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Factors affecting secondary metabolite production in plants: volatile components and essential oils

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ABSTRACT: The presence, yield and composition of secondary metabolites in plants, viz. the volatile components and those occurring in essential oils, can be affected in a number of ways, from their formation in the plant to their final isolation. Several of the factors of influence have been studied, in particular for commercially important crops, to optimize the cultivation conditions and time of harvest and to obtain higher yields of high-quality essential oils that fit market requirements. In addition to the commercial importance of the variability in yield and composition, the possible changes are also important when the essential oils and volatiles are used as chemotaxonomic tools. Knowledge of the factors that determine the chemical variability and yield for each species are thus very important. These include: (a) physiological variations; (b) environmental conditions; (c) geographic variations; (d) genetic factors and evolution; (e) political/social conditions; and also (f) amount of plant material/space and manual labour needs. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: secondary metabolites; essential oils; plant volatiles; chemotaxonomy; production factors

Introduction

Plants are known for their ornamental value and for providing oxygen, food, beverages, clothing, perfumes and building materials. In addition, plants are the source of an enormous number of compounds, known as secondary metabolites, with, for example, medicinal or pharmaceutical applications. The term 'secondary metabolites' has for a long time had an unprestigious meaning, since 'secondary' assumes that others, the 'primary' ones, are more important. This meaning is no longer acceptable and several examples show that the opposite may also be the case. The cell wall polymer lignin, clearly not a classical secondary metabolite, is without any doubt essential to terrestrial plants, but is also a product of such secondary metabolism. Although it is nowadays accepted that secondary metabolites play a major defensive and attractive role in the interactions between plants and their environment (with other plants, herbivores, pathogens and pollinators, among other things), their designation was maintained. Defining secondary metabolites, one has to consider that they: (a) have no direct implication on the growth and development of plants; (b) are often synthesized from primary metabolites; (c) have a distribution which is sometimes confined to a genus or species; (d) are often accumulated in high concentrations [1–3% fresh weight (f.w.)]; (e) may show high toxicity; (f) may have a marked biological effect on other organisms; (g) frequently have different production and accumulation sites; and (h) are sometimes accumulated in the vacuoles in a glycosidic form or accumulate in special secretory structures, e.g. trichomes, ducts, canals, laticifers.

Mixtures of volatiles and essential oils isolated from plants represent the 'essence' or odoriferous constituents of these plants; they have been used since early days, not only because of pharmaceutical properties but also as flavouring agents and in the manufacture of perfumes and cosmetics. The essential oils, which are isolated by steam or water distillation (citrus oils by cold pressing), and the mixtures of volatiles (volatile oils), which can be isolated by distillation, pressure or extraction, with organic solvents or supercritical carbon dioxide, are usually liquid at room temperature, slightly soluble in water and highly soluble in organic solvents. The volatiles present in a plant consist of a complex mixture of chemical compounds, each of which has certain chemical and physical properties which, in combination with their different proportions, give the oils distinctive characteristics. The differences in aroma among plant types result from differences in the volatility and quantity of the chemicals in the oils. The volatiles, which are mostly terpenoid substances (mono-, sesqui- and di-terpenes), are among the

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most valuable compounds produced by plants, side by side with alkaloids and phenolic substances, some of which are also volatiles, such as phenylpropanoids.

Within the past decades there has been a revival of interest in folk medicine, especially in herbal remedies, and in its conjunction with modern medicine. The same trend towards the use of natural raw materials is seen in the agro-, beverage, food, and flavour and fragrance industries.

The search for biologically active substances has encouraged, among other things, the use of essential oils and volatiles as antimicrobials and antioxidants in food and food products. The fact that essential oils and volatiles ally their aromatizing capacity to: (a) being natural and biodegradable; (b) having usually a low toxicity to mammals; and (c) being able to simultaneously accomplish the function of more than one of their synthetic equivalents, have all contributed to this. Furthermore, essential oils can be used in the protection of crops, and against pests and plagues, with the advantage of not accumulating in the environment and having a broad range of activities, which diminishes the risk of developing resistant pathogenic strains.

Some plant families, such as Apiaceae/Umbelliferae, Asteraceae/Compositae, Cupressaceae, Hypericaceae/Guttiferae, Lamiaceae/Labiatae, Lauraceae, Leguminosae/Fabaceae, Liliaceae, Malvaceae, Myrtaceae, Oleaceae, Pinaceae, Rosaceae and Rutaceae are particularly rich in secondary metabolites, viz. those present in volatile and essential oils. The recognition of the species potentially usable by producers can contribute to a more equilibrated usage of the rural spaces, encouraging the conservation of the forest heritage and potentiating its environmental influence, e.g. in the protection of hydric resources, limiting erosion phenomena and safeguarding biodiversity.

Essential oils as total mixtures, or some of their components, can also be used for chemotaxonomic purposes. However, the comparison of the essential oil composition described in different studies is sometimes difficult, if not impossible, not only because different methodologies of isolation and analysis may have been used, but also when different plant parts and various developmental stages are involved.

In order to fully recognize the best time of plant collection in terms of oil composition and/or yield, it is important to know the factors that influence production, and to know, for each particular case, their specific requirements. A previous review on the physiological aspects that affect essential oil production[1] made clear that many species show marked differences in composition, whereas others have a more or less stable composition. The present review re-evaluates the factors that influence the production of secondary metabolites and their composition, viz. those present in mixtures of volatiles and essential oils.

Factors that Influence the Production and Composition of Volatiles and Essential Oils

Flavours have long had a diverse and sophisticated role—the maceration of wormwood for the flavouring of Hebrew wines, the use of figs and roses in Persian and Assyrian wines, the development of the spice trade by the Greeks and Romans. Beyond flavouring properties, they also had a liturgical value: smoked and grilled meats were considered food of the Gods. Known since the Neolithic, honey is a flavouring that still keeps its importance, particularly in oriental civilizations. Likewise, hops have been to the present day associated with the production of beer. Some species of yarrow have also been used in the production of liqueurs. Since ancient times spices have been popular not only for their organoleptic properties but also for their food-preserving properties. Clove, cinnamon, garlic, thyme, oregano, anise and savoury are some of the spices that delay, decrease or inhibit the growth of fungi and prevent the production of aflatoxins or other mycotoxins.

Nevertheless, in nature several factors hinder a homogeneous and continuous production of secondary metabolites (Table 1) and this has led industry to search for alternative procedures to overcome these problems.

Physiological Variations

Organ development

The stage of development of the plant organ (leaf, flower and fruit ontogeny) can be a determinant for the composition of the volatiles.[1,2] In several cases, there is an

Table 1. Factors that influence the production and composition of secondary metabolites of plants

Physiological variations
Organ development
Pollinator activity cycle
Type of plant material (leaves, flowers, etc.)
Type of secretory structure
Seasonal variation
Mechanical or chemical injuries
Environmental conditions
Climate
Pollution
Diseases and pests
Edaphic factors
Geographic variation
Genetic factors and evolution
Storage
Political/social conditions
Amount of plant material/space and manual labour needs

Adapted from Figueiredo *et al.*[1]

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increase in the yield of the volatiles from the flower bud to the mature flower. Concomitantly, the composition can undergo major changes, some components varying from traces to 10% in the initial stages, to 50–70% in the full flowering stage. In other cases, the volatiles are largely accumulated before the organ is fully expanded. For *Ocimum sanctum*, it was shown that the relative amount of eugenol and methyleugenol decreased with the development of the leaves, which, according to the authors, could be explained by the use of these components in the synthesis of lignin and/or by further oxidation of phenol compounds catalysed by the increase of polyphenoloxidase and peroxidase activity.[3] According to Máñez *et al.*,[4] the changes in the composition of the volatiles with the maturation of the organ are directly related to higher rates of cyclization and dehydration of the compounds.

The essential oils isolated from *Achillea millefolium* flowerheads, growing in the Jardim Canecão de Almada, in different stages of development (Figure 1), showed a different coloration in relation to the developmental stages. The oil was blue in closed flowerheads (FF), blue-green in young open flowerheads (FJ), brown-yellow in fully open flowerheads (FA) and light-yellowish-brown in air-dried flowerheads (FS). This coloration change reflected the decrease in the relative amount of chamazulene with the maturation of the flowers (Figure 2). With regard to the main components of the oils, the relative amounts of camphor and 1,8-cineole increased with flower maturation, whereas β-pinene showed inverse behaviour (Figure 2).[5]

Diurnal fluctuations observed in the composition and/or the yield of essential oils, as well as changes in the composition with the development of the organ, or of the plant, have been considered as normal metabolic turnover. While in the case of diurnal fluctuations, the terpene turnover depends on photosynthesis and the utilization of photosynthates, in the changes related to the development, the monoterpene content decreases as a result of the turnover of the stored material. A study of two model systems (*Mentha × piperita* and *Salvia officinalis*) has shown that the catabolism of terpenes leads to the formation of glycosidic derivatives that are transported to the roots, where they are used in lipid synthesis.[6] The conversion of secondary metabolites into a glycosidic form is also an efficient detoxification system, to avoid autotoxicity, as it converts aglycones into water-soluble and inactive products.[7] It is also important to mention that differences in the yield and composition with organ development can be partly explained by the different types of secretory structures (see section on Type of secretory structure, below). Plants with external secretory structures can release secretions with maturation of the organ because of trichome cuticle disruption, whereas plants with internal secretory structures more often maintain a more stable yield and composition.

Pollinator activity cycle

Except for ornithophilous flowers, the great majority of zoophilic flowers are scented. Apart from the visual attraction (colour and shape), the odours are the most important cue for the attraction and orientation of pollinators, particularly for night insects. The kind of odour allows the insects to discriminate between flowers and to release the behavioural reactions necessary for pollination. In this respect, orchids are an example of a highly specialized system with pheromone-like production. They grow mostly in the Mediterranean and tropical areas and their pollinators are mostly bees. 1,8-Cineole, present in 60% of the scents of orchids analysed by Dodson *et al.*,[8] was found to attract the greatest number of pollinator bees (70%). In comparison, eugenol, methyl salicylate and methyl cinnamate attract fewer species. Experimental mixtures of these compounds in the proportions found in natural fragrances attracted the same kinds of bees as the natural odour.[8] Other examples in which the pheromones and flower scents affect the behaviour of insects are *Cassia fistulosa* (Leguminosae) and *Zieria smithii* (Rutaceae); these plants produce methyleugenol and, because of the striking attraction of this chemical to the fruit fly *Daucus dorsalis*, it has been proposed that *Cassia* blossoms and *Zieria* leaves could be employed in insect traps, when the fruit fly develops to pest proportions in fruit orchards (see references in Figueiredo *et al.*[1]).

Some orchid flowers mimic the receptive females of (usually) just one pollinator species (Pouyanne mimicry).[9–11] Males are attracted by the shape and odour of the flower, which resemble the virgin female bee, and transfer the pollinia during so-called 'pseudocopulation' with the flower labella. As an example, the orchid *Ophrys sphegodes* produces the same type of compounds and in similar relative amounts as those found in the sexual pheromones of the cuticle of the female virgin of the pollinator, the bee *Andrena nigroaenea* (Table 2). These flowers release only minute amounts of volatiles. This

Table 2. Comparison of the volatiles released by the orchid *Ophrys sphegodes* and the cuticle extracts of the female virgin of the pollinator, the bee *Andrena nigroaenea*

Compound	*Ophrys* (%)	*Andrena* ♀ (%)
Tricosane	31	29
Pentacosane	20	35
(*Z*)-11- + (*Z*)-12-Heptacosene	6	1
(*Z*)-9-Heptacosene	8	5
Heptacosane	12	11
(*Z*)-12 + (*Z*)-11-Nonacosene	7	4
(*Z*)-9-Nonacosene	9	7

Adapted from Schiestl *et al.*[9,10]

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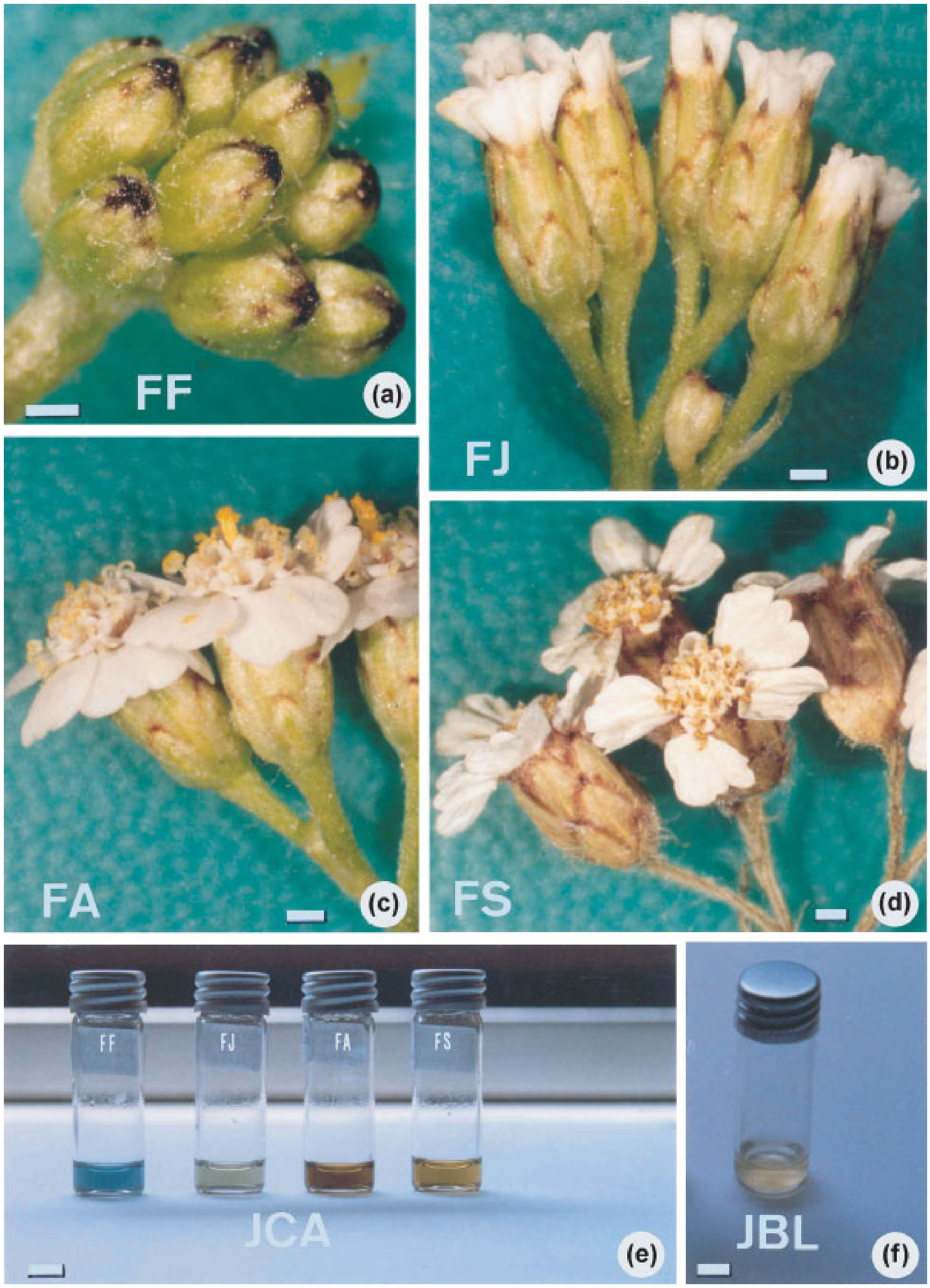

Figure 1. Aspects of the different stages of development of *Achillea millefolium* flowerheads used in essential oil isolation: (a) closed flowerheads (FF); (b) young open flowerheads (FJ); (c) fully open flowerheads (FA); (d) air-dried flowerheads (FS). (e, f) Coloration of the essential oils isolated from the flowerheads collected in the Jardim Canecão de Almada (JCA): FF, blue; FJ, blue–green; FA, brown–yellow; FS, light yellowish-brown) (e); and in the Jardim Botânico de Lisboa (JBL) (f), in different stages of development. From Figueiredo.[56] This figure is available in colour online at www.interscience.wiley.com/journal/ffj

orchid is known as the spider orchid and is distributed mostly in the Mediterranean area and Central Europe. The inflorescence possesses two to six flowers and is specifically pollinated by the males of *Andrena nigroaenea*. It is most interesting that the flower odour changes after pollination is complete, together with a diminution in the volatile production. Moreover, the relative amount of farnesyl hexanoate, a trace compound up to then, starts to increase. This compound is known to inhibit copulation when it is present in large amounts in the cuticle of the female. Together with other morphological changes that occur after pollination, the cessation of volatiles emission corresponds with basic functions. First, resources are conserved, since the synthesis of volatiles

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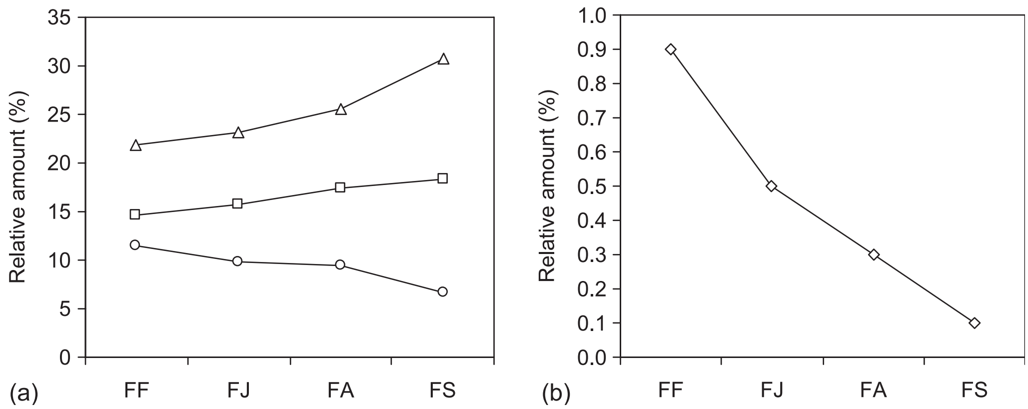

Figure 2. (a) Variation in the relative amounts of β-pinene (—○—), 1,8-cineole (—□—) and camphor (—△—) and (b) chamazulene (—◇—) during the development of the flowers of *Achillea millefolium*, collected in the Jardim Canecão de Almada. Adapted from Figueiredo *et al.*[5]

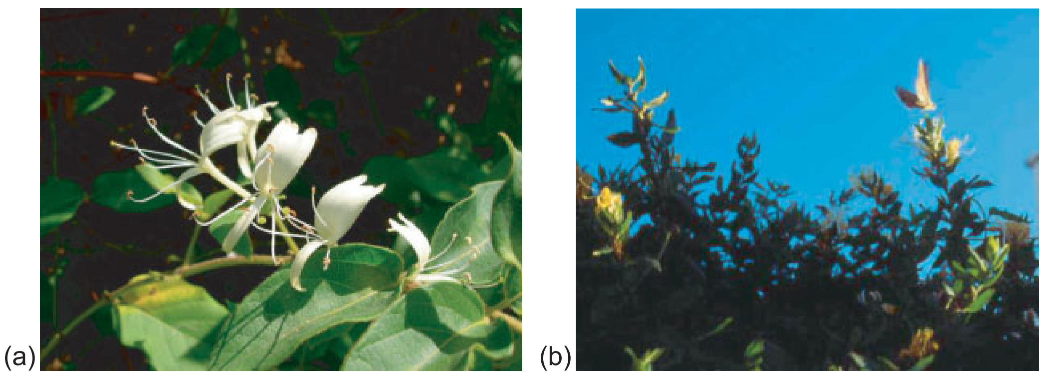

Figure 3. (a) *Lonicera japonica* and (b) the pollinator at the end of the day (20 h). This figure is available in colour online at www.interscience.wiley.com/journal/ffj

is a highly consuming process. Second, if the plant possesses more than one flower, scaling down the attraction of the already pollinated flowers directs the pollinators to the still unpollinated ones, which in turn increases the success of the pollination process.[9,10] Other examples of extreme specificity of floral scent towards the corresponding pollinator can also be found among aroids (*Arum* spp.) and figs (*Ficus* spp.). In all cases, this specificity of scents promotes the fidelity of the pollinator.

The scents of plants, together with nectar availability or pollen maturation, are thus in most cases related to the activity of the pollinator. So, in plants with diurnal pollinators, volatiles emission attains its maximum during the day, whereas those plants having night pollinators, such as bats, mice or nocturnal moths, show a maximum emission during night time. Several examples of diurnal or nocturnal rhythmic emission of volatiles can be found, e.g. *Hoya carnosa*, *Stephanotis floribunda*, *Odontoglossum constrictum*, *Citrus medica*, *Melaleuca alternifolia*, *Nicotiana sylvestris*, *N. suavolens* and *Trifolium repens* (see references in Figueiredo *et al.*[1]) and also for cacti.[12] The honeysuckle (*Lonicera japonica*; Figure 3) is well known to smell strongly at night, similar to jasmine and orange flowers. Ikeda *et al.*[13] have shown changes in the composition of the volatiles emitted from the living flowers of honeysuckle throughout the whole day; the strongest odour was found to be emitted between 19.30 and 07.30 hours, with a maximum between 23.30 and 3.30 hours (Figure 4).

Type of plant material

Although quite a few species yield a similar essential oil composition for their different organs, the composition can also be largely dependent on the plant part used: flowers, green parts (leaves and stems), bark, wood, whole fruits, pericarp or seed only, or roots.[1,14,15] Again, the variability can be particularly evident in entomophilous flowers, where volatiles can function as orientation clues, and thus the volatiles emitted by the flowers or flower parts are distinct from those of the other plant parts. Kuropka *et al.*[16] showed that in *Achillea ptarmica* the monoterpenes were almost non-existent in oils from the green parts and roots, while the contrary happened in the flowers. Máñez *et al.*[4] showed similar results for the essential oil from *Sideritis mugronensis*. According to

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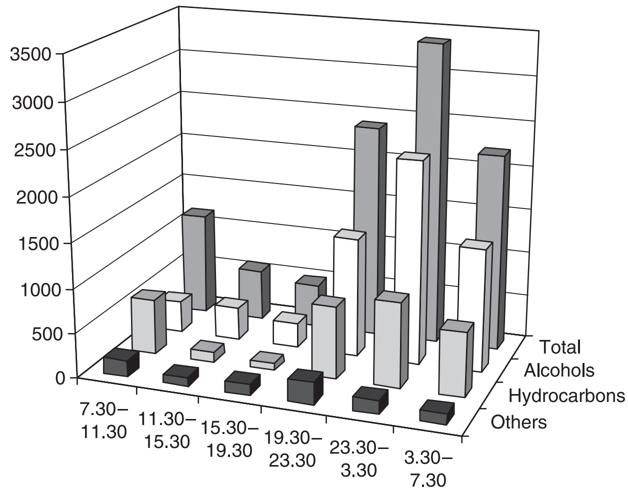

Figure 4. Variation of the composition of the volatiles emitted by flowers of honeysuckle during one day. Adapted from Ikeda *et al.*[13]

these authors, the larger amount of monoterpenes, particularly α-phellandrene, limonene and fenchone, in the floral oils was directly related to the pollinator's attraction. Likewise, analysis of the floral volatiles of *Lavandula pinnata*[17] showed differences in comparison with the volatiles from the leaves and stems collected in the flowering and vegetative phases (Table 3).

In general, terpenoid amounts are usually larger in reproductive structures and typically immediately before, and during, anthesis. Considering the defensive role of these components, it is also frequent that the higher levels of secondary metabolites are found in young organs rather than in old ones.

Type of secretory structure

The differences found in the composition of essential oils obtained from diverse plant parts can be partly explained by the existence of distinct secretory structures that are heterogeneously distributed over the plant body. Plant volatiles are produced in specialized secretory structures that minimize the risk of autotoxicity and simultaneously allow the presence of high levels of metabolic components at sites where their defensive and/or attractive role may be vital. The type and location of these structures is, mostly, characteristic of the plant family (Table 4). Nevertheless, there are several examples of plants with more than one type of secretory structure (e.g. trichomes and canals), as in *Achillea millefolium*[18] and *Pittosporum undulatum*.[19] In addition, these secretory structures do

Table 3. Variation of the relative amounts of the main components of the essential of *Lavandula pinnata* collected during different developmental phases

Components	*Lavandula pinnata*		
	Flowering phase		Vegetative phase
	Flowers	Leaves and stems	Leaves and stems
α-Pinene	6.5	3.5	3.7
Octen-3-ol	0.7	6.0	3.9
α-Phellandrene	15.9	6.3	10.8
Phenylacetaldehyde	1.3	5.9	9.2
β-Phellandrene	31.7	12.2	19.5
cis-β-Ocimene	8.9	4.2	6.5
Monoterpenes	82.0	39.2	57.8
Sesquiterpenes	13.0	21.8	13.6
Others	3.0	17.5	16.1

Adapted from Figueiredo *et al.*[17]

Table 4. Examples of different types of secretory structures occurring in some plant families

Secretory structures	Families*
External secretory structures	
Trichomes	Asteraceae, Cannabaceae, Geraniaceae, Lamiaceae, Plumbaginaceae, Rubiaceae, Rutaceae, Solanaceae, Verbenaceae
Osmophores	Araceae, Orchidaceae, Piperaceae
Internal secretory structures	
Idioblasts	Araceae, Aristolochiaceae, Calycanthaceae, Lauraceae, Magnoliaceae, Piperaceae, Saururaceae
Cavities	Hypericaceae, Leguminosae, Myrtaceae, Myoporaceae, Rutaceae
Ducts/canals	Anacardiaceae, Apiaceae, Asteraceae, Fabaceae, Hypericaceae, Myrtaceae, Pinaceae

* Apiaceae/Umbelliferae; Asteraceae/Compositae; Hypericaceae/Guttiferae; Lamiaceae/Labiatae; Fabaceae/Leguminosae.
Adapted from Figueiredo *et al.*[1]

Figure 5. Aspects of *Plectranthus madagascariensis* and detail of the lower leaf surface showing the bright orange peltate trichomes. This figure is available in colour online at www.interscience.wiley.com/journal/ffj

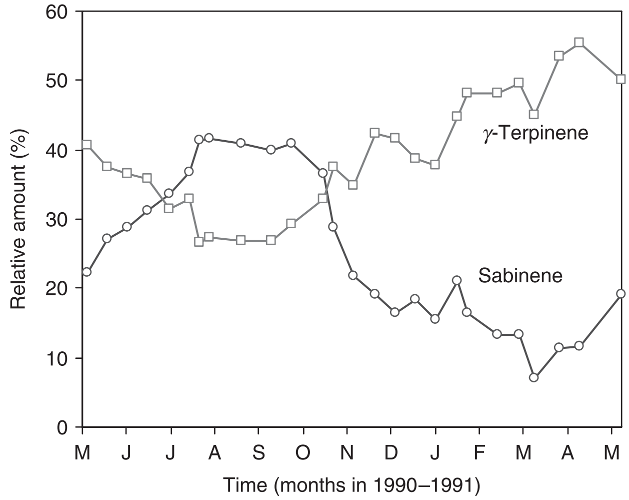

Figure 6. Seasonal variation of the relative amounts of sabinene (—○—) and γ-terpinene (—□—) in the essential oil of *Crithmum maritimum* between May 1990 and May 1991. Adapted from Barroso *et al.*[23]

not always develop in a synchronous way, do not always secrete the same types of compound and can have different secretory processes. Moreover, in some cases, i.e. in the taxa of a genus with internal secretory structures, the chemical composition of the secretion can be similar but the position of the secretory structure may define its final function for the plant, defensive or pollinator-attractive, as is the case for the genus *Dalechampia*.[20]

Morphological studies have shown a diverse distribution of trichomes in *Leonotis leonurus*.[21] Whereas peltate trichomes are abundant on both the leaves and the flowers, capitate trichomes are only numerous on the leaves and are rare or absent on the flowers. Moreover, capitate and peltate trichomes differ in their secretion processes. In peltate trichomes, the secretion remains accumulated in the subcuticular space unless an external factor disrupts the cuticle, whereas in capitate trichomes the secretion is probably released via micropores.

Different morphological and/or chemical techniques have been used for showing either a different (*Helianthus annuus*, *Geranium robertianum*, *Mentha* × *piperita*, *Poncirus trifoliata*) or a similar (*Salvia officinalis*) secretion in diverse glandular structures occurring in the same species (see references in Figueiredo *et al.*[1]). Also, in *Plectranthus ornatus*[22] and *P. madagascariensis*[21] there is a heterogeneous distribution of peltate and capitate trichomes in the reproductive and vegetative organs. In the latter species, there is an unusual type of capitate trichome restricted to the calyx. Moreover, in contrast to all the remaining colourless trichomes, there is one characteristic peltate type of trichome, with an orange to reddish colour, particularly concentrated at the leaf intervein areas; this type is visible to the naked eye (Figure 5). The main component of the volatiles of *P. madagascariensis* is 6,7-dehydroroyleanone, a diterpene, which has been isolated as orange to reddish crystals. The accumulation of this component seems to be restricted to the peltate type trichomes.

Seasonal variation

In some species, the composition of the essential oil also changes with the time of year, and thus the right time of harvest may be of major importance from an agronomic and economic point of view. For *Crithmum maritimum*, Barroso *et al.*[23] showed that during the flowering period the dominant component was sabinene, while γ-terpinene was the major component during the rest of the year (Figure 6). In *Achillea millefolium* the sesquiterpene hydrocarbons were the dominant components of the essential oil during the vegetative period, while in the flowering period the monoterpene hydrocarbons were the major constituents of the oil.[24]

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In the case of *Mentha* × *piperita*, a quality oil contains a relative amount of alcohols and menthol >50%, menthofuran <4% and pulegone <2%. Depending on the temperature, wind speed and soil humidity, among other factors, the irrigation is switched off 1–5 days before harvesting, so that the vigour of the crop and the oil quality is not affected. In 1 year plantations, although the yield tends to increase when flowering attains ≥50% (mid- to late August), the relative amount of menthofuran also increases. With over-maturation (September–October) the relative amount of menthol approaches 60% and the amount of menthofuran decreases, but the oil yield diminishes drastically, rendering the production too expensive.[25]

Apart from the monthly and annual fluctuations, or the changes according to the vegetative or flowering period of the plant, there may also be diurnal fluctuations that seem to be related to the activity of the pollinator, as previously mentioned. In other cases, the variations in the yield and composition of the oils were correlated with herbivory, weather parameters (day length, temperature and humidity) and to the attack of fungal pathogens, particularly in the months of rainfall (for references, see Figueiredo *et al.*[1]). In all cases, the harvesting time is species-specific and has to be determined according to the most favourable combination of oil composition and yield, from a commercial point of view. One such example is the choice of the right time to harvest *Vanilla* beans. The beans are harvested green and flavourless, and are then subjected to a curing process of 3–6 months. The purpose of curing is to create contact between the flavour precursors, located in the placental tissue surrounding the seeds, and the enzymes, mostly located in the outer fruit wall, that catalyse the hydrolysis of these compounds to flavour products, chiefly vanillin. If the beans are harvested too early, they are susceptible to mould and the vanillin content is lower, whereas over-ripening produces split beans of lower quality and lower price (for references, see Figueiredo *et al.*[1] Havkin-Frenkel *et al.*[26]).

Mechanical or chemical injuries

The emission of volatiles not only has a stimulant and/or attractive role but also works in a direct or indirect defensive way. Nevertheless, the effect of mechanical or chemical injuries, e.g. wounds, infestation by predators or treatment with herbicides, on the yield and composition of essential oils has been little studied.

The plants produce, under normal conditions (so-called healthy plants) a bulk of secondary metabolites, which is considered, in all, a constitutive production. When subjected to any type of traumatic injury, a *de novo* production can occur, i.e. new compounds not previously present in the plant can be produced, which is considered an induced production. The difference between constitutive and induced production can be ambiguous, since, for instance, the majority of the volatiles usually released by healthy plants become induced after any kind of injury. In most cases, the compounds are then produced in larger amounts and/or in different proportions. The induced response is not only a function of the species, but depends also on its developmental stage, the availability of water, the amount of light, etc.

The result of wounding, or infestation by predators, is particularly important in oleoresin-producing plants, which accumulate the resin in internal secretory structures, such as ducts or canals. For *Pinus pinaster* it has been shown that after perforation there was a 2.5-fold increase in oil yield, while inoculation with mycelium of *Verticicladiella* sp. led to a 60-fold enrichment in oil yield. Moreover, the addition of the mycelium had a more long-lasting effect on terpenes production than the wounding. Neither wounding nor addition of mycelium induced significant changes in the relative percentages of the main components. The foliar application of different herbicides and growth regulators in *Salvia officinalis* showed marked changes in the yield and composition of its essential oil, although no correlation could be established with the results obtained (for references, see Figueiredo *et al.*[1]). Likewise, Stahl and Wollensah[27] studied the effect of 11 herbicides on the development of the trichomes and the azulene production in *A. millefolium*. Several of these herbicides delayed glandular development and caused giant trichomes, with two to three times the number of cells. The production of azulene was also reduced, particularly when 2,4-D, Dicamba, Triazin, Chlorpropham and Brompyrazon were applied.

Conifers produce resins with ecological and commercial importance. Mono- and diterpenes in nearly similar amounts and lower amounts of sesquiterpenes occur in these resins, which are accumulated in specialized secretory structures, which can be as simple as blisters in *Abies* spp. (true fir) or more complex, such as the three-dimensional systems of canals that occur in *Picea* (spruce) and *Pinus* (pine) species. In *Picea* spp., the stem resin constitutively accumulates in axial resin canals in the cortex and, as a consequence of mechanical wounding, insect feeding or fungal injury, in traumatic resin ducts within the developing xylem. Using non-invasive methodology, Martin *et al.*[28] observed striking morphological changes 6–9 days after a methyl jasmonate (MeJA) spray treatment. Xylem cells adjacent to the cambium showed a denser cytoplasm and thinner walls, giving rise to the traumatic resin ducts, in addition to the already existing constitutive ones. Fifteen days after the treatment, the lumen of the traumatic canals was clearly visible, all together forming a ring within the youngest portion of the xylem. By this time, the lumen had begun to be filled with resin. Besides inducing the formation of new canals, the MeJA treatment increased the total

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accumulation of mono- and diterpenes, but the absolute amount of sesquiterpenes was unchanged.

As stressed or diseased plant material can develop induced larger amounts of volatiles, the situation can become problematic and create an allergic response in people handling this type of plant material, such as plant pickers and florists.

Environmental conditions

Climate

As mentioned by Reeve,[29] despite all kinds of technological advances, there is one element that remains far from human control, namely the climate. Essential oil production and that of secondary metabolites in general is extremely dependent on the weather conditions. Hurricanes, cyclones, floods and/or droughts are some of the atmospheric conditions that have had, and may have, more or less severe consequences for the aroma industry. According to Reeve,[29] the hurricanes that struck Florida in 2004 caused a 25% loss of the orange crops and a 63% reduction in the grapefruit harvest. The same author mentions also other examples, such as the effect of the annual Madagascar cyclones on vanilla availability and the consequent influence on the market prices, or the still negative effect of the floods in China and Argentina on geranium and lemon production, respectively. Europe does not escape from the weather effects, the floods and the unusually hot summers having a dramatic outcome on the coriander crop in Eastern Europe. In 'normal' years, the essential oil production from *Rosmarinus officinalis* of Tunisia is 60–70 tons, whereas, owing to droughts, the production fell to <20 tons in 2002 and 2003.[30] Other examples also show that in previous decades the environmental conditions had a damaging effect on the yield of commercially important plants. For instance, Indonesia lost about two-thirds of its pepper production due to drought, which therefore also negatively affected world production[31] (Figure 7).

Turtola *et al*.[32] showed that under induced stress conditions of drought the total amount of terpenes and resin acids increased, simultaneously with a decrease in the growth of *Pinus sylvestris* and *Picea abies* seedlings. Hydric stress seems to be directly related with an increase in the production of volatiles in some other species, such as *Anethum graveolens*, *Artemisia dracunculus* and *Ocimum basilicum*, whereas in *Artemisia annua*, *Coriandrum sativum* and *Thymus vulgaris* a yield increase was only attained with normal or higher irrigation. The method of irrigation, as well as the developmental stage of the plant for which this is more abundant, are also important for essential oil yield (for references, see Figueiredo *et al*.[1]).

Among other effects, drought stress can limit photosynthesis in plants and alter nutrient uptake and carbon,

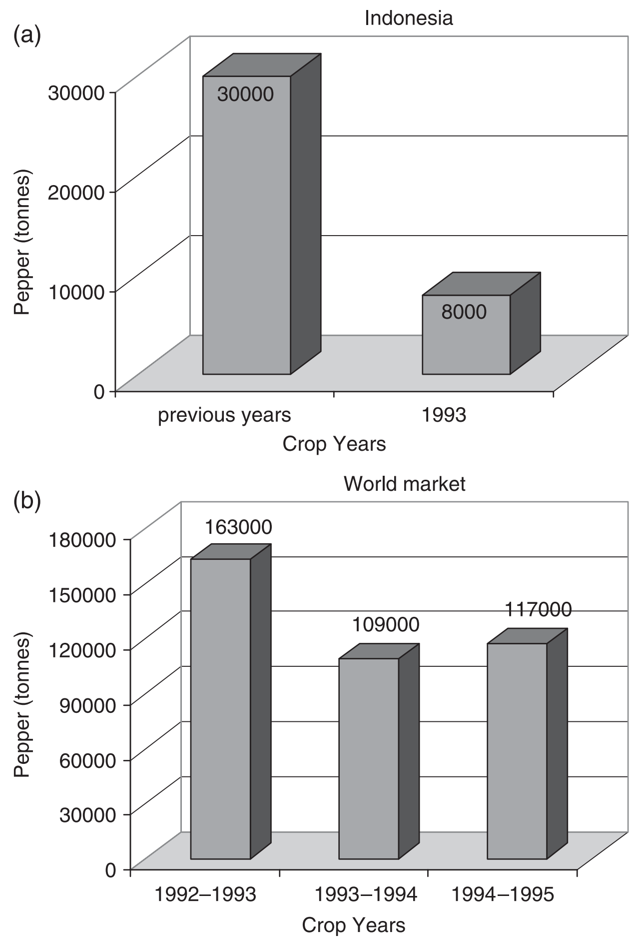

Figure 7. Consequences of drought for pepper production. Adapted from Weiss[31]

sugar, amino acid and inorganic ion fluxes. In addition, a plant under stress due to adverse weather conditions and/or limited nutrients is more prone to attacks by pathogens and herbivores.

During the months of lowest temperature and fewest hours of sunlight, there is an obvious decrease in volatiles production. In *Pinus elliotii*, monoterpene emission rates, particularly those of α- and β-pinene, myrcene, limonene and β-phellandrene, increased exponentially with an increase of temperature, between 20°C and 46°C.[33] However, according to the same authors, light had no influence on the emission rates of these components. A tropical climate is said to favour the formation of oxidized components in the oils.[34]

Pollution

Although known to occur, the detrimental effect of air pollution on secondary metabolites production, and particularly essential oil components, is difficult to access, since the consequences can be confused with the response to other types of stress. Ozone is considered one

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of the major plant pollutants. In addition, the effect of other pollutant agents, such as gases released by vehicles and fires, can be increased by factors such as wind, rain and temperature, among other things. Only a few studies have addressed the effect of air pollutants on essential oil components, and the results are sometimes contradictory[35] and differ according to the polluting agent.[36]

Dust pollution, such as near roadsides, cement factories and quarry works, causes gloom, stoma closure and diminished CO_2 flux. The comparative evaluation of the essential oil yield and composition of *Cistus albicans* and *Pinus pinea*, from quarries and rural fields, showed an increase in oil yield, whereas the chemical composition was unaltered in oil isolated from plants grown on quarry works (authors' unpublished results).

Diseases and pests

Several types of diseases and pests (mildew, rust and black spot, among other things) can cause major detrimental effects to the stability of the market supply. Some of these are a consequence of the introduction of new crops in places other than their original source. Thirty to forty percent of a pepper crop (*Piper nigrum*) can be lost as the result of an attack of the pepper flea beetle, *Longitarsus nigripennis*, and up to 50% of the shoots can be lost due to the top-shoot borer, *Laspeyresia hemidoxa*. In India, the injury is most damaging during periods of heavy shade and heavy rain, which hamper control measures.[31]

The biggest insect pest for tea tree (*Melaleuca alternifolia*) is the herbaceous pyrgo beetle, *Paropsisterna tigrina*, which can rapidly devastate a plantation by both adult and larval herbivory. The corresponding breeding programme nowadays selects for insect-resistant plants, by choosing those that produce the highest biomass.[37]

In the USA, the soil fungus *Verticillum dahliae* is considered one of the most limiting factors in *Mentha* × *piperita* production, by causing plant mulching. In Argentina, the plant shows higher susceptibility to root lice. In India, the crop is annual and it shows less vulnerability to these problems.[26]

Viral diseases such as cucumber mosaic virus (CMV), transmitted by aphids, have been detected in several medicinal and aromatic crops.[38] An infection of the giant hyssop (*Agastache anethiodora*) with CMV was found to induce an 88% reduction in the yield of the essential oil as well as changes in the relative amounts of the oil components.[39]

Edaphic factors

Several authors considered the type and composition of the soil as one of the determinant factors in secondary metabolites composition and that of volatiles in particular, in addition to other explanations for the differences found in oils of the same species. Given the high chemical variability of the essential oils isolated from four populations of *Thymus caespititius* collected in a range of 200 m on the same slope of Pico Verde (Azores), and the absence of morphological differences in the trichome indumenta, Pereira *et al*.[39] suggested that genetic and edaphic factors may be responsible for these differences.

The growth and survival of many plant species is severely depressed in poorly drained soils, thereby greatly reducing crop and essential oil yields as well as affecting the volatiles composition. According to Hornok,[40] the supplementation of the soil with three of the most important nutrients (nitogen, phosphorus and potassium) has generally shown an increase in the oil yields, although the separate addition of the same nutrients gave different results in the yield and composition of the same oils. Other authors have shown that, in contrast to the yield, the composition of the essential oil was not influenced by the concentration of nitrogen in the soil (for references, see Figueiredo *et al*.[1]). In addition, low Ca^{2+} levels may develop acidic soils that depress growth, and too-close row spacing can be detrimental, due to allelopathic factors or by mutual leaf and/or canopy shading.

Geographic Variation

There are countless examples of the occurrence of geographic variations of the yield and composition of volatiles, determining, for several species, the existence of distinct chemotypes/chemical races. The variations in the essential oils of *Zingiber officinale* and *Myristica fragans* of different origins can be given as examples (Tables 5 and 6). Other examples include the essential oils of *Crithmum maritimum*,[41,42] *Thymus carnosus*[43] and *T. caespititius* (Santos *et al*.[44] and references therein).

The study of Pateira *et al*.[41] concerning several populations of *C. maritimum* grown on the mainland of Portugal reported two chemotypes: chemotype 1 (15–47% dillapiole; 17–35% γ-terpinene; 10–18% methylthymol; 7–22% sabinene), and chemotype 2 (25–44% γ-terpinene;

Table 5. Variations in the composition of the essential oil of *Zingiber officinale* from different origins

Components	Relative amount (%)		
	Australia	India	Sri Lanka
Camphene	t–14	t	1–14
β-Bisabolene + β-farnesene	2–9	0.2–12	21–61
ar-Curcumene	6–10	19	6– 27
β-Sesquiphellandrene	3–11	12	t–0.3
Zingiberenes	4–28	7–36	0.4–2
Geranial	3–20	1	1–15
Neral	4	1	3–10
1,8-Cineole	8	0.3	2–12

Adapted from Pellerin[58] and Weiss.[31]

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Table 6. Variations in the composition of the essential oil of *Myristica fragrans* from different origins

Components	Relative amount (%)		
	West India	Sri Lanka	East India
α-Thujene	4.7	1.2	1.7
α-Pinene	11.5	14.8	22.3
Sabinene	28.2	41.8	19.5
β-Pinene	10.8	12.0	14.8
Terpinen-4-ol	9.5	2.2	4.3
Myristicin	0.5	4.1	10.2
Elemicin	0.9	2.1	0.4

Adapted from Reeve.[60]

17–34% sabinene; 10–18% methylthymol; not detected–6% dillapiole). The occurrence of a much higher γ-terpinene content, together with lower sabinene, dillapiole and methylthymol contents, recorded for Azorean oils, might suggest the existence of a distinct chemotype for plants growing on some Azorean islands.[42]

For *T. caespititius*, the essential oils from populations collected in mainland Portugal and on Madeira were found to be characterized by α-terpineol dominance, whereas those obtained from populations collected on nine Azorean islands showed a marked chemical variability, with carvacrol, thymol or α-terpineol as the dominant components.[44]

The different essential oil compositions of a species found for different origins reflect the different environmental conditions of each particular location and culture conditions (different altitudes, different solar exposition, different soil types, etc.). In addition, we can not forget that all these things mix together, so the differences in oil composition found for different geographical origins are also due to genetic differences. Moreover, the occurrence of different oils of a species from different geographical origins may also be determined by the processing of the material after harvest.

Genetic Factors and Evolution

Genetic and hybridization studies have shown that the composition of essential oils is under genetic control. Studies of the essential oils of several *Mentha* species have shown that CC or Cc genotypes promoted the conversion of α-terpineol into limonene, and the oxidation of the latter component to give carvone. On the other hand, the cc genotype produced a menthadiene that was converted into pulegone and menthol. Also for species of *Abies*, *Achillea*, *Clarkia*, *Cupressus*, *Juniperus*, *Perilla*, *Pinus*, *Salvia* and *Thymus*, the essential oil composition was shown to be genetically determined.[1,45–47]

Natural selection determines changes in the production of volatiles and the composition of the essential oils. As an example, the North American tree *Pinus ponderosa* is rich in volatile terpenes, viz. α-pinene, myrcene and limonene. The oleoresin of this tree attracts the female beetle *Dendroctonus brevicomis*, which then settles on the bark during its reproductive cycle, together with symbiotic fungal pathogens. The beetles employ α-pinene and myrcene from the oleoresin to synthesize their own pheromones, which are used during the reproductive cycle as attractants or repellents for the males. Studies of population variation in the composition of the oleoresin have shown that directional selection takes place for trees with high concentrations of limonene, which in large quantities is toxic to the beetle.[48]

The mechanisms that lead to the evolution of volatiles formation in plants include: (a) gene duplication followed by divergence, which retains the original enzymatic function, while a new function evolves from the duplicated gene; (b) convergent evolution, where new functions have arisen independently multiple times; (c) evolution of an existing gene, without duplication, resulting in a new enzymatic function, with loss of the original one; and (d) loss of enzymatic activity, caused by several factors, such as hybrid formation, mutations and/or chromosomal rearrangements.[49] In any of these cases, these processes lead to changes in gene expression. Additionally, functional enzymatic diversity can arise with very few changes in the enzyme structure and can be increased with the enzymes being exposed to variable environments, such as constitutive or inducible environments. If the production of new metabolites endows the plants with an adaptive advantage, then the production is maintained and/or increased. Changes in the protein expression may not necessarily lead to loss of the enzymatic activity, but may, for instance, cause the production of secondary metabolites to occur in a different cell, tissue or organ.

Storage

The relative amounts of secondary metabolites may also be affected by the storage method. Although drying can give rise to a number of negative physical and chemical modifications influencing the quality of the marketed plant, such as changes in appearance and aroma, due to the possible loss of volatile compounds, it may also reduce the growth of micro-organisms and prevent some negative biochemical reactions. Light, humidity, temperature, age of the material, contaminations, oxidation, resinification, etc., affect the yield and composition of the volatile fraction to a certain extent. As previously mentioned, the type of secretory structure is also of importance in this respect. Internal secretory structures are less prone to losses or transformations of volatiles, whereas external secretory structures (trichomes) can naturally release their secretion by cuticle disruption; these

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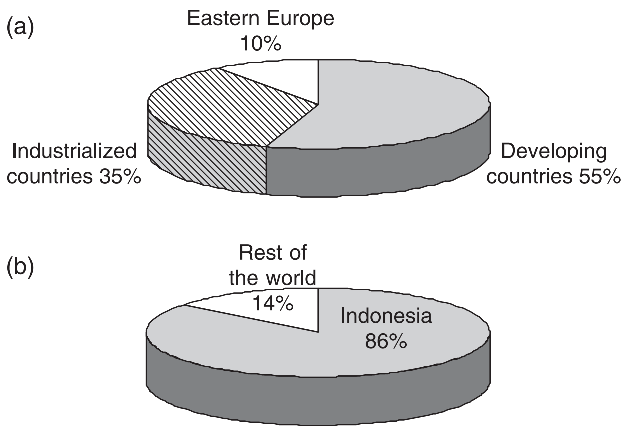

Figure 8. (a) Distribution of the essential oil production. Adapted from Verlet.[59] (b) World nutmeg oil production. Adapted from Reeve[60]

Table 7. Advantages and drawbacks of essential oil production in developing countries

Advantages	Drawbacks
Favourable climate for cultivation	Political instability
Weak competition from other crops	Insufficient capacity for investment in production and quality
Low-cost manual labour	Inadequate quality control
	Uncontrolled extraction and absence of a reforesting policy
	Embargo to exports

are thus more vulnerable to mechanical damage resulting from handwork, transport and/or crushing in piles. *Anethum graveolens*, *Cananga odorata*, *Carum carvi*, *Chamomila recutita*, *Jasminum grandiflorum*, *Narcisus poeticus*, *Ocimum basilicum*, *Sassafras albidum* and *Zingiber officinale* are some of the species that show a decrease in yield and a change in chemical composition of the volatiles with storage or drying. Conversely, storage and/or drying processes do not affect other species (for references, see Figueiredo *et al.*[1]). In still other cases, curing after the harvest is actually needed to develop the aroma, as is true for *Vanilla planifolia* beans. In this case, the hydrolytic enzymes (including β-glucosidase and other glycosyl hydrolases) and the substrates (glucovanillin and other flavour precursors) are spatially separated. The first ones are abundant in the outer fruit wall region, whereas the latter ones are profuse in the placental region around the seeds. Curing allows contact between the substrates and the enzymes that catalyse their hydrolysis, so that the flavour products, such as vanillin and other flavour constituents, are released.[26]

Political/Social Conditions

According to Corley,[50] the value of essential oils as ingredients is more than \$11 billion and grows at 8% per annum. About 30 000 tons of essential oils (65%) are derived from woody perennials (trees and bushes), which determines the limited elasticity of the suppliers. The remaining 35% come from herbaceous plants, mostly cultivated ones. If the production is from wild trees, the supply depends essentially on the abundance of the species and the costs of harvesting. Oil production from cultivated woody species involves considerable investments in plantations and slow reactions to market fluctuations. In addition, yields and costs vary with geographic area (depending on local conditions, climate and degree of mechanization), the economic climate and the level of productivity. The cultivation of some aromatic species, such as lavender or peppermint, is restricted to certain areas of the world, due to edapho-climatic conditions. Other species are more cosmopolitan, and as such have a wider distribution. Developing countries are responsible for 55% of the world production of essential oils (Figure 8a, b). According to Corley,[50] India remains the largest producer, with 17% of the world market, followed by China with 15% and the rest of the Pacific rim with 8%. Owing to the richness of their natural flora, together with low-cost manual labour, these countries are a big source of raw materials for essential oil isolation. However, political instability and insufficient capacity for investment in production and quality limit the steady supply needed by the firms from the consuming countries (Table 7). One of the consequences of this is the difference in prices of raw materials of different origins: while the price of the Egyptian, Indian and Moroccan jasmine was about 1€/kg in 1994, the same material bought in Grasse (France) amounted to 27€/kg.[51]

Another consequence of the restraint to one country as source of a certain plant material is the possible occurrence of environmental and/or political limiting factors that restrict the access to the plant raw material. As an example, due to uncontrolled extraction of the flora, harvesting of wild laurel leaf in Morocco requires a government permit, which is obtainable only at certain times and for specific areas.[52] Another example is that of *Cinnamomum camphora*. During the first four decades of the twentieth century the safrole production occurred by extraction of *C. camphora* of Chinese origin; by the Second World War, the European perfume industry was confronted with the impossibility of importing the Asian product, and the solution was to search for alternative raw material, namely, the Brazilian endemic *Ocotea cymbarum*. After 35 years of exploitation, the production dropped drastically because of uncontrolled extraction and the absence of a reforesting policy. This process

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Table 8. Amounts of plant material needed to obtain 1 kg essential oil

Plant	Country	Isolation procedure	Material	Amount (kg)
Aniba rosaeodora	Brazil	Hydrodistillation	Bark	100
Boswellia sacra	Somalia	Hydrodistillation	Resin	15
Cinnamomum zeylanicum	Sri Lanka	Hydrodistillation	Outer bark	80
Citrus aurantium (bitter orange petitgrain)	Paraguay	Hydrodistillation	Leaves/petioles	100
Citrus aurantium ssp. *bergamia* (bergamot)	Italy	Expression	Peel	200
Citrus aurantium (neroli)	Guinea	Hydrodistillation	Flowers	1000
Citrus limon (lemon)	Brazil	Expression	Peel	60–70
Citrus reticulata (mandarin)	Spain	Expression	Peel	50
Citrus sinensis (sweet orange)	USA	Expression	Peel	50
Citrus × *paradisi* (grapefruit)	USA	Expression	Peel	100
Cymbopogon citratus	China	Hydrodistillation	Leaves	30
Eucalyptus globulus	China	Hydrodistillation	Leaves	50
Jasminum grandiflorum	Morocco	Extraction with alcohol	Flowers	1000
Juniperus virginiana	USA	Hydrodistillation	Bark	30
Lavandula angustifolia	France	Hydrodistillation	Flowering tops	100
Mentha × *piperita*	China	Hydrodistillation	Leaves	50
Ocimum basilicum	Uganda	Hydrodistillation	Aerial parts	500–800
Pelargonium species	China	Hydrodistillation	Leaves	500
Pinus mugo	Canada	Hydrodistillation	Needles	200
Pogostemon cablin (patchouli)	Indonesia	Hydrodistillation	Leaves	35
Rosa damascena (rosa Turca)	Turkey	Hydrodistillation	Flowers	5000
Rosmarinus officinalis	Tunisia	Hydrodistillation	Aerial parts	50
Salvia sclarea	CIS*	Hydrodistillation	Flowering tops	1000
Santalum album	India	Hydrodistillation	Bark	20
Vanilla planifolia	Java, Madagascar	Extraction with alcohol	Beans	35
Vetiveria zizanioides	Indonesia	Hydrodistillation	Root	50

* Commonwealth of Independent States: political entity consisting of 11 former Soviet Union Republics.
Adapted from *Flora Perpetua*.[57]

culminated in a Brazilian government's Decree in 1991, prohibiting the felling of the tree, its trade and the production of its chemical derivatives. In response, the Brazilian companies started to import *C. camphora* oil from China and Vietnam, but these countries also ended up as victims of the uncontrolled exploitation of this species. Consequently, in recent years, studies have been undertaken to find alternative plant sources of a safrole-rich oil (≥80%), the Brazilian *Piper hispidinervium*.[55]

Market strategies are also important when considering the two main industrial goals: availability and stability (of quality and price). The natural vanilla industry lost 25–40% of its market in the last 3–4 years as a consequence of the excessively high price of vanilla beans, which led from the use of the expensive natural aroma to the cheaper synthetic substitutes. Nowadays, the vanilla market is confronted with the problem of again needing to stimulate the consumption of natural vanilla.[26,53]

Amount of Plant Material/Space and Manual Labour Needs

Although not directly affecting the production of volatiles, but with direct implications in its commercialization (availability and price), are the amounts of plant material needed for volatiles production and also the space and manual labour needs (Table 8). As an example, 1 kg jasmine flowers corresponds to 10 000 flowers, which imply 2 h work for a good collector.[51] Similarly, before the mechanization used nowadays, i.e. until the end of 1960s, the collection of *Narcisus poeticus* in France was performed manually in a period of 10 days–2 weeks during the flowering time in May. Although the data can vary considerably with the source, a good day of collection could yield up to 30 kg/person/day. Currently, the collection is semi-mechanized, being less laborious and more active, yielding about 300 kg/day. Nevertheless, the collection is less 'clean' than the manual collection, since leaves and stems are also collected. In this case, 450 kg flowers yields 1 kg concrete (prepared by extracting fresh plant material with non-polar solvents) and 350 g absolute (prepared by taking up concretes in ethanol).[54]

Conclusion

The future for aromatic and medicinal plants is strictly correlated to the high global competition and the resulting pressures. Medicinal and aromatic plants are of natural origin and, thus, their products are of variable composition. On the one hand, physiological, environmental, climatic and geopolitical conditions may negatively affect the supplies and prices, rendering them instable. This instability leads to the search for alternatives, which sometimes leads to synthetic products. On the other hand, many consumers still prefer natural products and that, allied to their diversified uses and the population's

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preferences, which are driven by fashion and the assimilation of new cultures, may be an additional reason for the use of natural products. Nevertheless, the search for natural products must not make immoderate use of the local flora. Moreover, the use of natural products also implies investing in quality, agricultural innovation and a combined effort of agriculture, industry and science for the control of diseases and pests, in the selection of the best cultivation sites, improved plant varieties, determination of the right time for collection and sustainable use of biodiversity. Practical application of this knowledge will be of the utmost importance, since some key compositional differences can be found in the essential oils and volatiles isolated from plant species.

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