Oregano

The genera *Origanum and Lippia*

Edited by Spiridon E. Kintzios

Medicinal and Aromatic Plants – Industrial Profiles

Oregano

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Dedicated to my mother, Stavroula

Contents

Preface to the series

There is increasing interest in industry, academia and the health sciences in medicinal and aromatic plants. In passing from plant production to the eventual product used by the public, many sciences are involved. This series brings together information which is currently scattered through an ever increasing number of journals. Each volume gives an in-depth look at one plant genus, about which an area specialist has assembled information ranging from the production of the plant to market trends and quality control.

Many industries are involved such as forestry, agriculture, chemical, food, flavour, beverage, pharmaceutical, cosmetic and fragrance. The plant raw materials are roots, rhizomes, bulbs, leaves, stems, barks, wood, flowers, fruits and seeds. These yield gums, resins, essential (volatile) oils, fixed oils, waxes, juices, extracts and spices for medicinal and aromatic purposes. All these commodities are traded worldwide. A dealer's market report for an item may say 'Drought in the country of origin has forced up prices'.

Natural products do not mean safe products and account of this has to be taken by the above industries, which are subject to regulation. For example, a number of plants which are approved for use in medicine must not be used in cosmetic products.

The assessment of safe to use starts with the harvested plant material which has to comply with an official monograph. This may require absence of, or prescribed limits of, radioactive material, heavy metals, aflatoxin, pesticide residue, as well as the required level of active principle. This analytical control is costly and tends to exclude small batches of plant material. Large scale contracted mechanized cultivation with designated seed or plantlets is now preferable.

Today, plant selection is not only for the yield of active principle, but for the plant's ability to overcome disease, climatic stress and the hazards caused by mankind. Such methods as *in vitro* fertilization, meristem cultures and somatic embryogenesis are used. The transfer of sections of DNA is giving rise to controversy in the case of some end-uses of the plant material.

Some suppliers of plant raw material are now able to certify that they are supplying organically-farmed medicinal plants, herbs and spices. The Economic Union directive (CVO/EU No 2092/91) details the specifications for the *obligatory* quality controls to be carried out at all stages of production and processing of organic products.

Fascinating plant folklore and ethnopharmacology leads to medicinal potential. Examples are the muscle relaxants based on the arrow poison, curare, from species of *Chondrodendron*, and the anti-malarials derived from species of *Cinchona* and *Artemisia*. The methods of detection of pharmacological activity have become increasingly reliable and specific, frequently involving enzymes in bioassays and avoiding the use of laboratory animals. By using bioassay linked fractionation of crude plant juices or extracts, compounds can be specifically targeted which, for example, inhibit blood platelet aggregation, or have antitumour, or antiviral, or any other required activity. With the assistance of robotic devices, all the members of a genus may be readily screened. However, the plant material must be *fully* authenticated by a specialist.

The medicinal traditions of ancient civilisations such as those of China and India have a large armamentaria of plants in their pharmacopoeias which are used throughout south-east Asia. A similar situation exists in Africa and South America. Thus, a very high percentage of the World's population relies on medicinal and aromatic plants for their medicine. Western medicine is also responding. Already in Germany all medical practitioners have to pass an examination in phytotherapy before being allowed to practise. It is noticeable that throughout Europe and the USA, medical, pharmacy and health related schools are increasingly offering training in phytotherapy.

Multinational pharmaceutical companies have become less enamoured of the single compound magic bullet cure. The high costs of such ventures and the endless competition from me too compounds from rival companies often discourage the attempt. Independent phytomedicine companies have been very strong in Germany. However, by the end of 1995, eleven (almost all) had been acquired by the multinational pharmaceutical firms, acknowledging the lay public's growing demand for phytomedicines in the Western World.

The business of dietary supplements in the Western World has expanded from the Health Store to the pharmacy. Alternative medicine includes plant-based products. Appropriate measures to ensure the quality, safety and efficacy of these either already exist or are being answered by greater legislative control by such bodies as the Food and Drug Administration of the USA and the recently created European Agency for the Evaluation of Medicinal Products, based in London.

In the USA, the Dietary Supplement and Health Education Act of 1994 recognized the class of phytotherapeutic agents derived from medicinal and aromatic plants. Furthermore, under public pressure, the US Congress set up an Office of Alternative Medicine and this office in 1994 assisted the filing of several Investigational New Drug (IND) applications, required for clinical trials of some Chinese herbal preparations. The significance of these applications was that each Chinese preparation involved several plants and yet was handled as a *single* IND. A demonstration of the contribution to efficacy, of *each* ingredient of *each* plant, was not required. This was a major step forward towards more sensible regulations in regard to phytomedicines.

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Roland Hardman

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It is possible that in the course of editing this volume, some reference material may have been used without knowledge, on my behalf, of a copyright ownership of it. In such a case, I apologize to any copyright holder whose rights may have been unwittingly infringed.

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Part 1

Introduction

1 Profile of the multifaceted prince of the herbs

Spiridon E. Kintzios

The scope of this volume is to offer an updated and analytic review on the currently available technical knowledge and market information of the world's commercially most valued spice – oregano. In addition, the book treats in detail various aspects of practical significance for the crop's industrialization – such as optimizing germplasm selection and utilization, novel cultivation methods and product processing, blending and uses in different countries, along with other market-related issues never included in previous reviews.

Oregano is the common name for a general aroma and flavor primarily derived from more than 60 plant species used all over the world as a spice. The majority of them belong to the Lamiaceae and Verbenaceae families, while a large distinction is made between the European (*Origanum* sp.) and Mexican (*Lippia* sp.) oregano.

European oregano is used as a flavoring in meat and sausage products, salads, stews, sauces, and soups. Prior to the introduction of hops, oregano was used to flavor ale and beer. The essential oil and oleoresin, used extensively in place of the plant material, are found in food products, cosmetics, and alcoholic liqueurs. Oregano is also a good salt replacement in tomato-containing recipes. Mexican oregano is used predominantly in flavoring Mexican foods, pizza, and barbecue sauces. Mexican oregano has a somewhat sharper and more pungent flavor than European oregano. The reader will find detailed information on the dietary properties of the spice and the various ways of adding it to foodstuff and beverage preparations. Issues of chemical stability and compatibility to diversified demand specifications will also be examined.

Most widely used is the genus *Origanum* (family Lamiaceae) (from the Greek words *oros* – mountain and hill and *ganos* – ornament). The taxonomy of the genus is rather complicated and a current issue of debate: indeed, *Origanum* sp. is characterized by a large (and still little investigated) morphological and chemical diversity resulting in the distinction of 49 taxa and 42 species. Respecting Ietswaart taxonomic revision there exist ten sections (*Amaracus* Bentham, *Anatolicon* Bentham, *Brevifilamentum* Ietswaart, *Longitubus* Ietswaart, *Chilocalyx* Ietswaart, *Majorana* Bentham, *Campanula ticalyx* Ietswaart, *Elongatispica* Ietswaart, *Origanum* Ietswaart, *Prolaticorolla* Ietswaart). Since Ietswaart's publication, five more species and one more hybrid have been recognized, raising the number of species to 43 and the number of hybrids to 18.

More than 300 scientific names have been given, during the last 150 years, to not more than 70 presently recognized *Origanum* species, subspecies, varieties and hybrids. This plethora of different names reflects the extent of morphological variation the genus exhibits in nature. The overwhelming majority of the taxa are locally distributed within the Mediterranean region, with nine species being located in Greece and 21 in

Turkey. Sixty per cent of all *Origanum* taxa are recorded to grow in Turkey, indicating this country as the gene center of *Origanum*. In addition, 17 hybrids between different species have been described, some of which are known only from artificial crosses. Very complex in their taxonomy, *Origanum* biotypes vary in respect of either the content of essential oil in aerial parts of the plant or essential oil composition. Essential oil 'rich' taxa with essential oil content of more than 2 per cent (most commercially known oregano plants), are mainly characterized either by the dominant occurrence of carvacrol and/or thymol (together with considerable amounts of γ-terpinene and *p*-cymene) or by linalool, terpinene-4-ol and sabinene hydrate as main components. The two most well commercially known 'oregano' species are *O. vulgare* subsp. *hirtum* (Greek oregano) (as well as winter sweet marjoram or pot marjoram, which is derived from *O. heracleoticum* and *O. onites* (Turkish oregano), each having an essential oil content of more than 2 per cent.

The genus *Lippia* (family Verbenaceae) is the most well known of several plants in Mexico that bear a resemblance to the Mediterranean oregano in terms of flavor and aroma, and the leaves of which have long been established by trade practice to be oregano (curly leaf oregano, Mexican sage, origan, oregamon, wild marjoram, Mexican marjoram, or Mexican wild sage). The genus *Lippia* Houst. consists of approximately 200 species of which 46 have been chemically examined.

The species mainly used are either *Lippia graveolens* or *L. berlandieri*. Because most of the species are aromatic, the studies on the chemistry of this genus are mostly related with the composition of the essential oils and only a very few ones devoted to the non-volatile constituents. An outstanding feature of *Lippia* is the difference observed in the essential oil composition reported for the same species from different geographic origins. The mono- and sesquiterpenoids found in the essential oils for all but two of the *Lippia* species investigated so far are quite common and widespread in the plant kingdom, the exceptions being *L. integrifolia* (Gris.) Hieron. which produces ketones based on the unique sesquiterpene skeletons named lippifoliane and integrifoliane and *L. dulcis* Trev. which contains (+)-hernandulcin, a sesquiterpenoid 1500 times sweeter than sucrose. Iridoids glucosides, phenylpropanoids, naphthoquinoids and flavonoids are the four types of significant non-volatile secondary metabolites reported in *Lippia*.

The *Origanum* species are perennial herbs native to the dry, rocky calcareous soils in the mountainous areas of southern Europe and south-west Asia, and the Mediterranean countries. The perennial, erect plants reach a height of 0.8 to 1 m and have pubescent stems, ovate, dark green leaves, and white or purple flowers. The root structure of oregano is such that it binds the soil and keeps it from washing away on steep slopes. European oregano is primarily produced in Greece, Italy, Spain, Turkey, and the United States. The *Lippia* species are small shrubs with larger leaves than the *Origanum* species and come primarily from Mexico.

The reported life zone for *Origanum vulgare* is 5–28 °C with an annual precipitation of 0.4–2.7 m and a soil pH of 4.5–8.7. Although much of the commercial material is collected from wild plants, fields can be seeded or established from transplants on light, dry, well-drained soils that are somewhat alkaline. Harvesting can take place two to six times per year. The *Lippia* species are predominantly collected as wild plants in Mexico.

Information is provided, in a very detailed manner, on structural dynamics of *Origanum* in order for the reader to get a picture about the construction and operation of the specific tissues composing the plant fundamental organs (leaf, stem, root).

Particular attention is given to glandular (peltate, capitate Type I and II) hairs and nonglandular hairs, their ontogenetic patterns from a protodermal initial cell and the topology of essential oil biosynthesis. Such data (e.g. knowing the structural components participating in a certain metabolic process as well as their temporal alterations and modifications) can be utilized for other fields of *Origanum* research.

There are a number of publications referring to the chemisty of *Origanum* which is known widely in the world of herbs and spices for its volatile oils. Oregano is the commercial name of those species that are rich in the phenolic monoterpenoids, mainly carvacrol, occasionally thymol, while marjoram is the commercial name of those that are rich in bicyclic monoterpenoids *cis*- and *trans*-sabinene hydrate. Besides the qualitative variation of the volatile compounds at the infrageneric level, there is considerable quantitative variation at the infraspecific level. Remarkable chemical variations have not only been observed between but also within populations and accessions. The seasonal variation of essential oil yield and composition has received less attention. There is yet limited knowledge in the biosynthesis of the essential oil compounds and their inheritance, which would be useful for a more effective selection and establishing a targeted breeding program. For all these reasons, wild collection accompanied with quality and species maintaining assurance systems (sustainability, GHP) and/or field production of reliable genotypes are the future methods of choice for quality products. The enormous inter- and infraspecific chemical polymorphism of *Oregano* sp. offers a wide range for selection towards the production of specific monoterpenes as fine chemicals, new odor and flavor profiles a.s.o. In the present book, a detailed chemotaxonomic account of *Origanum* taxa is presented by group and by section. *Origanum* species are rich in other compounds as well, such as various phenolics, lipids and fatty acids, flavonoids and anthocyanids. In a separate chapter, main breeding targets and quality criteria are set out and various breeding methods are discussed.

It is generally accepted that the Greek oregano has the best essential oil quality, the main constituents of which are carvacrol (the compound responsible for characterizing a plant as of the oregano type) and/or thymol, accompanied by *p*-cymene and γ-terpinene. Mexican oregano oil contains approximately equal amounts of carvacrol and thymol and smaller amounts of 1,8-cineole and other compounds. The basic composition of the oil varies with the plant source and geographical growth area.

Although oregano has been known and used for centuries, it gained only lately mass popularity, largely due to its relationship to marjoram (*O. marjorana*), the popular and botanical terms for both species having long been confused. And while sweet marjoram was one of the most popular herbs during the Middle Ages, oregano was scarcely cultivated, probably due to the plant's tendency to compete against other plants growing nearby. On the other hand, wild oregano has been traditionally collected in Mediterranean countries and in Mexico for use in many of the favorite dishes (e.g. for tomato-based sauces, lamb, seafood, chili peppers and almost any garlic flavored dish). The rest of the world discovered oregano after Second World War, with the expansion of pizza consumption (and in a lesser-degree, Mexican-style foods). Oregano consumption boomed from almost nil to a consumption volume of over 500 000 tons, demonstrating a per capita increase of importation into the United States of 3800 per cent from 1940 to 1985. Product prices depend heavily on quality. The overall market of oregano is expanding becoming thus by far the largest selling herb today. Latest estimates put worldwide production of oregano at about 10 000 tons. Turkey has a dominant position in the worldwide trade of oregano (over 2/3 of the total production), followed by Mexico, Greece and other Mediterranean countries. Greece has long been a leading source and its product has traditionally commanded the highest prices, nevertheless not always sufficiently meeting demand. Though Italy harvests large amounts of oregano, most of it is consumed domestically. The Mediterranean-type of product, as compared to the Mexican, is a smaller leaf of somewhat lighter green color and milder, sweeter flavor. As compared to sweet marjoram, however, it is much strongly flavored. The harvesting and processing of oregano is similar in Mediterranean and Mexican areas. In a separate chapter, a comparative demonstration is given of cultivation practices of oregano in different producing countries, such as Turkey, Hungary, Germany, Israel, Slovenia, the Federal Republic of Yugoslavia and Albania.

Cultivation of oregano can be a profitable business. Growers currently enjoy increased market prices due to the limited product availability, as a result of the exhaustion of wild oregano populations due to intensive collection. A recent survey in Greece (Papanagiotou *et al.*, 2001) indicated that for a given average yield of 1850 kg per hectare and an average product price of 4.1 € per kg, the net profit for the grower is 2500 € per hectare, a value considerably higher then for most crop and horticultural species. Labor (1260 man-hours/hectare) was estimated to reach 64 per cent of the total production cost.

The herb is often sold by mesh size, indicating average particle size. In United States oregano imports are roughly equal from both Mediterranean and Mexican species. The Mexican Oregano is a much stronger, more robustly, ‘wild’ flavored oregano. After cleaning, the leaves of Mediterranean oregano come into a size of 30 or 60 mesh, with larger leaf particles giving the choicest, more refined appearance. In Mexico, shippers often refer to their most refined product as ‘Greek cut.’ In the United States the herb is offered as ground or whole leaf oregano (although not always in the original whole form). Beyond that, various mesh sizes may also be available, each being the most appropriate choice for a particular use. Other important species collected and marketed as European oregano include *Thymus capitatus* (Spanish oregano), *Origanum syriacum* (*Origanum maru* – Syrian marjoram or zatar) and *Origanum virens*. Additional species used in Mexican oregano include *Lippia palmeri* and *Lippia origanoides*.

The original fresh material is the essential factor determining the quality of the dried herbs. Nevertheless, the drying method, type of packaging, and storage conditions also has clear effects on the microbiological quality of the herbs. An exemplary investigation is the Finnish Herb Study, presented in detail in this volume.

As a medicinal plant, European oregano has traditionally been used as a carminative, diaphoretic, expectorant, emmenagogue, stimulant, stomachic, and tonic. In addition, it has been used as a folk remedy against colic, coughs, headaches, nervousness, toothaches, and irregular menstrual cycles. Turkish villagers have traditionally used *kekik water*, the aromatic water obtained after removing essential oil from the distillate of oregano herbs, which has in recent years become a commercial commodity. *Origanum* oil is a powerful disinfectant, and carvacrol and thymol are considered to be anthelmintic and antifungal agents. The documented insecticidal and allelopathic properties of the species suggest its potential value as a biological control agent. Antibacterial/fungicidal properties are reviewed in detailed as is the use of the genus as a food ingredient, either for its antioxidant properties or its distinctive flavor. Especially, the monoterpenic phenol carvacrol, the main constituent of commercial oreganos should be given special emphasis since its low toxicity and surprisingly high and diverse biological activities render this simple molecule a promising lead for the development of novel medicines not only for humans but also for animals and plants. Carvacrol-rich oils are also used for their high potency especially as antibacterial and antifungal agents. The use of carvacrol-rich oils as ingredients in animal feed and for the preservation of food against bacterial or fungal spoilage and as antioxidants appears to increase. The *Lippia* species of oregano are generally recognized as safe for human consumption as natural flavorings/seasonings, and the *Origanum* species are generally recognized as safe as natural extracts/essential oils. However, oregano is one of the most common foodstuffs, which have the ability to cause aversions during pregnancy. In addition, and according to some reports, oregano intake can cause systemic allergic reactions. These items are particularly treated with.

Although the monograph documentation of *O. vulgare* was submitted to German Ministry of Health, the stuff responsible for phytotherapeutic medicinal domain – Commission E evaluated *Origani vulgaris herba* negatively (Banz. Nr. 122 from 6th July 1988), because of lack of scientific proofs for a number of indication areas (Blumenthal, 1998). Nevertheless, many of the studies confirmed benefit effects of oregano for human health. In this volume, extensive reference is provided on the bioactive/pharmacological properties of the *Origanum* genus, such as antioxidant and antimicrobial action and its use for the treatment of a vast list of ailments, such as respiratory tract disorders like cough or bronchial catarrh (as expectorant and spasmolitic agent), in gastrointestinal disorders (as choleretic, digestive, eupeptic and spasmolitic agent), as an oral antiseptic, in urinary tract disorders (as diuretic and antiseptic) and in dermatological affections (alleviation of itching, healing crusts, insect stings), viral infections and even cancer. Oregano, its essential oil or isolated compounds have been studied also from the point of possible applications in plant protection, in post-harvest crop/fruit protection or in apiculture, where species specific fungi endanger the production systems. Finally, *Origanum* taxa, especially those that are rich in essential oils, have been extensively studied for their insect-pollinating or nectar yielding effects.

In spite of its commercial importance *Origanum* sp. is still an underutilized plant species and very few commercial oregano cultivars exist. Even today, oregano production from cultivation is less than production from the wild, which results to an apparent danger of genetic erosion. This situation can probably change due to the increased knowledge on cultivation technology (such as fertilization, irrigation, pest management and hydroponics) and the exploitation of the natural variability, either towards the selection and introduction to cultivation of new biotypes or the creation of new cultivars through breeding. For this purpose, a deeper, updated knowledge of the plant's biosynthetic pathways, in relation to its ecophysiological behavior and biology is essential. Equally important is the construction of appropriate germplasm databases, an updated account of which is presented in this volume. Finally, the possible use of waste products from oregano processing, such as *cyclone powder* (Oregano lipids) in shampoos and other cosmetic preparations is evaluated.

Several *Origanum* species are cultivated as culinary herbs and as garden or medicinal plants. Hereby most of the genetic diversity of the genus is concentrated in collections of individual growers, which contain about 600 accessions. Different approaches have been undertaken for the conservation of oregano germplasm, and the activity of international seed- and germplasm banks and affiliated organizations (such as the Oregano Genetic Resources Network) is reviewed.

Both *Origanum* and *Lippia* species are yet a novel target for biotechnology, though some interesting progress has been achieved towards the micropropagation of certain species, such as *O. vulgare*, *O. bastetanum* and *L. junelliana*. The current focus of such applications is related to the establishment of clonal populations, which could offer a means to overcome the problems associated with germplasm variability. Further perspectives include the use of cell cultures for the scaled-up production of useful pharmaceuticals and the enhancement of breeding activities (e.g. *in vitro* selection for disease resistance, exploitation of somaclonal variation).

Monographs on specific life sciences related topics frequently forego certain aspects of methodology. When carrying out comprehensive Origanum- or Lippia-related searches, more than one database should be consulted. In Origanum some 54 per cent of all investigated references can retrieved by one (most inclusive) database what is not enough. By investigating several different bibliographic databases and references/records on genera *Lippia* and *Origanum*, one can identify a total of 1001 references published in 476 journals during 1981–1998. It is evident that single databases, or information sources, cannot serve as good examples to illustrate some principal directions in the field of medicinal plants. Prior to setting up a research and conducting database searches the end-users should gather comprehensive instructions on each particular information service and get familiar with its construction and maintenance characteristics, as extensively described in this book.

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Part 2

Botany

2 Structural features of *Origanum* sp.

Artemios M. Bosabalidis

INTRODUCTION

The genus *Origanum* (Lamiaceae) includes 39 species widely distributed in the Mediterranean region (Vokou *et al.*, 1993). The species *Origanum vulgare* predominates in occurrence, while *Origanum dictamnus* is endemic of the island of Crete in southern Greece. The plants are perennial herbs spontaneously growing in calcareous substrates. One of the striking morphological characteristics of the *Origanum* plants is the presence of glandular and nonglandular hairs covering the aerial organs. Both types of hairs originate from epidermal (protodermal) cells. Every epidermal cell, according to Netolitzky (1932), practically possesses the potentiality to develop into a hair. A number of external and internal factors, however, determine which ones of the typical epidermal cells will develop into hairs, and furthermore, which morphological pattern they will follow.

The glandular hairs are numerous on the vegetative organs (stems, leaves, bracts), while their density becomes reduced on the reproductive organs (calyces, corollas). On the stamens and the gynoecium of the flowers, no glandular hairs have been reported to occur (Werker *et al.*, 1985b). The glandular hairs secrete an essential oil with a characteristic odour, mainly due to the major components of the oil, the monoterpenes carvacrol and thymol. The essential oils of *Origanum* have been found to develop strong antioxidant, antimicrobial, insecticidal and genotoxic activities (Scheffer *et al.*, 1986; Sivropoulou *et al.*, 1996; Karpouhtsis *et al.*, 1998).

Though descriptions of the morphology of *Origanum* species are quite often given in the literature on plant taxonomy (Ietswaart, 1980; Kokkini *et al.*, 1991; Circella and D'Andrea, 1993), relevant reports on the anatomy and cytology of the various *Origanum* organs, are limited. In the present article, an attempt was made to partly bridge this gap by providing information on the structural features of *Origanum*.

STRUCTURE OF THE FUNDAMENTAL ORGANS

The leaf

Morphology of the leaf

The leaves of *Origanum* species may differ in size, shape and thickness, as well as in the density, type and size of the nonglandular and glandular hairs covering their surfaces. The leaf of *O. dictamnus* is nearly rounded with a mean side surface area of 358.75 mm^2 (Bosabalidis, 1990a). It is densely covered with branched nonglandular hairs which lend to it a velvety appearance (Figure 2.1). Hairs are more numerous on the lower leaf side, which, thus, becomes brighter. The distribution of the nonglandular hairs is uniform all over the leaf surface with no particular accumulation at a certain area. The leaf also bears glandular hairs which appear as small transparent droplets scattered throughout (Figure 2.1, arrows). Their population is remarkably lower than that of the nonglandular hairs.

The leaf of *Origanum* × *intercedens* (hybrid from *O. onites* and *O. vulgare* subsp. *hirtum*) is ovate in shape, petiolate, with a serrate margin. At full expansion, each leaf side was measured to display a mean surface area of 207.83 mm^2 and a thickness of 203.38 μm (Bosabalidis and Exarchou, 1995). The pubescence is composed of glandular and nonglandular hairs as well (Figure 2.2). The glandular hairs are uniformly distributed and their number and size are higher on the upper leaf side. The nonglandular hairs are multicellular uniseriate and their tips face the leaf apex. They are more numerous on the abaxial leaf side veins and their density and length increase toward the leaf base.

The leaf of *O. vulgare* subsp. *hirtum* does not significantly differ from that of *Origanum* × *intercedens*, as concerns the size, shape and thickness, as well as the density of the glandular and nonglandular hairs.

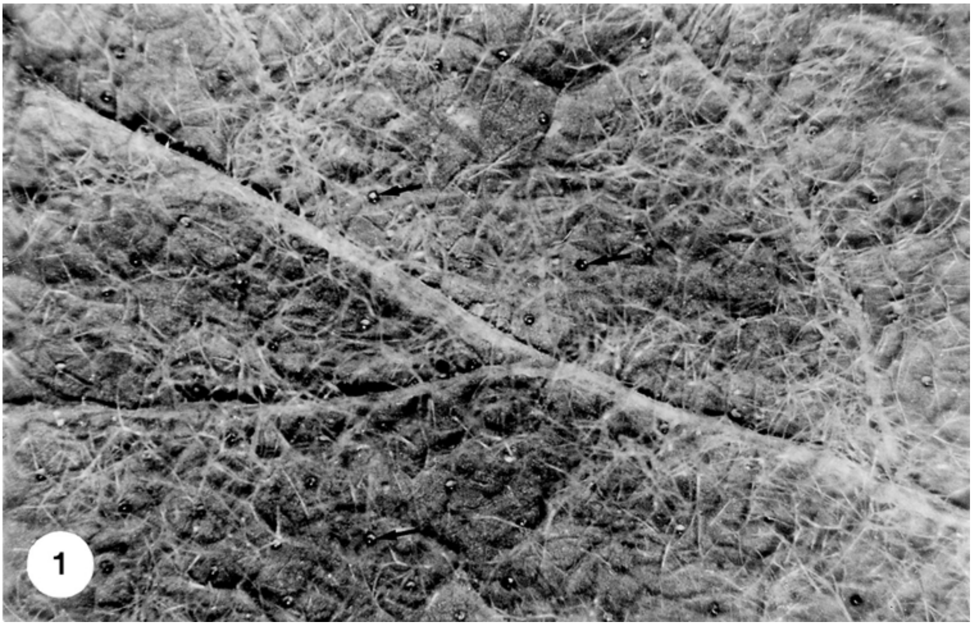
Anatomy of the leaf

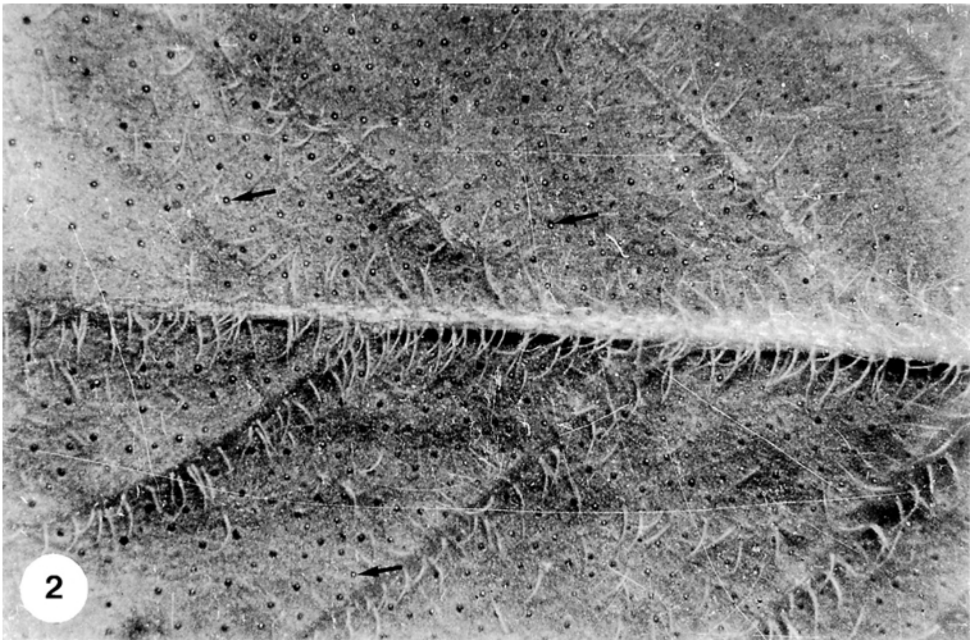
Though a great number of publications on leaves of aromatic plants exists, most of them deal with the glandular hairs and their secretions, and only a few have proceeded with the study of leaf anatomy (Henderson *et al.*, 1970; Yamaura *et al.*, 1992; Werker *et al.*, 1993; Bosabalidis and Exarchou, 1995; Bosabalidis and Kokkini, 1997; Bosabalidis and Skoula, 1998b; Gavalas *et al.*, 1998).

In a cross section of a fully grown leaf of *O. vulgare* subsp. *hirtum*, the cells of the upper epidermis appear much more larger than those of the lower one and covered with a relatively thick cuticular layer (Figure 2.3). Stomata exist on both leaf epidermises, but their number is significantly higher on the lower epidermis (Table 2.5). The chlorenchyma cells of the palisade parenchyma are elongated and ordinarily arranged in one layer. The chloroplasts are numerous, usually located at the parietal cytoplasm. The cells of the spongy parenchyma mostly exhibit a round profile and they leave between them large intercellular spaces. At the positions on both leaf surfaces where peltate glandular hairs occur, the epidermis forms more or less deep depressions (not shown in Figure 2.3). Morphometric assessments conducted on leaf cross sections of *O. vulgare* subsp. *hirtum* resulted in the estimation of the relative volumes of the partial histological components (Table 2.1).

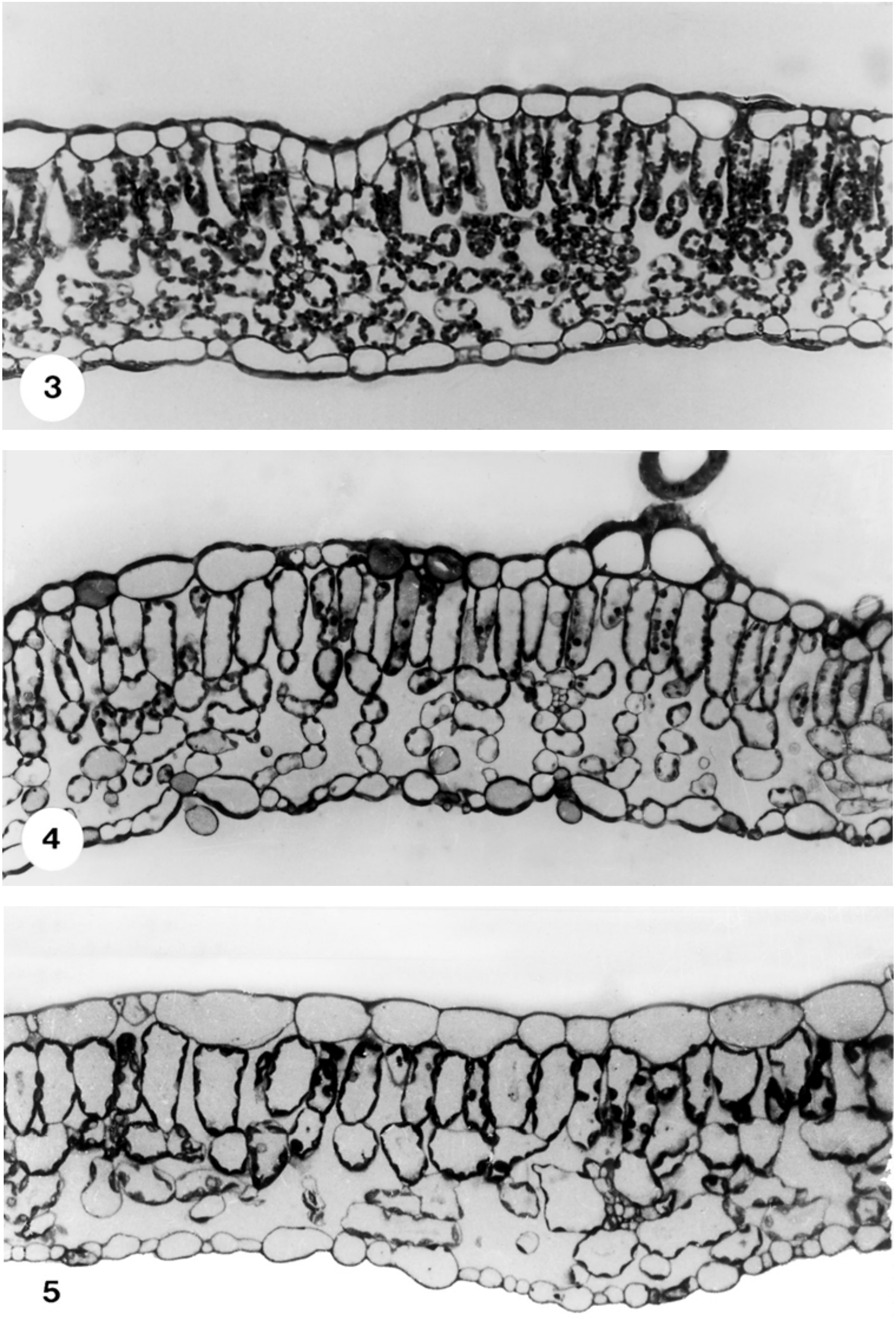
Table 2.1 Relative volumes (±standard deviation) of the leaf histological components in *Origanum vulgare* subsp. *hirtum*

Component	*n*	*RV (%)*
Upper epidermis	24	9.3 ± 1.8
Palisade parenchyma cells	24	27.4 ± 12.6
Palisade parenchyma intercellular spaces	24	6.8 ± 1.9
Spongy parenchyma cells	24	28.8 ± 3.1
Spongy parenchyma intercellular cells	24	15.1 ± 2.8
Lower epidermis	24	7.7 ± 1.6





Figures 2.1 and 2.2 Partial view of the morphology of the leaf lower surface in *Origanum dictamnus* (Figure 2.1 [×19]) and *Origanum* × *intercedens* (Figure 2.2 [×11]). Arrows point to glandular hairs.



Figures 2.3–2.5 Cross sections of leaves from *Origanum vulgare* subsp. *hirtum* (Figure 2.3), *Origanum* × *intercedens* (Figure 2.4) and *Origanum dictamnus* (Figure 2.5) to show corresponding leaf anatomy (×200).

Cross sections of leaves of *Origanum* × *intercedens* (Figure 2.4) as well as those of *O. dictamnus* (Figure 2.5) have disclosed no significant anatomical differences, compared to *O. vulgare* subsp. *hirtum*. Worthy differences mainly comprise the length and width of the palisade parenchyma cells and the density of chloroplasts.

The epidermis

TYPICAL EPIDERMAL CELLS

In leaf cross sections of *Origanum* species, the typical epidermal cells appear to differ in size and number between the upper and lower leaf sides (Figures 2.3–2.5). Leaf paradermal sections reveal that epidermal cells are in close contact with each other leaving between no intercellular spaces (Figure 2.6). The cell outlines (anticlinal walls) are irregular in the form of successive foldings, which contribute to a better adhesion of the cells. This holds true for both the upper and lower leaf surfaces (Figures 2.6–2.7).

At early development, the epidermal cells appear in ultrathin sections rich in protoplasm with large nuclei, ordinarily located at the proximal cell region (Figure 2.8). Vacuoles are relatively small and they occupy the distal cell region. None of the typical cell organelles proliferates in volume or number. The leucoplasts have an irregular shape and they contain large globular inclusions which are amorphous and of high electron density (Figures 2.8 arrows, 10). Similar intraplastidal inclusions have been also encountered in the initial cells of the glandular hairs (peltate and capitate), as well as in the basal cells of the differentiated glandular hairs.

At advanced development, the epidermal cells become occupied at their greatest portion by a large vacuole with an electron translucent content (Figure 2.9) (Bosabalidis, 1987b). The cytoplasm is limited at the periphery of the cells forming a thin perietal layer in which plastids, small mitochondria, short elements of the rough endoplasmic reticulum and a few dictyosomes, are embedded. Quantitative estimations on the relative volumes of the cytoplasm, the central vacuole and the nucleus of dittany leaf epidermal cells, are presented in Table 2.2. The leucoplasts still contain large globular inclusions, which, however, this time have a fine granular appearance (Figures 2.9 arrow, 11). The inclusions are bordered by a single membrane and they occupy about 54 per cent of the plastidal volume (Table 2.3).

Morphometric assessments conducted on the epidermal cell plastids (and their inclusions) gave specific values associated with volume and surface area (Table 2.4) (Bosabalidis, 1987b). Leucoplast membrane-bound inclusions with a circular outline and an amorphous internal structure, have been also observed in the cambial cells of *Fraxinus americana* (Srivastava, 1966), the tissue culture of *Daucus carota* (Israel and Steward, 1967), the vascular parenchyma of *Callitriche stagnalis* (Favali and Patrignani, 1973), the mesophyll of *Phaseolus aureus* (Hurkman and Kennedy, 1976), the laticifers of *Papaver somniferum* (Nessler and Mahlberg, 1979), etc.

The chemical nature of the plastidal inclusions is proteinaceous, as cytochemical tests with pepsin incubation have shown (Bosabalidis, 1987a). Pepsin resulted in a progressive digestion of the inclusion bodies (Figures 2.12, 2.13). Similar results for plastidal membrane-limited inclusions have been also obtained in the chlorenchyma cells of palms (Gailhofer and Thaler, 1974) and of tobacco (Ames and Pivorun, 1974) by using the same proteolytic enzyme. Treatment of ultrathin sections with diaminobenzidine (DAB) further resulted in an intense staining of the inclusions (Figure 2.14). The great