MIRAAF to finance the first year of experiments of the new project entitled 'Increase in the production of MAPs'.

The project moves in the same direction as the previous project which ended in 1995, but with new objectives as follows:

- involvement in the research of those regions which are interested in participating in the research of the Project in coordination with the Operational Units financed by the Ministry;
- in addition to aromatic plants, the insertion of certain MAPs such as valerian (*Valeriana* spp.), *Echinacea pallida*, common melilot (*Melilotus officinalis*) and witch hazel (*Hamamelis virginiana*);
- the development of experimental work on insecticides and fungicide plants, bearing in mind the extreme interest and topicality of the subject and of the expectations that a large part of biological farmers show for these plants.

On the whole, the project aims to obtain results with an immediate effect on the agricultural sector involved which, although relatively small in scale (2300 ha), is constantly evolving and currently seems to be heading towards sustained growth.

**Annex I.** Objectives of the MIRAAF Project on 'Increase Production of Medicinal and Aromatic and Pesticide Plants (IPPO)'.

1. Study crop management techniques of some species in order to increase the production of medicinal and aromatic plants in Italy;
2. Contribute to decreasing the surplus of food crops by diversifying agricultural productions and contribute to increasing farmers' incomes;
3. Promote sustainable agricultural systems and investigate new natural insecticides and fungicide products for crop protection.

**Annex II.** List of species to be studied by the IPPO Project and its market promotion initiatives.

#### **Aromatic plants**

|                                       |                     |
|---------------------------------------|---------------------|
| <i>Salvia officinalis</i> L.          | sage                |
| <i>Chamomilla recutita</i> Rauschaert | chamomile           |
| <i>Foenolicum vulgare</i> L.          | fennel              |
| <i>Origanum</i> spp.                  | meridional marjoram |
| <i>Myrtus communis</i> L.             | myrtle              |
| <i>Rosmarinus officinalis</i>         | rosemary            |

#### **Medicinal plants**

|  |                        |
|--|------------------------|
| <i>Melilotus officinalis</i> (L.) Pallas | common melilot         |
| <i>Echinacea</i> spp.                    | palepurple cone flower |
| <i>Valeriana officinalis</i> L.          | valerian               |
| <i>Hamamelis virginiana</i> L.           | witch hazel            |

#### **Insecticidal and pesticidal plants**

|   |                     |
|---|---------------------|
| <i>Melia azadarach</i> L.   | Persian lilac       |
| <i>Chrysanthemum cinerarifolium</i> L.                            | Dalmatian pyrethrum |
| <i>Reynoutria sachalinensis</i> (F. Sch.) Nakai                   | –                   |
| <i>Phytolacca dodecandra</i> L'Herit and<br><i>P. decandra</i> L. | pokeweed            |

#### **Promotion initiatives**

- Market studies
- Information and promulgation
- Institution of a permanent Inter-professional Committee (farmers, researchers, traders, industrialists).

## Cultivating oregano in Italy: The case of 'Bioagricola A. Bosco', a Sicilian firm

**Domenico Chiapparo**

Bioagricola A. Bosco, Favara (Agrigento), Italy

### Introduction

This presentation aims to describe to the participants of this workshop the cultivation of oregano on my farm, the Bioagricola A. Bosco, which was established in the Agrigento hinterland in 1992.

Several well-known experts have already spoken today about oregano with regard to various agronomic and scientific issues.

As we all know, oregano is a crop that attracts great interest for many reasons, economic potential and risk of genetic erosion being among the most important ones. As far as my experience in Sicily is concerned, I can say that in this region several varieties of oregano can be found. In the province of Agrigento alone, it is possible to count six of them, including *Origanum heracleoticum*. This particular variety is the one that I have decided to cultivate because of its very unique aroma and easier cultivation practices.

### The farm and the habitat

The farm is located some 30 km north of Agrigento (Agro di Aragona loc.) at 300 m asl. The soil is highly calcareous (pH 7.9), with a north-south exposure. With regard to exposure, the best results in the cultivation of oregano are obtained on lands exposed to the north. In January 1992, the year I started my activities, I collected some 20 plants in the wild from a nearby mountain and planted them in a parcel of land 150 m<sup>2</sup>, by distributing them regularly in this space. The following year, thanks to artificial irrigation during the hottest periods, each of these plants produced a little bush of ca. 1 m in diameter. From these bushes I was able to obtain an average of 180 plantlets, which were then used to establish a new larger cultivation of 6 ha. This field is structured as follows: 115 cm distance between rows and 50 cm distance within rows. Owing to the natural development of the plants, at maturity the field is composed of compact rows 50-60 cm apart. Such a distance allows for the cultivation of the field with *ad hoc* machinery. It might be asked whether this system is rather expensive, and whether it would not have been better instead to use seeds directly. The answer to this is simple: we would have obtained a very heterogeneous field!

However, other techniques allowing for the conservation of the genetic integrity of the plant throughout the field include cuttings and micropropagation. From personal experience, this cutting procedure has been used with very good results during the vegetative stage in January, with the herbaceous stem and also in the open field. The micropropagation technique *in vitro* necessitates specialised laboratories.

Because my farm operates according to the rules of the EU regulation 2092/91 regarding the biological control of pest and diseases, weed control is only done mechanically (at least four times a year). The most common weeds in the field are the *Avena* species. In this field, particularly suitable for oregano cultivation, I have

never used either chemical or biological fertilizers. According to old local farmers, who have been growing oregano for domestic consumption in little patches, the life time of an oregano cultivation can last for several decades.

#### **Agronomic results obtained in the 2nd year after the establishment of the cultivation**

Each bush of oregano in the field produces about 0.6 kg of green product, which multiplied by the 17 000 bushes present in 1 ha, gives a total of 10.2 t. This amount reduces to 2.75 t in the final product, as the 'pot herb cut' will undergo a loss of weight from drying and stem removal of about 73%. The drying process takes place in environments protected from the sun, at a temperature no higher than 29°C. I leave it to you to consider the economic impact of this production.

My farm aims at the commercialization of this product in bundles, placed in cellophane bags. However, the commercialization of this type of product is not easy. I found great difficulties in marketing it. Indeed the market niche for this type of product is very narrow.

Another possibility for increasing the economic returns of this cultivation is to extract essential oils for pharmaceutical and cosmetic uses. Personally, I have distilled, just as an experiment, a certain amount of oregano. However, even though the quantity of oil obtained was reasonably good (2.33%), I do not wish to undertake this type of activity.

## VI. International Cooperation

### Introduction

The report of the first meeting of the Oregano Genetic Resources Network was drafted by S. Padulosi and circulated on 7 June 1996 to all participants for further comments and amendments. This represents its final version endorsed by all the participants.

The meeting took place from 9.00-12.00 am at Callà 2 Hotel, Policoro, southern Italy. It was chaired by S. Padulosi and was attended by the following: D. Baricevic, J. Bernáth, G. De Mastro, K. Hammer, W. Junghanns, S. Kokkini, E. Kitiki, V. Marzi, I. Morone Fortunato, J. Novak, G. Olivier, U. Paap, P. Perrino, E. Putievsky, C. Rey, G. Sarli, M. Skoula, P. Spada and L. Xhuveli.

Unable to attend were M. Bacchi, C. Baser, B. Benjilali, A. Bezzi, D. Chiapparo, C. Franz, F. D'Antuono, E. Leadley, C. Leto, G. Logozzo, E. Lombardo, B. Pasquier, V. Picci, L. Qani and A. Salamone.

This report was distributed by mail on 23 July 1996 to both attending and non-attending persons mentioned above.

### The discussion

#### • Bernáth

If we recall the various presentations given in the Workshop, it is clear that there are a number of urgent actions that need to be taken to promote better conservation and use of *Origanum* species:

- more attention to botanical aspects;
- need to establish a central herbarium to use as a point of reference for taxonomic studies and queries regarding the classification of these species;
- need to have reference material for plants that are being analysed chemically;
- more research on seed physiology and other biological aspects.

In addition, it is paramount to foster collaboration among scientists involved in oregano research to facilitate the exchange of information on biochemical and botanical findings.

#### • Putievsky

To make a good impact with our Network we should be realistic. I would therefore suggest concentrating our efforts on a limited number of crucial actions:

- germplasm collecting;
- establishment of a reference herbarium collection;
- carry out studies on phenological aspects.

In the beginning, these activities could be limited to only a few sites and material and later on, after having established the work, extended to a wider area and to a wider representation of genetic diversity.

In my opinion, support should first of all be given to promoting all the herbarium collection. Having said that, I feel it would be wise to use the money that the UMS project would allocate for the 1997 Network meeting to support the

activities mentioned. We can then meet in 1998 and thus present some concrete output of our Networking activities.

With regard to research, it would be very important to invest in cold storage and germination requirement studies, as very little is known on this subject.

- **Marzi**

We should consider that the degree of variation found in local populations of *Origanum* is incredibly high (e.g. the case of Italian material). Therefore, to facilitate the work of the breeder, it would be very important to concentrate on a number of populations and carry out investigations on (1) aroma, (2) colour and (3) yields.

In fact, it is rather difficult to know which of the material currently maintained in our collections would best meet the requirements of the industrial sector.

- **Putievsky**

What Marzi says is true, but we should not just look at industrial application in our goals. We need to have a seedbank for conservation of the whole variation and not just what might be of interest to the industrial sector. For example, one day we might discover that Italian material could grow very well in Turkey and therefore we should work to ensure that this material is conserved and made available to users of that country. In any case, any adequate strategy on germplasm use will need to study the whole genepool as a first step in promoting better use of the crop.

- **Kitiki**

Yes, indeed we should collect as much genetic diversity as possible to facilitate breeding work. We should collect every distinct population as we do not know what genetic traits they might carry. Once this step has been taken, we should then characterize the material. In short, we should indeed aim at the conservation and use of plant genetic resources of oregano in its full sense. Users will then use the material according to their necessities.

With regard to the herbarium collection, it will be important to have at least two reference collections. I would suggest establishing one in Greece and another in Turkey.

- **Perrino**

In the plant genetic resources field there are different approaches for best conserving germplasm material. For our case, I would recommend first concentrating our efforts on collecting those populations which are most endangered. For instance, there are some species which are endemic only to a limited area and they might well be seriously threatened with genetic erosion, as we heard in some presentations during the Workshop. To complement *ex situ* conservation activities, it will be important to identify those sites where *in situ* conservation measures also should be applied. Of course, species which are distributed over a wide area will have lower priority in a collecting strategy.

- **Olivier**

What exactly do we mean when we talk about utilization?

- **Marzi**

Regarding use, it is important to bear in mind that similarly to what has happened for major crops (like tomatoes, for instance), farmers have selected many interesting local types of oregano over the years. This material has scarcely been collected and

studied. It is very important, therefore, to have a representation of this diversity that is of great usefulness to breeders and to users in general.

- **Perrino**

I would like to stress that *in situ* cannot replace *ex situ* conservation. The *in situ* approach is meant to be applied particularly to endemic populations. I also would like to remark that if the plant genetic resources community had reacted more quickly to create projects like the UMS devoted to minor/underutilized species, we would not have lost so much of the genetic diversity of these species as we have.

- **Hammer**

The issue of choosing the best way to preserve genetic diversity is a complex one. There are pros and cons in both systems and we should go for a harmonised approach. Long-term conservation of germplasm without monitoring the viability of material is deleterious, as can be said for excessive rejuvenation which causes genetic drift. Long-term conservation is also a costly commitment. The *in situ* conservation of oregano germplasm is advantageous; nevertheless, we should also consider the importance of having available seed and easy access to it, which is limited when we use *in situ* conservation.

- **Bernáth**

Still on collecting priorities, I would like to recall the case of Hungary where 180 medicinal species have been gathered from wild vegetation over 20 years, with the aim of promoting their better exploitation. Today we estimate that 20 species from this group have become endangered. We should remember this when we are setting priorities for *Origanum*.

- **Perrino**

Genebanks do complement very well the role played by *in situ* conservation, and they are not meant to replace these activities.

- **Kitiki**

*In situ* conservation is not a cheap activity! Moreover it is a relatively new discipline; more research is still needed on this.

- **Kokkini**

We should also say that the conservation of plant genetic resources is often done not just for marketing purposes, but also for cultural, traditional or emotional reasons. Regarding conservation, we should concentrate for the time being on a few species, as it will be unrealistic to look after all of them. The University of Thessaloniki holds some 1000 herbarium specimens of *Origanum* species; we should try to share this wealth with others, try to make this material available for further knowledge on these plants. Furthermore, I would like to stress that any genebank entry should be always accompanied by its correspondent herbarium specimen, to be used as a reference material.

- **Perrino**

Another very important aspect that we have not mentioned yet is data-basing. The setting up of a database is also a priority action. This will provide users with a clear picture of what is available in Herbaria and genebanks on oregano. We should of

course be careful when inputting data to the database not to identify the material incorrectly.

- **Baricevic**

Databases are very useful tools. In Slovenia, the University of Ljubljana is setting up a relational database that, once ready, will be able to combine taxonomic information with data referring to environment and characterization and evaluation works. This type of database will be very valuable for enhancing the use of oregano.

- **Putievsky**

In a scale of priorities out of all the actions mentioned, I feel that the database is not such an urgent task. A more urgent task would be that of preparing guidelines on how to collect oregano populations, how to best store the seeds, etc.

- **Xhuveli**

Oregano is a very popular crop in Albania. In spite of that, we do not have adequate information on its distribution in the country and how to best safeguard its diversity. For instance, what should be the best *in situ* conservation strategy for those indigenous populations exploited commercially? I would indicate therefore a strategy for oregano comprising three main steps: (1) distribution survey; (2) collection of genetic diversity; (3) formulation of a strategy for the exploitation of wild populations.

- **Putievsky**

What Xhuveli says is very important; however, regulation of exploitation of natural resources, particularly those under threat of erosion, is something that should be done by the National Authorities. In Israel, for example, there are laws that limit the exploitation from the wild of *O. syriacum*, which is an endangered species. It is indeed a problem of education of public opinion as well as regulation of commercial exploitation through national laws.

- **Junghanns**

Working on wild material of oregano is not a simple job. In my company we have experienced how difficult this task is. A major problem is the standardisation of quality of material taken from the wild that is being brought into cultivation for commercialization.

With regard to a database, for the private sector it is very important to have this tool because the market preferences change often, and we need to continuously review our strategy in terms of germplasm to be used and a database would greatly help that.

- **Skoula**

Investigations on the requirements for carrying out seed conservation of oregano species should receive proper attention.

### The Network Working Plan

Following this discussion, Padulosi stressed that the meeting should provide some specific recommendations that the Network should pursue to meet the goal of better conservation and use of oregano species. Many actions were suggested; however, to have an impact in the short term, it would be wise to first concentrate on a few important activities considered by everybody as basic needs.

Padulosi suggested that if the Network agrees, the financial support that UMS has allocated for the meeting in 1997 be used instead to sustain activities identified for that year.

Participants unanimously agreed that the following three activities should receive top priority by the Network and that it would be wise to use the 1997 support to promote those actions:

1. establishment of a seed germplasm collection;
2. establishment of a herbarium reference collection;
3. establishment of a database.

The following institutions indicated their interest to be focal points for these activities:

- The Aegean Agricultural Research Institute, Izmir, Turkey: **seed conservation** (attention: Dr Kitiki)
- The University of Thessaloniki, School of Biology, Lab. of Systematic Botany and Phytogeography, Thessaloniki, Greece: **herbarium collection** (attention: Prof. Kokkini)
- The University of Horticulture and Food Industry, Dept. of Medicinal Plant Production, Budapest, Hungary: **database** (attention: Prof. Bernáth).

#### Actions

- submit to Padulosi by the end of July 1996 the final version of their paper for the Workshop proceedings (Attention: **ALL**);
- contact the three institutions and work out details on how to implement the above-mentioned initiatives in collaboration with other Network members (Attention: **PADULOSI**).

#### Election of Chairperson

The participants elected Dr Eli Putievsky, Chairperson of the Oregano Genetic Resources Network. Ms Melpo Skoula and Dr Giuseppe De Mastro were also elected as Co-Chairpersons.

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## VIII. Useful Bibliography

This additional bibliography is meant to complement the one already provided in each of the papers delivered at this Workshop. References have been grouped under the main topic dealt with in the investigation. Unless specified, all publications are in English.

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### Biology

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## IX. List of Experts

The following list of experts is meant to complement the one provided in Appendix VII. These names have been provided directly to IPGRI, gathered from Internet on WWW, or from results of a questionnaire survey carried out by the IPGRI UMS project in 1993 and involving Mediterranean Research Institutes. All individuals/companies listed here deal at various levels with aromatic, medicinal, herb and spice plants including oregano (\* indicates that the area of interest is aromatic species in general).

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 CHAMOV, D.  
 GUTEVA, Y.  
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### Canada

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 Saskatoon Research Centre  
 107 Science Place  
 Saskatoon SK S7N 0X2  
 Tel. (+1) 306 956 72 00  
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 Delhi ON N4B 2W9  
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### France

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 Jardin Botanique de la Ville de Nice  
 78 avenue de la Corniche Fleurie  
 06200 Nice

### Germany

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 06484 Quedlinburg  
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**Spain**

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## X. List of Associations

Following is a list of associations dealing at various levels with the conservation, cultivation, use and marketing of medicinal and aromatic plants, including oregano.

A more extensive one accompanied with detailed information on the scope of each association and activities promoted by their members (meetings, newsletters, etc.) can be found at the Herbnet URL address:

<http://HerbNet.com/associations.html>.

### Australia

Australian Commercial Herb Growers  
Association  
PO Box 470  
Kerang 3579, Victoria

Australian Herb Society Inc.  
Maria E. Schotrt  
PO Box 110  
Mapleton, Queensland 4560

Australian Traditional Medicine  
Society  
PO Box 442  
Ryde 2112

Herb Society of South Australia  
PO Box 140  
Eastwood 5063

Manning Valley Herb Association  
c/o Ann Stevens  
PO Box 1028  
Taree, NSW 2430  
Tel. (+61) 65- 59 17 71

National Herbalists Association of  
Australia  
c/o Robyn Kirby  
PO Box 61  
Broadway, NSW 2007  
Tel. (+61) 2 211 64 37  
Fax. (+61) 2 211 64 52

Organic Herb Growers of Australia  
Inc.  
PO Box 171  
South Lismore, NSW 2480

Queensland Herb Society  
Barbara Wickes  
26 Rupicola Place  
Chapel Hill, 4069, Queensland  
Tel. (+61) 7 378 20 75

Singleton and Hunter Herb Society  
Inc.  
PO Box 34  
Singleton NSW 2330  
Tel. (+61) 65 74 72 84

Wildflower Society of Western  
Australia  
Box 64  
Nedlands, WA 6009

### Belgium

International Society for Horticultural  
Science (ISHS)  
Kardinaal Mercierlaan 92  
3001 Leuven, Belgium

### Canada

Canadian Association of Herbal  
Practitioners  
921 17th Ave. SW  
Calgary AB T2T 0A4

Canadian Federation of  
Aromatherapists  
868 Markham Road, Ste 109  
Scarborough ON M1H 2Y2  
Tel. (+1) 416439 19 51

Ontario Herbalists' Association  
11 Winthrop Place  
Stoney Creek ON L8G 3M  
Tel. (+1) 416 536 15 09  
Fax. (+1) 416 536 15 09

**Italy**

Assoerbe  
Via Serbelloni 7  
20121 Milan  
Tel. (+39) 2 79 56 01

**United Kingdom**

Aromatherapy Organizations Council  
3 Latymer Close  
Braybrooke Market Harborough  
Leicester LE16 8LN

**British Herb Trade Association**

Rachel Moseley, NFU  
164 Shaftesbury Avenue  
London W2H 8HL  
Tel. (+44) 171 331 74 15  
Fax (+44) 171 331 74 10

**British Herbal Medicine Association**

Old Coach House  
Southborough Rd.  
Surbiton, Surrey

Essential Oil Trades Association Ltd  
61 Clinton Lane  
Kenilworth, Warwickshire CV8 1AS

**Herb Society**

The Caroline Holmes  
134 Buckingham Palace Rd.  
London, SW1W 9SA  
Fax. (+44) 296- 62 51 26

**International Federation of**

Aromatherapists  
204 Chiswick High Road  
London, W4  
Tel. (+44) 181 742 26 05

**National Institute of Medical  
Herbalists**

56 Longbrook Street  
Exeter, Devon EX4 6AH  
Tel. (+44) 1392 42 60 22  
Fax. (+44) 1392 49 89 63

**United States of America**

American Botanical Council  
Mark Blumenthal  
PO Box 201660  
Austin TX 78720-1660  
Tel. (+1) 512 331 19 24  
(+1) 512 331 88 68

American Herb Association  
PO Box 1673  
Nevada City CA 95959-1673  
Tel. (+1) 916 265 95 52

American Herbal Products  
Association  
PO Box 30585  
Bethesda MD 20824  
Tel. (+1) 301 951 32 07

**American Herbalists Guild**

PO Box 746555  
Arvada CO 80006-6555  
Tel. (+1) 303 423 88 00  
Fax. (+1) 303 423 88 28  
email herbs@got.net  
ahg@earthlink.net

**American Society for Horticultural  
Science**

701 North Saint Asaph Street  
Alexandria VA 22314  
Tel. (+1) 703 836 46 06

**American Spice Trade Association**

PO Box 1267  
580 Sylvan Ave.  
Englewood Cliffs NJ 07632  
Tel. (+1) 201 568 21 63  
Fax. (+1) 201 568 73 18

**Arizona Vegetable Growers  
Association**

Henry L. Giclas, E.D.  
3120 N 19th Street, Suite 190  
Phoenix, AZ 85015  
Tel. (+1) 602 266 62 25

Association for the Promotion of  
Herbal Healing  
George Weissman  
PO Box 7011  
Berkeley CA 94707  
Tel. (+1) 415 526 62 50

Association of Natural Medicine  
Pharmacists  
8369 Camps de Elysses  
Forestville CA 95436  
Tel. (+1) 707 887 13 51  
Fax. (+1) 707 887 90 94

Delaware Herb Growers and  
Marketers Association  
Delaware State University  
Dept. of Ag. and Nat. Resources  
Dover DE 19901

Fragrance Foundation  
c/o Annette Green  
145 East 32nd St.  
New York NY 10016

Garden Writers of America  
Association  
10210 Leatherleaf Court  
Manassas VA 22111  
Tel. (+1) 703 257 10 32  
Fax. (+1) 703 257 02 13

Great Northern Botanicals  
Association  
PO Box 362  
Helena MT 59624

Hawaii Herb Association  
Alice Y. Kadowaki  
PO Box 62150  
Honolulu HI 96839  
Tel. (+1) 808- 988 66 64

Herb Growing & Marketing Network  
c/o Maureen Rogers  
PO Box 245  
Silver Spring, PA 17575-0245  
Tel. (+1) 717 393 32 93  
Fax. (+1) 717 393 92 61  
email herbworld@aol.com  
<http://HerbNet.com/associations.html>.

Herb Research Foundation  
c/o Rob McCaleb  
1007 Pearl St., Suite 200  
Boulder CO 80302  
Tel. (+1) 303 449 22 65  
Fax. (+1) 303 449 78 49

Herb Society of America  
c/o Michelle Milks  
9019 Kirtland-Chardon Rd.  
Kirtland OH 44094  
Tel. (+1) 216 256 05 14  
Fax. (+1) 216 256 05 14

Herb Society of Central Florida  
PO Box 533958  
Orlando FL 32853-3958  
Tel. (+1) 407 896 32 03

Illinois Herb Association  
c/o Lowell Lenschow  
1701 Towanda Ave.  
Bloomington IL 61701  
Tel. (+1) 309 557 21 07  
Fax. (+1) 309 557 25 59

International Aromatherapy and Herb  
Association  
c/o Jeffrey Schiller  
3541 West Acapulco Lane  
Phoenix AZ 85023  
Tel. (+1) 602 938 44 39  
email [jeffreys@aztec.asu.edu](mailto:jeffreys@aztec.asu.edu)

International Herb Association  
Cathy Sebastian  
PO Box 317  
Mundelein IL 60060-0317  
Tel. (+1) 847 949 43 72  
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Kentucky Herb Association  
PO Box 123  
Washington KY 41096

Longfellow Herb Society  
PO Box 309  
Kingfield ME 04947  
Tel. (+1) 207 265 56 33  
Fax. (+1) 207 639 20 12

Madison Herb Society  
PO Box 8733  
Madison WI 53708

Maryland Herb Association  
14119A Peddicord Rd.  
Mt Airy MD 21771-8436  
Tel. (+1) 301 829 14 78

Michigan Herb Business Association  
c/o Judy Larison  
135 East 120th St.  
Grant MI 49327  
Tel. (+1) 616 834 54 81

Michigan Marketing Association  
c/o Christopher Steele  
618 Seymour  
Lansing MI 48933  
Tel. (+1) 517 371 24 11

National Association for Holistic  
Aromatherapy  
219 Carl St.  
San Francisco CA 94117  
Tel. (+1) 415 564 67 35  
Fax. (+1) 415 564 67 99

National Nutritional Foods  
Association  
3931 Mac Arthur Blvd, Ste 101  
Newport Beach CA 92660  
Tel. (+1) 714 622 62 72  
Fax. (+1) 714 622 62 66

National Oils Research Association  
894H Route 52  
Beacon NY 12508  
Tel. (+1) 914 838 43 40  
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emailnorassoc@aol.com  
Natural Food Associates  
PO Box 210  
Atlanta, TX 75551

New Age Publishing and Retailing  
Association (NAPRA)  
PO Box 9  
Eastsound WA 98245  
Tel. (+1) 206 376 27 02

New York State Farmers Direct  
Marketing Association  
119 Ford Hill Rd.  
Whitney Point NY 13862

New York State Vegetable Growers  
Association  
c/o Jean Warholic  
PO Box 356  
Ithaca NY 14852-0356  
Tel. (+1) 607 539 76 48

North Carolina Herb Association  
c/o Jeanine M. Davis  
2016 Fanning Bridge Rd.  
Fletcher NC 28732  
Tel. (+1) 704 684 35 62

Ohio Ecological Food & Farm  
Association  
PO Box 02234  
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Oklahoma Herb Growers &  
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Tulsa OK 74170

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Texas Herb Growers & Marketers  
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United Plant Savers  
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West Virginia Herb Association  
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