23RD INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

BOOK OF ABSTRACTS

23RD INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

SCOTTISH AGRICULTURAL COLLEGE
AUCHINCRUIVE AYR SCOTLAND
SEPTEMBER 9-12 1992

THURSDAY SEPTEMBER 10, 1992

08:45 - 09:00 **Opening Ceremony**

Session I: Use of medicinal plants in traditional and modern ethnopharmacy

Chaired by: E H Graven and J J C Scheffer

09:00 - 09:30 Plenary Presentation ***I-1***

J Bernáth - Research Institute for Medicinal Plants, Budakalász, Hungary.
Systematics of species characterised by essential oil accumulation and their application in traditional and up-to-date therapy.

I-2
09:30 - 09:50 *E Sezik* - Gazi University, Ankara, Turkey.
Volatile oil containing plants used in traditional medicine in Turkey.

I-3
09:50 - 10:10 *K Oosterhaven* - ATO-Agrotechnologie, Wageningen, Netherlands
Effect of s-carvone on potato sprout growth.

I-4
10:10 - 10:30 *G Buchbauer* - Vienna University, Vienna, Austria.
Fragrance compounds and essential oils in aromatherapy research.

10:30 - 11:10 Coffee Break.

I-5
11:10 - 11:30 *S Demissew* - Addis Ababa University, Addis Ababa, Ethiopia.
Botanical studies of some essential oil bearing plants in Ethiopia and their indigenous uses.

I-6
11:30 - 11:50 *G Tumen* - Anadolu University, Eskisehir, Turkey.
Essential oil composition of four *Origanum vulgare* subspecies of Anatolian origin.

I-7
11:50 - 12:10 *E Sarer* - Ankara University, Ankara, Turkey.
Investigations on volatile oils of some *Teucrium* species from Turkey.

I-8
12:10 - 12:30 *M Lis-Balchin* - South Bank University, London, UK.
Agrochemical usage of essential oils of the Geraniaceae family.

12:30 - 13:45 **LUNCH** and Informal Poster Session.

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Session II: Factors influencing plant essential oil formation

Chaired by: C Bicchi and B V Charlwood

13:45 - 14:15	Plenary Presentation	*II-1*
	Ch Franz - Vienna University, Vienna, Austria. **Genetics and breeding of essential oil crops.**	
14:15 - 14:35	*A M Bosabalidis* - Aristotle University, Thessaloniki, Greece. **Development, essential oil secretion and morphometric character istics of glandular trichomes in *Origanum dictamnus* L.**	*II-2*
14:35 - 14:55	*R R Carlton* - Schering Agrochemicals, Saffron Walden, UK. **Variation in volatile oil from the leaves of *Myrica gale*.**	*II-3*
14:55 - 15:15	*T A van Beek* - Agricultural University, Wageningen, Netherlands. **Analysis of the essential oil of the so-called *Mentha mirennae* Bruno. A comparative study with *Mentha* species.**	*II-4*
15:15 - 15:50	Coffee Break.	
15:50 - 16:10	*U Ravid* - Agricultural Research Organisation, Haifa, Israel. **The enantiomeric composition of some monoterpene ketones and alcohols in various essential oils.**	*II-5*
16:10 - 16:30	*H J Bouwmeester* - DLO, Centre for Agrobiological Research, Wageningen, Netherlands. **Effects of environmental factors on caraway [*Carum carvi* L.] essential oil production.**	*II-6*
16:30 - 16:50	*K G Tkachenko* - Kamarov Botanical Inst., St. Petersberg, Russia. **The essential oil of *Mentha* species from St. Petersberg.**	*II-7*
16:50 - 17:10	*G Ruberto* - CNR, Valverde, Italy. **New essential oils from new *Citrus* hybrids.**	*II-8*
17:10 - 19:30	**Formal Poster Session.**	
19:30 - 20:00	**Kyle & Carrick District Council CIVIC RECEPTION**	
20:00 - 24:00	**SCOTTISH EVENING**	

FRIDAY SEPTEMBER 11, 1992

Session III: Chemical analysis of plant secondary metabolites

Chaired by: J B Harborne and E Stahl-Biskup

09:00 - 09:30	Plenary Presentation *M Lindström* - Firmenich SA, Geneva, Switzerland. **Alkaloids, bases and essential oils**	***III-1***
09:30 - 09:50	*L Maignial* - Nestec Ltd, Lusanne, Switzerland. **Simultaneous distillation extraction under static vacuum: isolation of volatile compounds at room temperature.**	***III-2***
09:50 - 10:10	*P Weyerstahl* - Technical University of Berlin, Berlin, Germany. **Constituents of the essential oil of the rhizomes of *Hedychium gardnerianum*.**	***III-3***
10:10 - 10:30	*E Wilhelm* - Hamburg University, Hamburg, Germany. **Essential oil analysis in the European pharmacopoeia.**	***III-4***
10:30 - 11:10	Coffee Break.	
11:10 - 11:30	*N Perry* - Otago University, Dunedin, New Zealand. **Computer assisted analyses of rose oils from New Zealand.**	***III-5***
11:30 - 11:50	*J Holthuijzen* - Hamburg University, Hamburg, Germany. **Some practical experience of analysis of glycosidically bound volatiles.**	***III-6***
11:50 - 12:10	*D A Moyler* - Universal Flavours Ltd, Bletchley, UK. **Extraction of essential oils with CO_2.**	***III-7***
12:10 -12:40	*C Bicchi* - Torino University, Torino, Italy. **The use of cyclodextrin derivatives in the enantiomeric separation of volatile compounds.**	***III-8***
12:40 - 13:45	**LUNCH** and Informal Poster Session.	

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Session IV: Biotechnology of plant secondary metabolites: New developments

Chaired by: R K M Hay and R Hiltunen

13:45 - 14:15 Plenary Presentation *IV-1*

M J C Rhodes - AFRC Institute of Food Research, Norwich, UK.
New approaches to the study of essential oil accumulation.

IV-2
14:15 - 14:35 *M Humphrey* - Bush Boake Allen Ltd, London, UK.
Observations on essential oil distillation in the laboratory.

IV-3
14:35 - 14:55 *E Werker* - The Hebrew University, Jerusalem, Israel.
Glandular hairs in some genera of the *Lamiaceae*: structure, location and production.

IV-4
14:55 - 15:15 *H Becker* - Saarlandes University, Saarbrücken, Germany.
Production of terpenoids in aseptic cultures of liverwort.

15:15 - 15:50 Coffee Break.

IV-5
15:50 - 16:10 *A Henderson* - Strathclyde University, Glasgow, UK.
Mode of action of methyl jasmonate.

IV-6
16:10 - 16:30 *P D Reüdi* - Zurich University, Zurich, Switzerland.
Biological activities of n-alkylphenols and -catechols from *Plectranthus albidus* (Labiatae).

IV-7
16:30 - 16:50 *J R J Paré* - Environment Canada, Ottawa, Canada.
Microwave-Assisted Process [MAP]: Applications to dried spices.

IV-8
16:50 - 17:10 *W R Abraham* - Gesellsch. Biotechnol, Braunschweig, Germany.
Biotransformation of sesquiterpenes and a statistical approach towards intelligent screening.

17:30 - 18:00 **Group Photograph and Tree Planting**

19:45 - 20:15 **Sherry Reception**

20:15 - 22:30 **SYMPOSIUM DINNER**

I-1 Systematics of Species Characterised by Essential Oil Accumulation and Their Application in Traditional and Up-To-Date Therapy

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The ancient records on utilisation of aromatic plants - including essential oils - date back approximately 2600 to 2100 BC in Egypt. At the same historical time the "divine" value of these medicines was drawn into myths by the Chinese. The utilisation of essential oils in Europe started much later and as "quinta essentia" were defined by Paracelsus (1495-1541), and became the part of therapy (Dispensatorium Pharmacopolarum 1546) very soon. Up to now about 1400 different species are utilised for essential oil production.

According to the ***chemotaxonomical investigations*** among the gymnosperms, it is the Coniferopsida and the Taxopsida which store essential oils and balms in schizogenous exocretory organs and canals. In angiosperms essential oil containing taxa are widespread conforming to its whole range of 111 families. The presence and accumulation of essential oils attribute to the chemotaxonomic evaluation [1]. However from the economical point of view only 67 families and 187 genera are utilised as a source of essential oils, concretes and absolutes [2].

Based on the ***therapeutic activity*** of above mentioned compounds, the way of traditional application is very common and unchanged even nowadays. It is proven by some Far East examples. On the basis of the scientific establishments, the essential oil became the tool of up-to-date therapy and used against many diseases: fungal and bacterial infections, asthma, bronchitis, rheumatism, insomnia, neuralgia, migraine, hyper-, and hypotension, dyspepsia, etc.

The popularised form of application is known as 'Aromatherapy' (the nomenclature introduced by Gattafosse in 1928), while the scientific way reflecting in the descriptions of Pharmacopoeia and registrated products of pharmaceutical factories.

Using essential oils and their products - as in the case of all biological active compounds - we have not to be blind to the drawbacks. According to the recent results the ***adverse effects*** of essential oils must be considered [3]. Even in the case of wildly used genera as Cymbopogon, Eucalyptus, Foeniculum, Mentha, we have to count on disadvantageous phenomena.

References:
[1] Tétényi, P., 1986. Craker, L.E., Simon, J.E., Herbs, spices and medicinal plants. Vol 1. Oryx Press: 11-32.
[2] Tucker, A.O. and Lawrence, B.M.,1987. Craker, L.E., Simon, J.E., Herbs, spices and medicinal plants. Vol 2. Oryx Press: 183-240.
[3] De Smet, P.A.G.M., Keller, K., Hansel, R. and Chandler, R.F., 1992. Adverse effect of herbal drugs. 1. Springer-Verlag: 105-178.

I-2 Volatile Oil Containing Plants Used in Traditional Medicine in Turkey

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In Turkey, the usage of folk medicines are widespread. In villages, inhabitants generally try to cure their simple disorders by themselves using available plants. In some cases, they consult the elder people in their village or nearby villages for their illness. Some semi-religious people (called sheih or shih in Turkish) also recommend plants for the treatment of some diseases. Traditional practitioners (halk hekimi in Turkish) also use local plant remedies for treatments of their patients.

The plants growing in the vicinity of villages are utilised as folk medicine in simple pharmaceutical forms (infusion, decoction, poultice, etc.) for certain diseases (diarrhoea, hemorroids, abscess, kidney stones, indigestion, etc.).

The plants used as folk medicine mostly contain volatile oils as *Mentha spicata, M. longifolia, Origanum vulgare, O. majorana, O. onites, O. minutiflorum, O. spyleum, Micromeria myrtifolia, Stachys lavandulifolia* ssp. *lavandulifolia, Achillea vermicularis, A. millefolium, Teucrium polium, T. chamaedrys, Helichrysum* spec., *Anthemis* spec., *Pinus* spec., *Juniperus* spec., etc. The investigations carried out on the chemistry of the volatile oils of the plants used as folk medicine in Turkey are also described in this paper.

I-3 Effect of S-Carvone on Potato Sprout Growth

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Caraway seeds contain 4-6% essential oils, with S-carvone as the predominant compound. S-carvone is a common constituent in food and perfumery products. In addition to these applications, S-carvone can be used as an antifungal as well as a potato sprout inhibiting agent.

The mechanism by which S-carvone acts on potato sprouting is not known. We investigated the posiible role of S-carvone in the inhibition of potato sprout growth.

The effect of S-carvone on potato (*Solanum tuberosum* cv. Bintje) sprout growth was studied in a semi *in vivo* system using potato eye pieces. S-carvone inhibited the sprout growth within 2 days.

An extensive loss of the activity of 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMG CoA reductase; E.C. 1.1.1.34), a key-enzyme in the mevalonate pathway, occurred concomitantly with the inhibition of sprout growth. The enzyme activity decreased to less than 3% of the control after 4 days of treatment. The loss of HMG CoA reductase activity could not be attributed to a direct effect of S-carvone on the enzyme nor to an effect on *in vitro* translation processes. Northern analysis of the mRNA population at various time intervals during the treatment, using a specific potato HMG CoA reductase probe, revealed a considerable hybridisation signal after 4 days of treatment. This indicated that the effect of S-carvone on the activity of HMG CoA reductase in potato sprouts is not regulated by transcriptional events, but by as yet unknown mechanisms.

I-4 Fragrance Compounds and Essential Oils in Aromatherapy Research

Buchbauer, G.,[1], Jäger, W.,[1] Jirovetz, L.[1] and Dietrich, H.[2]

[1] Institute of Pharmaceutical Chemistry, University of Vienna, Währingerstr. 10, A-1090 Vienna, AUSTRIA.

[2] Central Laboratory Animal Experiments, Medicinal Faculty, University of Innsbruck, A-6020 Innsbruck, AUSTRIA.

Inhalation experiments with mice were performed using pure fragrance compounds and essential oils ascribed stimulating effects in order to control their properties also in an aromatherapeutical dosage. Thymol, carvone, eugenol, isoeugenol, camphor and the essential oils of peppermint, clove, eucalyptus, thyme, basil and jasmine and various others but also some more fragrances were investigated by this animal model. After the inhalation experiments, blood was taken from the animals and the serum (after plasma extraction by using bond-elut-columns) analysed by means of chromatographic-spectroscopic systems (GC-FID, GC-MS and GC-FTIR) [1,2] to find out the active compounds responsible for the observed effects.

In additional testings, the effects of the afforementioned fragrances after an induced sedation by an i.p.-dosage of diazepam were determined in the same way.

Also some preliminary results of human experiments will be discussed.

References:

[1] Buchbauer, G., Jirovetz, L., Jäger, W., Dietrich, H., Plank, C. and Karamet, E., 1991. Z. Naturforsch (46C): 1067-1072.

[2] Jirovetz, L., Buchbauer, G., Jäger, W., Raverdino, V. and Nikiforov, A., 1990. Fresenius J. Anal. Chem. (338):922-923.

I-5 Botanical Studies of Some Essential Oil Bearing Plants in Ethioia and Their Indigenous Uses

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Ethiopia in NE Africa has a wide range of climate, geological and topographic conditions, ranging from an altitude of 115m below sea level at the Afar Depression to Ras Dejen reaching up to 4620m. This diverse habitat harbours between 6500 and 7000 species of vascular plants. Some of these plants which are used locally for various purposes produce essential oils.

The leaves of *Artemisia rehan* in the family Compositae and the resins from various species of *Boswellia* and *Commiphora* in the family Burseraceae are used locally as fragrance materials. *Boswellia* and *Commiphora* species produce incense and myrrh respectively. The rhizomes of *Echinops kebericho* in the family Compositae are used as a fumigant after child birth. The leaves of *Lippia adoensis* and *Premna schimperi* in the family Verbenaceae are used to clean kitchen utensils to impart fresh fragrance. The leaves and flowers of many species in the genera *Ocimum*, *Satureja* and *Thymus* in the family Labiatae; *Lippia* species in the family Verbenacceae; the fruits of *Trachyspermum copticum* in the family umbelliferae; the rhizomes of *Aframomum korrorima* and *Zingiber officinalle* in the family Zingiberaceae and others are traditionally used as condiments in the preparations of coffee, tea and various types of food.

A number of other essential oil yielding plants are used in traditional medicine. Examples include: *Ajuga remota* the leaf extract of which is used against intestinal complaints and the leaf juice of *Ocimum lamiifolium* is used to relieve fever and headaches. Both species belong to the family Labiatae.

Chemical investigations on the essential oils of some of the indigenous species indicate that the oils comprise constituents which are important in pharmaceuticals, flavour and fragrance industries. For example, the essential oil of Z. *officinale* contain (36% zingiberene, 12% ß-bisabolene, 12% ß-sesquiphelandrene and 8% ar-curcumene); *A. rehan* (23.5% camphor); *Lippia adoensis* (up to 85% linalool); *Ocimum canum* (17.5% terpinen-4-ol, 15% camphor and 15% linalool), *O. forskolei* (up to 55% of methylchavicol); *O. urticifolium* (55-57% eugenol); *Thymus serrulatus* (over 50% thymol) and *T. copticum* (69% carvicol). Some species of *Boswellia* and *Commiphora* are known to have oils rich in monoterpenes (limonene, paracymene, α-terpineol) and sesquiterpenes (ß-bisabolene). *Premna schimperi* is known to have antiseptic properties.

I-6 Essential Oil Composition of Four *Origanum vulgare* subspecies of Anatolian Origin

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[2] Department of Biology, Faculty of Education, Uladag University, 10100 Balikesir, TURKEY.
[3] Medicinal Plants Research Centre, Anadolu University, 26470 Eskisehir, TURKEY.

Essential oil compositions of four subspecies of *Origanum vulgare* growing wild in Turkey have been examined by GC and GC/MS analysis. The essential oils of *O. vulgare* ssp. *hirtum*, ssp. *gracile*, ssp. *vulgare* and ssp. *viride* have been compared for their components. Carvacrol is the main component of Turkish *Origanum vulgare* ssp. *hirtum*.

I-6 Improvement Studies on Cultivation of Java Citronella (*Cymbopogon winterianus* Jowitt) in Himalayan Hills of Darjeeling, India

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The present paper deals with the agrotechnology, oil yield and oil composition of the commercially grown essential oil yielding plant, *Cymbopogon winterianus* Jowitt, growing in Darjeeling hills, India. Reproductive stage of development recording minimum growth rate permitted the biogenesis of essential oil as well as aldehyde content most effectively. The study has established specific requirements of agronomical inputs for success in cultivation of Citronella involving altitude effects, spacing trials, harvesting times, NPK fertilisation as well as foliar application of mineral nutrients. Of the growth parameters studied, the rate of leaf and tiller formation revealed a close agreement with the rates of essential oil biogenesis whereas the relationship between expansion growth and essential oil synthesis could not be established in this plant species. Analysis of biochemical fractions during different development stages of the plant revealed that there is an intimate involvement of total nitrogen, soluble nitrogen and total carbohydrate contents with the essential oil synthesis.

I-7 Investigations on Volatile Oils of Some Teucrium Species From Turkey

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In recent years there has been considerable interest in genus Teucrium. Many species are currently used in folk medicine of many countries. Little has been published on the composition of the volatile oils of Teucrium species. 27 species of Teucrium from 8 sections are found in Turkey, which are used in folk medicine for the treatment of some illnesses.

In previous studies we investigated the volatile oils of *T. polium* and the varieties of *T. chamaedrys* from Turkey [1,2].

In the present study, the chemical composition of the volatile oils of *T. creticum* L., *T. montbretii Bentham* ssp. pamphylicum P.H. Davis, *T. montbretii Bentham.* ssp. montbretii and *T. kotschyanum* Poech from different sections were analysed.

The plant materials were collected from Southern Turkey and the volatile oils were obtained by hydrodistillation of the materials. The oil yields were 0.36%, 0.60%, 0.39% and 0.14% respectively. By GC and GC-MS analyses of the oil samples 47 compounds were detected. The oils were composed mainly of sesquiterpenes. The major compounds of the oil of *T. creticum* were: ß-caryophyllene (12.71%), linalool (12.53%); *T. monbretii* ssp. pamphylicum: ß-caryophyllene (47.85%), α-humulene (19.08%); *T. montbretii* ssp. montbretii: ß-caryophyllene (36.91%), caryophyllene oxide (8.95%); *T. kotschyanum*: ß-caryophyllene (18.87%), and caryophyllene oxide (11.46%). Results of the analysis will be discussed in detail.

References:

[1] Sarer, E., Konuklugil, B. and Doga, C., 1987. 11 (2): 317-325
[2] Sarer, E., Yenen, M., 1991. 22nd Int. Symposium on Essential Oils Abstracts, 70.

I-8 Agrochemical Usage of the Essential Oils of the Geraniaceae Family

Lis-Balchin, M.,[1] Deans, S.G.[2] and Simmonds, M.[3]

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[3] Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

Hexane extracts of a number of Geraniaceae species and cultivars were tested for their insecticidal and antimicrobial efficacy. Finely cut fresh leaves were shaken with cold hexane and the hexane extract was then reduced using a rotary evaporator below 40°C to give approximately the equivalent of 15g leaf ml^{-1}. Insecticidal action was determined by antifeedant studies using *Spodoptera littoralis* larvae. Antimicrobial studies were conducted on 25 micro-organisms using the agar well diffusion method. The results were correlated with the essential oil composition obtained by gas chromatography and mass spectrometry.

II-1 Genetics and Breeding of Essential Oil Crops

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Essential oils show an enormous diversity of compounds and compositions with different olfactorial and pharmacological properties. The documentation of this variation in secondary products itself, however, is of limited value: at least as important is to understand the biosynthesis and the genetic control of essential oil formation. This knowledge opens possibilities of breeding genetically tailored plant types having desired essential oil composition and concentration.

Already from the large inter- and infraspecific variations it was assumed that the formation of those substances is mainly genetically controlled. As their biosynthesis derives from two metabolic pathways only, the mevalonic acid pathway for terpenes and the shikimic acid pathway for phenylpropanoids, taxonomically distant species can contain the same substances, as infraspecific taxa may show distant patterns. For instance the monoterpene fenchone is not only typical for *Foeniculum vulgare*, but was detected also in a chemotype of *Thymus pulegioides*, and myristicin, a phenylpropanoid, may be found in as distant species as *Myristica fragrans* (Myristicaceae), *Petroselinum hortense* (Apiaceae) and *Perilla frutescens* (Lamiaceae).

Progress in biochemical systematics and the inheritance of biochemical characters depends on extensive single plant investigations of taxa. Within the Lamiaceae family not only the genus *Mentha*, but also *Ocimum sp.*, *Thymus vulgaris* and *Perilla frutescens* have been studied extensively. Some more recent results are known from *Salvia sp.* and *Origanum sp.* On the other hand, *Matricaria recutita* and *Achillea millefolium* are examples of the Asteraceae family with at least partially elucidated inheritance of chemical characters as well as recently bred chemocultivars.

Besides the knowledge of biosynthesis also that of flower and reproduction biology is an important prerequisite of practical significance for the plant breeder to choose the appropriate method for breeding new cultivars, eg. F - hybrids.

II-2 Development, Essential Oil Secretion and Morphometric Characteristics of Glandular Trichomes in *Origanum dictamnus* L.

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Origanum dictamnus (Lamiaceae) is an endemic aromatic plant of the island of Crete, Greece. The aerial organs and particularly the leaves are covered with numerous glandular and non-glandular hairs giving to them a velvetous appearance. Non-glandular hairs are simple (not branched) and composed of several linearly aligned cells. Glandular hairs are of two types. The first corresponds to short hairs with a unicellular foot and stalk and an also unicellular small pear-like head. The second type is represented by large peltate hairs with a voluminous multicellular head ellipsoidal in shape. The latter hairs are considered to majorly involve in the process of essential oil secretion and for that reason our research interest was focussed on them in the first place.

The development of a peltate hair initiates from a single protodermal cell which is bigger than the neighbouring cells. This cell first undergoes two successive periclinal and asymmetrical divisions to give the future foot and stalk cells of the hair and the mother cell of the head. Divisions in the head region further proceed anticlinally giving rise to 12 cells, from which 4 cells are small and centrally located, whereas 8 cells are large and peripherically arranged. Paradermal sections cut from the very tip of the peltate hairs up to the lowest part of their basis, provided evidence that the stalk and foot regions of the hairs are unicellular. Histochemical treatment of the peltate hairs with specific biological dyes disclosed that the essential oil is produced in the head cells (not in the foot and stalk cells) and accumulates in an extraplasmic chamber formed between the apical walls of the head cells and the cuticle.

In order to identify the intracellular components responsible for the biosynthesis of the essential oil and the manner it secretes, studies with electron microscope have been conducted. These studies showed that at the stage when glandular peltate hairs start to secrete, the ground plasm of the head cells appears to contain a great population of essential oil droplets. Essential oil droplets were not observed to occur in cell organelles and particularly in the plastids and the ER-elements which are generally thought to participate in the secretion of essential oils. The entire population of oil droplets gradually migrates toward the tip of the head cells and after it passes through the apical walls it ultimately enters the subcuticular oil-accumulating chamber. Conclusion of secretion is followed by disintegration of the head cells.

Application of morphometric methods further showed that a developed leaf of *O. dictamnus* bears 2680 peltate hairs (1210 on the upper side and 1470 on the lower one) with a head diameter of about 90μm. The subcuticular space of a peltate hair was calculated to contain 1.81 x 10^5 μm^3 of essential oil and the 12 head cells 0.37 x 10^5 μm^3. The theoretical essential oil yield of mature leaves was estimated to be 1.4% v/w. The changes in the relative volume of the ground plasm, plastids, mitochondria, ER, dictyosomes, nucleus, vacuoles and essential oil accumulated in the cytoplasm of the head cells, were further measured at six stages of peltate hair development. Values for the ground plasm varied from 25% to 53% of the total secretory cell volume. Corresponding values for the nucleus progressively decreased from 23% to 6%. The plastids, mitochondria, ER and dictyosomes did not exceed 10% during all stages. The average volume percentage of the cytoplasmic essential oil was estimated to reach 38% at the stage of peltate hair maturity.

II-3 Variation in Volatile Oil From the Leaves of *Myrica gale*

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[1] *Present address*: Environmental Science Department, Schering Agrochemicals Ltd, Chesterford Park Research Station, Saffron Walden, Essex, CB10 1XL.

Bog myrtle (*Myrica gale* L., Myricaceae) is an aromatic shrub which grows in temperate wetlands of western Europe and North America. It grows extensively throughout Scotland and is common on Flanders Moss, a raised peat bog west of Stirling in Central Scotland. The volatile oil of bog myrtle has attracted the attention of biologists and chemists for more than 80 years. In the last 25 years reports of the composition of oils from Canada, USA, Spain, Switzerland and the Netherlands have been reported. In this study plants from the Flanders Moss population were investigated.

In June 1987 leaves from 10 plants were collected, the volatile oil from each was extracted and analysed. The data was reviewed to assess variation in the composition of the oil within the population. In August 1987 leaves from 5 plants in the same group were further sampled and the oil was analysed to assess changes during the summer. These results were also compared with earlier reports to give an indication of geographical variation.

The oil was extracted by mechanically rupturing the glands while the leaves were submerged in n-pentane. The pentane was driven off under nitrogen and each sample was analysed by gas liquid chromatography (GLC). Monoterpenes were identified by a combination of retention time comparison with standards and gas chromatography-mass spectroscopy (GC-MS). Other constituents were identified by GC-MS.

The volatile oil was a complex mixture of terpenes which also contained at least two dihydrochalcones. The major constituents (> 5%) common to all plants sampled were α-pinene and 1,8-cineol. In June ß-elemenone was a major component in 9 of the plants. Germacrone constituted more than 15% of the oil in 5 plants and less than 1.5% in the other 5 plants. This represented the clearest source of variation within the group although many minor constituents were not detected in all plants.

The clearest source of seasonal variation also involved sesquiterpenes. In August the relative importance of ß-elemenone had diminished more than 5-fold. In those plants where germacrone was a major oil component its importance diminished, but to a much lesser extent than ß-elemenone. The reduction in sesquiterpenes during the summer is unlikely to be passive (ie. due to evaporation). These compounds are biologically active and their presence in June is likely to form an effective defence against both herbivores and pathogens during the period of maximum growth. Their reduction through the summer may be a strategy to avoid adaptation by herbivores to these defenses.

When these results and earlier reports were compared differences along geographical lines became apparent. In European populations α-pinene is the major monoterpene whereas myrcene fills this position in both North American populations. 1,8-cineol is also important in European plants but is a minor constituent in American populations. Within Europe the greatest similarity appears to exist between populations in the Netherlands and Scotland.

II-4 Analysis of the Essential Oil of the So-Called *Mentha mirennae* Bruno. A Comparative Study with *Mentha* Species

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In 1924 Bruno reported for the first time on a new species of the genus *Mentha* of the Labiatae family [1]. The species was named *Mentha mirennae*, after Father Mirenna of Loutre from the island of Tinos in the Greek Archipelago. Since then only a few more publications have appeared. Rovesti and Rovesti in 1927 [2] and Sorgès in 1931 [3] published data on the properties and composition of *M. mirennae* oil. The oil was shown to possess a high linalool content which is of commercial interest. This *Mentha* species might be a source of natural linalool in the Mediterranean region. However, the plant was never authenticated as a new species and no report is known in the botanical literature.

On the request of Prof. Mattei in Palermo plant material of *M. mirennae* from Tinos was cultivated in the botanical garden of the University of Palermo. During the second world war the cultivation was destroyed and the herbarium specimens were lost. Only samples of the essential oil in sealed vials were saved. As part of the present study the oil was reanalysed with modern chromatographic and spectroscopic methods. This was done to confirm the high content of linalool and to compare the chemical composition of the oil with that of other *Mentha* oils. Thus it might be possible to identify the species taxonomically.

The analysis showed that mainly oxygenated monoterpenes are present. The main component is (-)-linalool (70.2%). Other minor compounds are limonene (1.5%), (E)-linalool oxide (furanoid) (1.1%), linalyl acetate (9.8%), ß-elemene (1.1%), α-terpineol (1.4%), geranyl acetate (1.0%), 3,7-dimethyl-1,5-octadien-3,7-diol (1.4%) and caryophyllene oxide (1.3%). In total 40 compounds were identified. Thus the earlier findings concerning the high linalool content are confirmed.

After a chemical comparison of the oil composition with that of other *Mentha* species and a study of local flora we have come to the conclusion that the composition of the essential oil of *M. mirennae* agrees best with that of a variety of *M. x citrata* rich in linalool [4]. *M. citrata* plants are frequently cultivated in gardens on Aegean islands and contain linalool or linalyl acetate as main constituents [5].

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[5] Private communication of Prof. Stella Kokkini, Aristotelian University of Thessaloniki,

II-5 The Enantiomeric Composition of Some Monoterpene Ketones and Alcohols in Various Essential Oils

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Several herbs, such as geranium, fennel, caraway, dill, spearmint and marjoram, were grown by modern agronomic techniques for maximum yield of chiral monoterpene ketones and alcohols. The natural chiral oxygenated compounds were collected from the essential oils by preparative gas chromatography using a modified Brownlee-Silverstein thermal gradient collector. The enantiomeric composition was determined by gas chromatography using commercially available chiral fused-silica capillary columns with modified ß- and gamma- cyclodextrin phase. It was found that carvone and fenchone isolated from various herbs and spices were enantiomerically pure in most cases while terpinen-4-ol and citronellol were mixtures of enantiomers. The enantiomeric distribution of the monoterpenes may be useful in essential oil analysis as well as in chemotaxonomic investigations.

II-6 Effects of Environmental Factors on Caraway (*Carum carvi* L.) Essential Oil Production

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The potential of caraway (*Carum carvi* L.) as an industrial crop is investigated in the Netherlands in a national research program. Caraway fruits (usually referred to as seeds) contain an essential oil which consists of 95-99% of the monoterpenes (+)-carvone and (+)-limonene. Its capacity to inhibit sprouting of potatoes and its fungicidal activity make caraway essential oil and particularly carvone an attractive candidate for saver pesticides. However, the use of caraway essential oil for industrial applications is hindered by the large variability in essential oil yield. This is caused by variation in both seed yield and the essential oil content of the seeds resulting in up to six-fold differences in essential oil yield between years and individual farmers. The present paper focuses on the weather-related variations in essential oil yield between years.

Analysis of data of farmers suggested that there is a positive relationship between solar radiation from flowering to harvest and essential oil content and a negative relationship between average wind speed during seed ripening and essential oil content.

Volatilisation of caraway essential oil has been suggested before [1] and it seems likely that this process is stimulated by wind. In a greenhouse experiment, exposure of fruit-bearing umbels to wind produced by ventilators resulted in over 80% lower essential oil content. This effect of wind however, may also be due to inhibition of photosynthesis (closure of stomata) which reduces the availability of substrate for essential oil synthesis instead of or in combination with stimulation of volatilisation. Experiments to assess possible losses due to volatilisation are presented.

It was shown in field trials that essential oil content was decreased by shading during seed development, which suggests a possible effect of solar radiation. In a greenhouse experiment, a positive relationship between plant sugar content and essential oil accumulation was found. *In-vitro* experiments supported the conclusion from these experiments that essential oil synthesis depends on sugar availability. There was a positive relationship between sucrose concentration in the culture medium and essential oil accumulation in caraway fruits [2].

Essential oil content in caraway fruits appears to be the outcome of synthesis - which depends on assimilate availability - and possibly volatilisation which may be affected by wind. A better understandind of these processes may enable agronomists to improve crop management to increase essential oil production.

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As variaç. [illegible] causadas por factores genéticos mas são ambientais 1x q qd colocados em idênticas condições ("green-house") o seu comporta/ é idêntico em casos

II-7 The Essential Oil of Some *Mentha* Species from St. Petersburg

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[2] St. Petersburg State University, Russia, CIS.

Mentha crispa L., *M. longifolia* (L.) Huds. and *M. piperita* L. "Multimentha" were cultivated from the Botanical Gardens. The essential oil of the fresh leaves of mints growing in Russia, were produced by water distillation. The essential oil yields obtained were 0.23% from *M. crispa*, 0.16% from *M. longifolia* and 0.4% from *M. piperita*.

The quantitative amounts of the identified principal components of the leaf *M. crispa* oil are as follows: santene (0.06%), α-thujone (0.13%), α-pinene (0.8%), ß-pinene (4.3%), myrcene (2.2%), p-cymene (2.8%), 1,8-cineole (17.3%), ocimene Y (6.1%), gamma-terpinene (1.3%), linalool (19.6%), menthol (1.6%), pulegon (11.4%), carvacrol (1.6%), $C_{15}H_{24}$ (0.8%), caryophyllene (3.0%) and $C_{15}H_{24}$ (3.1%); *M. longifolia* - α-thujone (0.4%), ß-pinene (1.1%), myrcene (1.2%), p-cymene (5.2%), ocimene X (6.6%), unidentified (1141 Kovach's index, M^+ 166, 23.3%), menthol (12.5%), thymol (3.7%), carvacrol (3.3%), $C_{15}H_{24}$ (0.1%), caryophyllene (3.0%) and $C_{15}H_{24}$ (1.1%); *M. piperita* - α-pinene (0.1%), ß-pinene with sabinene (0.3%), myrcene (0.5%), 3-octanol (0.9%), limonene with 1,8-cyneole (4.4%), ocymene X (0.6%), ocymene Y (0.2%), gamma-terpinene (0.4%), linalool (0.3%), 3-octil acetate (0.5%), menthon (17.2%), isomenthon (4.2%), unidentified (1145 Kovach's index, M^+ 166, 3.4%), isomenthol (3.2%), menthol (54.0%), pulegone (3.4%), menthil acetate (2.1%) and caryophyllene (1.4%).

The results of the identification of the components is based on the GC and GC-MS data in combination with their partition coefficients in the hexane/acetonitrile system.

II-8 New Essential Oils From New *Citrus* Hybrids

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Citrus production is one of the most important economic resources for Southern Italy. Nevertheless as worldwide demand for fresh fruits, and mainly for citrus juice products (frozen concentrate, aqueous essences, essential oils) is increasing, the Italian production and experts are undergoing a crisis, partially due to competition arising from the worldwide spread of citrus cultivation.

This downward trend could be overcome by improving the quality of the fruit, for both the fresh-fruit market and the processing industry.

In this context the introduction of new hybrids obtained by crossing the extant species could prove useful.

As part of phytochemical studies of new citrus crosses obtained in Sicily, we wish to report the results obtained from comparative analyses of the essential oils of clementine (*Citrus clementina*) and grapefruit (*Citrus paradisi*), and those of their seven new hybrids.

Furthermore, the essential oil of a new mandarin hybrid, the 'Cami', a very promising cross between "Comune" clementine (*Citrus clementina*) x "Avana" mandarin (*Citrus deliciosa*) used as seed parent and "Mapo" tangelo (*Citrus deliciosa* x *Citrus paradisi*) as pollen parent, will be discussed.

III-1 Alkaloids, Bases and Essential Oils

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The association of alkaloids or bases (the distinction is not clear) with essential oils dates at least from the isolation of piperine in 1820. A summary of the more recent work on bases, particularly pyridines, in our laboratories will be given, and I shall describe some of the more unusual and novel naturally occurring bases we have found in patchouli oil, jonquil absolute, juniper oil and orange oil. The organoleptic effect of these pyridines is sometimes important. The possible biogenetic pathway of some of the bases will be examined.

†March 20, 1992.

III-3 Constituents of the Essential Oil of the Rhizomes of *Hedychium gardnerianum*

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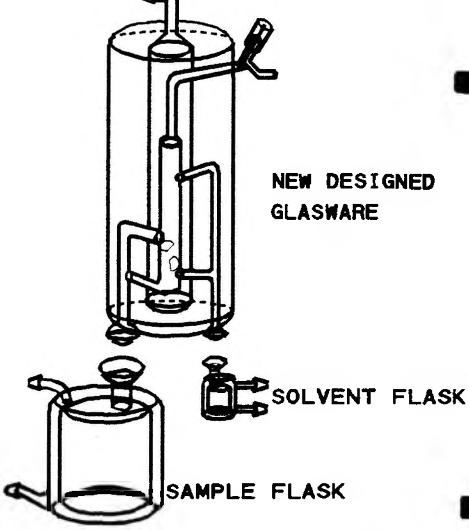
From the rhizomes of *Hedychium gardnerianum* (Zingiberaceae) growing in the eastern Himalayan region of India an essential oil was obtained with a fresh and eucalyptus top note and a base note like Labdanum oil. Isolation of several functionalised trimethyldecalins including some aldehydes was possible by flash chromatography of the high boiling fraction. Most important are 11-nor-drim-8-en-12-al and drim-8(12)-en-11-al (-bicyclofarnesal). For olfactory comparison purposes both these aldehydes have been synthesised. They show a strong woody, ambergris odour.

Acknowledgements:
We are grateful to Dr B.P. Pradhan, Department of Chemistry, University of North Bengal, 734430 Darjeeling, and to G.C. Subba, Govt. Diosgenin Factory, Gairibas, Darjeeling, for supplying the essential oil.

III-2

SIMULTANEOUS DISTILLATION-EXTRACTION UNDER STATIC VACUUM: AN ISOLATION OF VOLATILE COMPOUNDS AT ROOM TEMPERATURE.

L. MAIGNIAL - NESTEC RESEARCH CENTER, LAUSANNE SWITZERLAND.

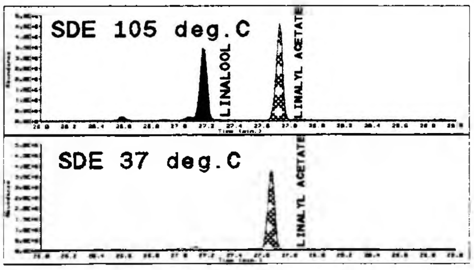


■ VARIOUS SOLVENTS CAN BE USED ALLOWING TO EXTRACT UNDER THE FOLLOWING TEMPERATURES:

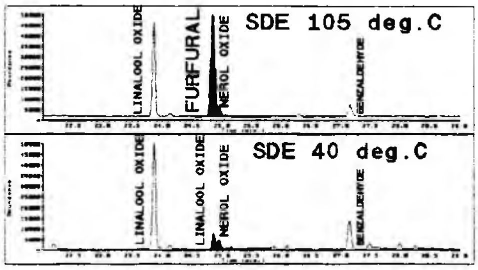
	SAMPLE T deg.C	SOLVENT T deg.C
n-OCTANE	19	26
2-PENTANONE	25	17
TOLUENE	30	20
HEPTANE	30	17
ISOOCTANE	37	20
HEXANE	50	22

■ COMPARISON BETWEEN STANDARD AND STATIC VACUUM S.D.E.:

Distillation-extraction of LINALYL-ACETATE



Distillation-extraction of MEXICAN HONEY



Recovery yields of various molecules (%) (Internal standard quantitation).

COMPOUND	ATM. SDE	VACUUM SDE
ETHYL BUTYRATE	118 / 13	92 / 13
LIMONENE	109 / 2	103 / 2
2-E-HEXENAL	91 / 3	76 / 4
2-ACETYL PYRAZINE	55 / 2	44 / 2
E-ANETHOL	102 / 2	92 / 2
DODECANOL	97 / 2	92 / 2

■ CONCLUSION :

- Selective extraction of volatile compounds in a single step;
- Thermal artefacts discarded;
- Choice among several solvents allows to optimize each application;
- One sample can be analysed with several techniques (GC / FID, MS, FTIR, Sniffing);
- Efficient extraction yields;
- Accurate quantitative measurements (ISTD).

III-4 Essential Oil Analysis in the European Pharmacopoeia

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In view of the extension of Europe and an expected enlargement of the European Community it is of topical interest that this paper deals with the European Pharmacopoeia. It reports on the range of the current European Pharmacopoeia within Europe. A summary is given on the existence of national pharmacopoeias besides the European one.

Our main point of view is the essential oils and the essential oil bearing plants. The methods used for the identification, determination of purity and content of essential oil or essential oil constituents, respectively, are described in detail. Additionally, we compare the analytical instructions given in the different monographs. The choice of methods dealing with essential oil analysis is discussed. Furthermore, we summarise the supplementary instructions of the German national Pharmacopoeia (DAB 10) and the DAC.

III-5 Computer-Assisted Analyses of Rose Oils From New Zealand

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Rose oils, from *Rosa damascena* grown in Central Otago, are being investigated as a potential new high-value product for New Zealand. Detailed studies of the effects of various cultivation, harvesting and extraction conditions require gas chromatographic (GC) analyses of large numbers of rose oil samples. These analyses are computer-assisted at most stages.

Component peaks in GC analyses are identified by their Kovats retention indices, by searching a computerised data base. An autosampler GC instrument carries out analyses of multiple samples without an operator.

Chromatographic software recognises major peaks and corrects retention times to produce standardised result files for each sample. These result files are summarised and analysed by using a statistical software package. In particular, principal components analysis is very useful in picking out patterns from this multivariate data.

Principal components analysis of 45 rose oils, concretes and an absolute separated the different extraction methods very clearly (Figure 1).

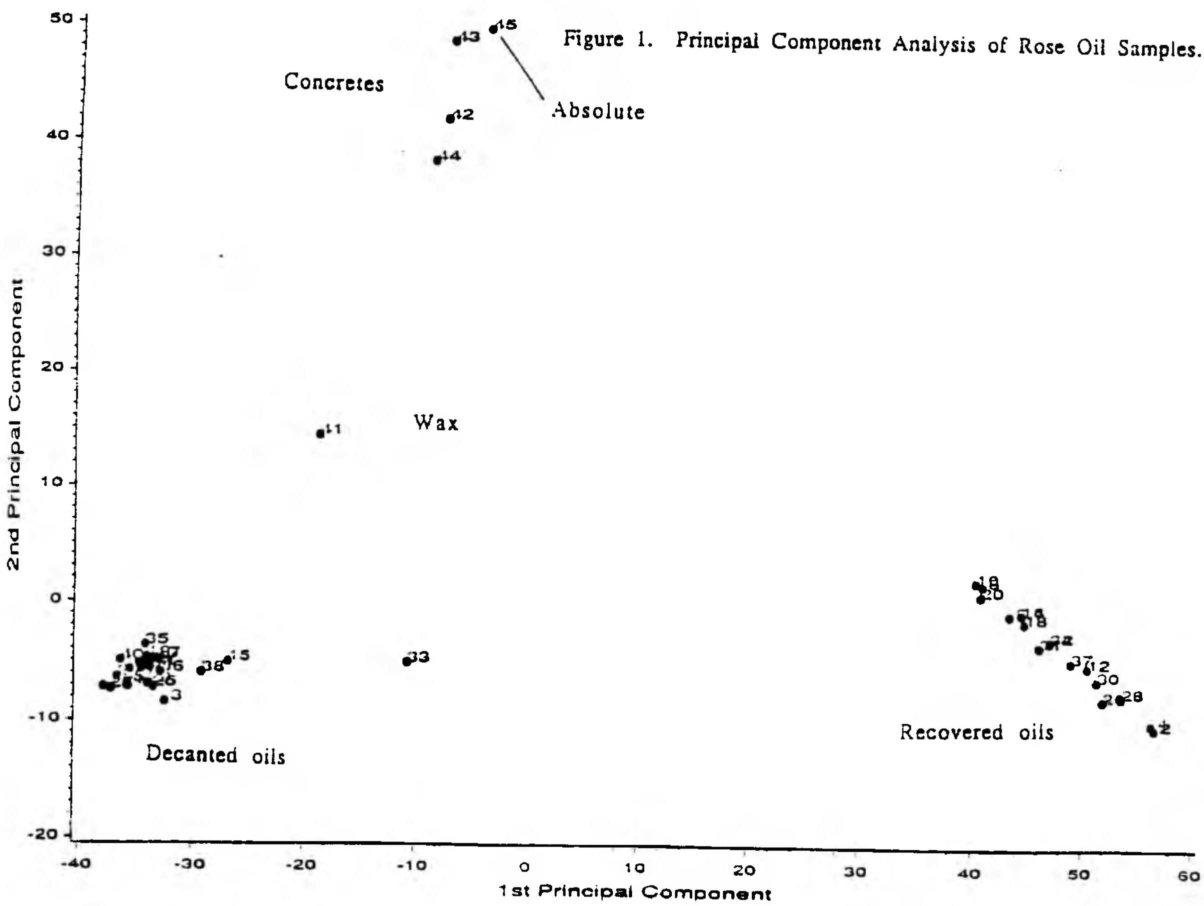

Figure 1. Principal Component Analysis of Rose Oil Samples.

III-6 Some Practical Experience of the Analysis of Glycosidically Bound Volatiles

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In the course of our examination of glycosidically bound volatiles of Lamiaceae we had to solve some difficulties concerning the handling of the glycosidic fractions, eg. the sample clean-up, and the following separation and identification of the glycosides. In principle two different methods were tried and tested:

1. The enzymatic hydrolysis of the glycosidic fraction and identification of the aglycones by means of the GC and GC-MS.

2. The derivation of intact glycosides and subsequent analysis by GC and GC-MS according to common methods in sugar analysis.

Both ways have been described in literature and we intend to demonstrate the bounds of possibility of them. For this purpose results of our investigation into the glycosidic fractions from *T. vulgaris*, *T. praecox* ssp. *arcticus* and *T.* x *citriodorus* will be discussed.

III-7 Extraction of Essential Oils With CO_2

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The use of compressed carbon dioxide to extract essential oils from primary botanicals has been an established practice for ten years. The technology involves high pressures and low temperatures, in an energy efficient process which is environmentally friendly because the source of CO_2 is from a by-product of fermentations, not combustion of fossil fuels.

A comparison of the properties of CO_2 to traditional extraction solvents will be made together with a description of the processes used on an industrial scale.

Comparative studies of the steam distillation and CO_2 extracted essential oils of a selection of oils from the large range available, will be made using free choice odour profiling and standardised fingerprint GLC analysis.

III-8 The Use of Differently Derivatised Cyclodextrins in the GC Separation of Racemic Mixtures of Volatiles

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The behaviour of differently derivatised alpha-, beta- and gamma- cyclodextrins in the separation of racemic mixtures of volatiles are discussed. Columns were prepared following Schurig's method by mixing the derivatised cyclodextrin with OV-1701 or OV-1701-OH terminated. About 150 racemates with widely differing structures were used to test the performance of 2,3,6 permethylated alpha-, beta- and gamma-cyclodextrins and 2,6-dimethyl-3-trifluoro-acetyl alpha-, beta- and gamma-cyclodextrins mixed with OV-1701 or OV-1701-OH terminated in different ratios. Column performances were evaluated through a test mixture containing 10 compounds with highly different structures. The influence of column characteristics (film thickness, cyclodextrin/OV1701 ratio, column length and so on) and operating chromatographic conditions (carrier gas, flow-rate and operative temperatures) on the resolution of racemates is discussed, as on the consistency and reproducibility in column performances over time and under different storing conditions.

IV-1 New Approaches to the Study of Essential Oil Accumulation

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Modern molecular biological techniques provide powerful tools for study of the environmental and developmental factors which control the expression of plant secondary metabolic pathways. The application of these techniques to the genetic manipulation of the expression of such pathways offers exciting opportunities to alter the accumulation of compounds which may an important role in the defence of the plant. These methods are beginning to be applied to the manipulation of the accumulation of individual compounds but problems remain in their application to complex mixtures of compounds such as the essential oils.

An initial problem is the lack of efficient, reproducible methods for the transformation and regeneration of essential oil-producing plant species. More importantly an understanding of biochemical route and enzymology of formation of many flavour components is lacking and most of the important genes coding for enzymes controlling the levels of the flavour compounds have yet to be isolated. Although difficulties remain in each of these areas, progress towards solving some of these problems has been made with a number of essential oil-producing plants. The present paper will review progress towards manipulating secondary metabolism in *in vitro* culture using examples from a number of different systems to illustrate how the complex problems of highly branched pathways might be tackled.

One area in which substantial progress has been made is the development of transformed plant organ cultures which produce essential oils which reflect at least qualitatively the aromatic plant from which they were derived. Transformed organ cultures have the advantage that their formation involves transfer of DNA from an *Agrobacterium*-vector and its integration and expression in the plant genome. The use of such cultures thus facilitates the manipulation of metabolic pathways by allowing integration of genes coding for biosynthetic enzymes into the cells of the culture. The present paper will describe methods for the transformation of *Mentha spp* and the properties of the transformed shoot tissue in relation to growth and monoterpene production.

IV-2 Observations on Essential Oil Distillation in the Laboratory

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The determination of the essential oil content of plant materials as well as the collection of oil for subsequent examination is now a routine laboratory procedure.

It is usually done by water distillation and the use of a specially designed and calibrated apparatus referred to as a 'trap'. This enables the distilled oil to be collected whilst at the same time returning the aqueous 'condensate to the still. Whilst this procedure of 'cohobation' has been practised for centuries by the use of Florentine flasks their application to laboratory bench equipment is relatively recent.

What, at first sight, seems to be a simple process turns out to be more complex and evidence of the difficulties encountered is shown by the large number of different pieces of apparatus which have been proposed for use. Variations in design and principle are very wide and it is not possible to combine all the best features into a single piece of apparatus.

Several of these designs are discussed and the evolution of the current apparatus of the European Pharmacopoeia is traced. A new apparatus is presented which includes several novel features. It can be used for quality control purposes as well as for research and a description of its application and method of use is given.

Clevenger

IV-3 Function of Glandular Hairs in the Labiatae at Different Stages of Plant Development

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The benefits of glandular hairs on young and mature organs of the Labiatae, during the vegetative and reproductive developmental stages of the plant, are discussed. 'Short term' glandular hairs, that start and end secretion rapidly, serve for the protection of young organs. All glandular hairs are produced at an early stage of organ development, and their density is therefore smaller on mature leaves, which had completed their expansion than on younger leaves, which are more vulnerable to predators. Reproductive and vegetative organs, and the various reproductive organs may differ in the location, density, relative distribution of types, and in the composition of the essential oils of their glandular hairs. It is suggested that when flowers are young, they are protected by glandular hairs on their calyx, while at anthesis, glandular hairs on the corolla and stamens, and possibly also on the calyx, serve to attract pollinators.

IV-4 Production of Terpenoids by *in vitro* Cultures of Liverworts

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The accumulation of lower terpenoids is restricted to special accumulation sites, eg. oil cells, oil glands, resin ducts. In undifferentiated tissue cultures such accumulation sites are lacking and as a consequence, lower terpenoids are not found or are only present in minimal amounts. Differentiated cultures do produce lower terpenoids to a certain extent, but these cultures are not easy to handle in large amounts.

Liverworts, one of three classes of bryophytes, contain lower terpenes in oil cells or subcellular structures, the so-called oil bodies. The structure of the respective terpenes found in liverworts is partly the same as in higher plants. In some cases, the terpenes are unique and not known in other plant species; in other cases they are enantiomeric to known structures. Among the hitherto isolated terpenes are quite a number exerting biological activity. However, in many cases it is difficult to collect enough plant material in nature to carry out structure elucidation and especially isolate compounds for biological tests. *In vitro* culture of liverworts may overcome this difficulty. It has been shown that different species can be grown as differentiated plants and undifferentiated cells. In both cases, the terpenes accumulated from field collected plants are the same as those from *in vitro* cultures.

Although in literature it has been reported from *Calypogeia azura* [1] that undifferentiated cultures produce the same amount of terpenes than differentiated ones we have found in our cultures [2,3] that the amount of undifferentiated cultures is less. Some cultures show a fair growth rate with boubling times as low as four days, others have longer doubling times. So far, little work has been done to optimise these cultures.

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IV-5 Mode of Action of Methyl Jasmonate

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Methyl jasmonate is a lipid derived cyclopentanone widely distributed throughout the higher plants. The last decade has seen interest in the biological activities of methyl jasmonate on plant tissues greatly increase. Applied as an exogenous solution methyl jasmonate can induce senescence, growth inhibition, tuberisation, stomatal closure and the synthesis of specific proteins. Many of these properties are in common with abscisic acid (ABA). Methyl jasmonate is also a volatile and several effects attributed to the vapour have been reported recently. Among these is the synthesis of proteinase inhibitors as a means of plant defence and the synthesis of vegetative storage protein to act as a nitrogen sink. All of these effects taken together suggest that methyl jasmonate may be a new form of plant growth regulator. However the mode of action behind these effects to date has been largely unexplored.

The senescence of excised barley cotyledons provides a system to investigate the mode of action of methyl jasmonate vapour. Cotyledons exposed to the vapour rapidly undergo chlorophyll degradation and proteolysis, which are indicative of natural senescence seen in plant tissues. Lipoxygenase activity is also rapidly enhanced within hours of exposure to the vapour. This enzyme has been implicated in the senescence of membranes and is known to have chlorophyll degrading properties. It is also an important enzyme for the biosynthesis of methyl jasmonate. Results indicate that low levels of methyl jasmonate vapour may act as a signal for the onset of senescence and that the regulation of lipoxygenase activity may be involved in the mode of action. Possible explanations for the raised lipoxygenase activity may include enhanced gene expression, direct modulation of enzyme activity by methyl jasmonate or activation of a membrane to provide a pool of fatty acid substrate.

IV-6 Biological Activities of n-Alkylphenols and -catechols from *Plectranthus albidus* BAKER (*Labiatae*)

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Activity guided fractionation of extracts of the title plant and HPLC separation of main fractions showing significant antioxidative activity, yielded a series of novel long-chain alkylphenols and catechols. Their structures have been established by spectroscopic methods and total synthesis, the aliphatic part consisting of even numbered saturated and olefinic 4-(5'-oxo-n-C_{10}-C_{22}) chains [1].

All the compounds are *in vitro* strong antioxidants, the phenols inhibiting the Fe^{2+}-catalysed autoxidation of linoleic acid in the same order of magnitude as α-tocopherol, whereas the effects of the catechols are comparable to the commercial antioxidants BHA and BHT, respectively.

Several compounds are potent inhibitors of enzymes of the arachidonic acid metabolism [2]. The dose-dependent inhibitory effects (IC_{50}) on 5-lipoxygenase (5-LO) and cyclooxygenase (CO) of the most effective ones are in the sub-micromole range (52nM for *(E,E)*-1-(3,4-dihydroxyphenyl)deca-1,3-diene on 5-LO, and 0.36 μM for 1-(3,4-dihydroxyphenyl)octadecane-5-one on CO), hence being more potent than the structurally closely related contact allergens urushiol and card(an)ol (μM). A structure-activity relationship cannot be established yet because the two enzymes show inconsistent effects concerning chain length and the position and number of double bonds.

Furthermore, the potential effects of selected compounds on DNA strand scission are discussed.

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IV-7 Microwave-Assisted Process (MAP): Applications to Dried Spices

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The microwave-assisted process (MAP) has been used to produce extracts from various dried spices. Although MAP was used to produce extracts from fresh herbs, this is the first report ever of MAP extracts from such dried materials. Operating conditions have been developed to obtain extracts identical in nature to currently commercially available products. Novel extracts have also been produced from selected dried spices in order to extend the flavourists' palette. These extracts demonstrate the greater versatility of MAP over conventional extraction processes. Yields have been optimised and the novel extracts have been characterised by gas chromatography/mass spectrometry analysis.

IV-8 Biotransformation of Sesquiterpenoids and a Statistical Approach Towards Intelligent Screening

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Biotransformations have the advantage of proceeding under mild conditions and with high regio- and enantioselectivity without producing toxic wastes. One of the main drawbacks in biotransformation however is the necessity to screen. Up to now a prediction of the most suitable strain or groups of strains for a desired transformation is very vague and depends on the experience of the experimenter. Because of these difficulties often a huge number of strains need to be tested in a screen which can be very time consuming.

Our idea was to group the strains of our collection into groups of similar biotransformation capacity. Such a grouping of strains will strongly reduce the screening effort: For a desired biotransformation a small number of strains of each group will be tested in a pre-screen revealing the most active group in this particular biotransformation. With this information in hand the main screen will be focussed to the members of the most active group. Such a procedure will drastically reduce the screening effort.

Because of the complexity of microorganisms containing a large number of different enzymes a single product (univariate analyses) is not sufficient for the grouping in such an approach, instead many products were used (multivariate analyses). We tested 12 substrates, mainly mono- and sesquiterpenoids, with 100 strains, determined the R_f values of the products and used these data in multivariate analyses.

The analysis revealed that the taxonomic position of the strains is mirrored in their biotransformation capability. Fungi and bacteria could be discerned as well as the Basidiomycotina, Ascomycotina, Deueromycotina and Zygomycotina and Gram-positive and -negative bacteria. A discriminant analysis yielded five groups of strains with distinct biotransformation activity. Such clustering will allow a more efficient screening, entering the field of a real intelligent screening.

I-P1 Novel Essential Oils from Southern Africa

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The Centoil (Ciskei Essential Oils) Project is directed at using the development of local aromatic plant species as a vehicle for rural development. Research attention has been devoted to the identification of promising species, the selection and cloning of desirable chemotypes, the development of field management procedures for the expoitation of the crops and the investigation of potential markets. The poster deals with the following new essential oil crops:

Artemisia afra is the prime medicinal plant in Southern Africa. The oil has an attractive blue green colour and a clean strong "medicinal" fragrance. *A. afra* appears to have potential for use in soaps, perfumes and external medicines. Although thujone is the most abundant component in the oil, both high and low thujone clonal selections have been made.

Eriocephalus punctulatus yields a dark blue oil (chamazulene) with an attractive fruity fragrance. By all accounts, the oil has promise for use in both perfumery and flavouring. Oil production has been very limited as the wild plants are sensitive to excessive defoliation. With the recent development of clonal material and effective harvesting systems it would appear that there are good prospects for producing commercial qualities of oil.

Pteronia incana is an aromatic weed which is invading large areas of pasture land in Ciskei, and from which favourable yields of oil can be obtained. The oil has a fragrance somewhat reminiscent of Juniper berries and could probably be used in industrial perfumes etc.

Salvia stenophylla yields an oil which contains up to 50% of *a*-epi-bisobolol and which may have potential as a component in skin cremes, etc.

Lippia javanica is a well known medicinal herb from which an oil can be extracted that has a high (50%) content of ipsdienone. This compound is closely related to ipsdienol which is one of the pheromone components for European pine bark beetles (*Ips typomophus*). Encouraging results have been achieved where the oil *per se* has been used for the protection of forest logs.

Oil samples will be available for olfactory evaluation.

I-P2 Essential Oils in *Hyptis suaveolins* (L.) Poit. (Lamiaceae) from Bangladesh: Its Medicinal and Pharmacological Significance

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The genus *Hyptis* Jacq. (Lamiaceae) has over 400 species distributed throughout tropical America. Three species including *H. suaveolins* (L.) Poit. a native of tropical America, naturalised in all tropical countries and the only strongly aromatic species from Bangladesh. It grows in dry, open localities, along streams, road sides, coconut plantations, tobacco and rubber estates. This species is listed and considered as a potential anti-cancer agent (Hartley 1969; Mabberley 1990). In Java it is used as a medicinal plant and said to promote lactation in women. In Thailand the tip of the shoots are added to food as a flavouring agent. The leaves are also used as a stimulant, useful against catarrh and skin complaints (Burk 1935). In the Philippines leaves are used as an antiseptic for wounds and skin diseases. The first report of essential oils analysis and identification by GC/MS of *H. suaveolins* from Bangladesh is in the hope to test and find ways of their uses in medicine, biology and food industry. Mechanism of action was studied on *in vitro* guinea-pig ileum preparations to show pharmalogical significance.

II-P3 Structure and Ontogeny of the Peltate Oil Glands of *Monarda citriodora* Var *Citriodora*

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Light microscopy and transmission and low-temperature scanning electron microscopy have been used to study the peltate oil glands of *Monarda citriodora* Cerv. ex Lag. ssp. *citriodora* var. *citriodora*.

Gland morphology is typical of that found in other members of the Labiatae and consists of basal epidermal cells. In young glands a thickened cuticular layer is closely appressed to the secretory cell walls. As glands age a large subcuticular space develops between the cuticular layer and secretory cell walls. Oil accumulates in the sub-cuticular space.

The fine structure of the basal epidermal cells changes little during gland development, remaining highly vacuolate and retaining apparently active cytoplasm. The cytoplasm of the stalk and secretory cells in young glands is characteristic of cells in a highly active metabolic state. In particular, these cells contain a highly developed endoplasmic reticulum, lobed leucoplasts and numerous vesicles of varying size. Under appropriate fixation conditions the vesicles contain electron-opaque material believed to be terpenoid oils. In older glands which have a subcuticular space filled with oil, the cytoplasm of both stalk cells and secretory cells becomes very electron-opaque. Membrane systems swell forming many small vesicles and organelles degenerate, although leucoplasts remain distinguishable.

The peltate glands are formed at an early stage in the development of the leaves. Complete young glands are present on leaf primordia within 100μ of the leaf meristem. The major region of gland development occurs on the edge of leaf primordia approximately $100\text{-}150\mu$ from the meristematic tip. Gland initiation is first evident as a protrusion on the epidermis. At an early stage an anticlinal division bisects the cell in the longitudinal plane. Periclinal division occurs to produce the basal and stalk cells. Further anticlinal divisions then produce the eight secretory cells. The secretory cells appear to flatten into a disc, possibly by radial enlargement. Oil filling need not occur immediately as mature, unfilled glands are present on mature leaves.

II-P4 Herbivore Induced Changes in Leaf Chemistry: Does Herbivory Elicit Disease Resistance in Plants?

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The effect of herbivory on the leaf chemistry of *Myrica gale* (bog myrtle/sweetgale) was investigated. Bog myrtle is an aromatic shrub which grows in temperate wetlands of western Europe and North America. It grows extensively throughout Scotland and is common on Flanders Moss, a raised peat bog west of Stirling in Central Scotland. In summer it rarely suffers vertebrate herbivory but is host to a number of insect herbivores including capsid bugs and tortricid moth caterpillars.

Bog myrtle invests in an array of secondary metabolites including glandular volatile oil and phenolics. In a field experiment a group of plants on Flanders Moss were kept free of insect herbivores and a second set were allowed to sustain natural herbivory. Leaves were collected from both sets on a weekly basis and the surface densities of leaf oil glands were determined. Volatile oils were extracted into pentane and profiled using gas liquid chromatography. Leaf phenolics were extracted into aqueous methanol and profiled with high performance liquid chromatography.

Herbivory became evident towards the end of May and was widespread by the first week in June. Early instars of *Lygocoris spinolai*, a capsid bug, accounted for almost all herbivory observed on the plants under investigation. Herbivory elicited a significant increase in the density of oil glands on wounded leaves although the density on neighbouring undamaged leaves did not change. No effect on the composition of the oil was observed. Herbivory also elicited a qualitative change in the phenolic content of leaves through enhanced production of a single flavonoid, kaempferol-3-(2,3-diacetoxy-4-p-coumaroyl)rhamnoside. There was no evidence that the concentrations of other flavonoids were affected by herbivory.

The volatile oil and the induced flavonoid were tested for fungal growth inhibitory properties using fungi isolated from bog myrtle on Flanders Moss. Both were found to have marked antifungal activity at low concentrations. When tested on the generalist lepidopteran herbivore *Spodoptera littoralis* the flavonoid exhibited no antifeedant or growth restricting properties. Although it is likely that the volatile oil would be toxic to many insects the plant's strategy of increasing gland density on wounded leaves alone suggests that this phenomenon is unlikely to deter further herbivory by *L. spinolai*, the early instars of which are flightless but highly mobile. However, the wounds caused by herbivory are a potential site of fungal infection and any increase in local concentrations of antifungal secondary metabolites would enhance bog myrtle's defenses against pathogens.

These results suggest that herbivore induced changes in leaf chemistry help defend bog myrtle against pathogen challenges associated with herbivore damage.

II-P5 Influence of Environmental Conditions and Organ Development on the Essential Oil Composition from the Flowerheads of Two Populations of *Achillea millefolium* L. ssp. *millefolium*

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In a previous study we analysed the composition of the essential oils of the flowers and of the leaves of *A. millefolium* ssp. *millefolium* growing in the Botanical Garden of Lisbon (BGL) [1]. Later, a different composition of the essential oil of the same subspecies, growing in Canecao Garden of Almada (CGA), was found. Several data have been published in the literature showing that the essential oil composition of the yield are influenced by genetic, climatological and soil conditions, as well as by organ development, method of propagation, and the time of collection of the plant material. Therefore samples of flowerheads of *A. millefolium* ssp. *millefolium* in different stages of development were collected at the same time in both gardens, and the composition of their oils was studied by GC and GC-MS.

Although the monoterpene fraction was dominant in all the oils analysed (*ca* 80%), the main components differed: sabinene and 1,8-cineole were dominant in the oils isolated from the plants growing in the BGL, whereas camphor, ß-pinene and 1,8-cineole were the main components of the oils obtained from CGA. Germacrene-D was the major constituent of the sesquiterpene fraction of all the oils analysed. All of these compounds showed fluctuations in their relative amounts during the flowerheads development. It is noteworthy that chamazulene was only detected in the oils from the flowerheads collected in CGA, its amount decreasing throughout the flowerheads development.

According to Maffei *et al.* [2], the presence or absence of several compounds, and their relative amounts, in the essential oils of *Achillea* depend on genetic and environmental factors. Since the plants growing in the CGA were obtained from those growing in BGL, and thus were genetically similar, analyses of soil composition and meteorological data from both gardens, at the time of harvest, were obtained, in order to examine whether the differences between these oils could be related to climate or soil conditions. The meteorological data did not reveal significant differences in temperature, humidity and global radiation with regard to both gardens. Only the insolation was four hours longer in the CGA than in BGL. The analysis of the composition of the soils from the two gardens revealed that both soils were sandy-loam, but BGL had higher levels of $CaCO_3$. Generally the soil of CGA was richer in cations readily usable for exchange (Ca, Mg, K and Na), and had higher levels of assimilable P and K. The amounts of total nitrogen and carbon were not very different between the two soils.Considering that other authors reported that changes in oil composition and yield can be due to climate and edaphic conditions, it is likely that a correlation does exist between the differences found in the composition of the essential oils analysed and the levels of the different nutrients in the soil and the number of insolation.

The results of this study indicate that some caution should be taken when chemical data are used for systematic purposes within the *A. millefolium* aggregate, not only because differences can be found within the same population owing to different stages of otgan development, but also because differences can exist between populations due to environmental conditions.

References and Acknowledgements:
This study was granted by Junta Nacional de Investigaçao Cientifica (Invotan)
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II-P6 Season Influence on the Chemical Composition of Various Chemotypes of Niaouli Essential Oils From Madagascar

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Fifty trees of niaouli (*Melaleuca quinquenervia*) have been chosen for following the range of variation in the chemical composition of the essential oils during three seasons (March, July and December). Among the forty seven compounds characterised by GC/MS and relative retention times, twenty four have been retained for the characterization of various chemotypes. The chemical composition of 150 samples have been used in an attempt of chemotaxonomic classification using Principal Component Analysis and Factorial Discriminant Analysis. Four chemotypes have been observed:

- a cineole type
- a cineole + Delta-3 carene type
- a viridifloral type
- a nerolidol type

No significant change in chemical composition was detected for these chemotypes during the three seasons investigated.

II-P7 Essential Oils and Cold Stress in *Rosmarinus officinalis* L.

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Essential oils were distilled during winter 1991-92 from *Rosmarinus officinalis* L. plants growing in experimental plots of the Botanical Gardens of the University of Turin. Oils were analysed by means of gas chromatography and the quantitative and qualitative composition of several monoterpenes and sesquiterpenes was estimated. Temperatures were detected and correlated with oil content and composition. Figure 1 shows a comparison of oil content and temperatures, while Figure 2 depicts the variations in borneol, bornyl acetate and camphor (the main monoterpenes) along with limonene and germacrene D (the main sesquiterpene); in both figures the linear regression of temperatures is also shown. The data was also statistically processed by using multivariate methods of analysis (Cluster analysis and Principal Component Analysis).

The results obtained indicated that borneol and bornyl acetate were particularly affected by low temperatures. On the other hand limonene and germacrene D showed their maxima when temperatures were low. Camphor did not show any particular correlation with temperature variations. The poster discusses also the biochemical relationships among borneols and camphor.

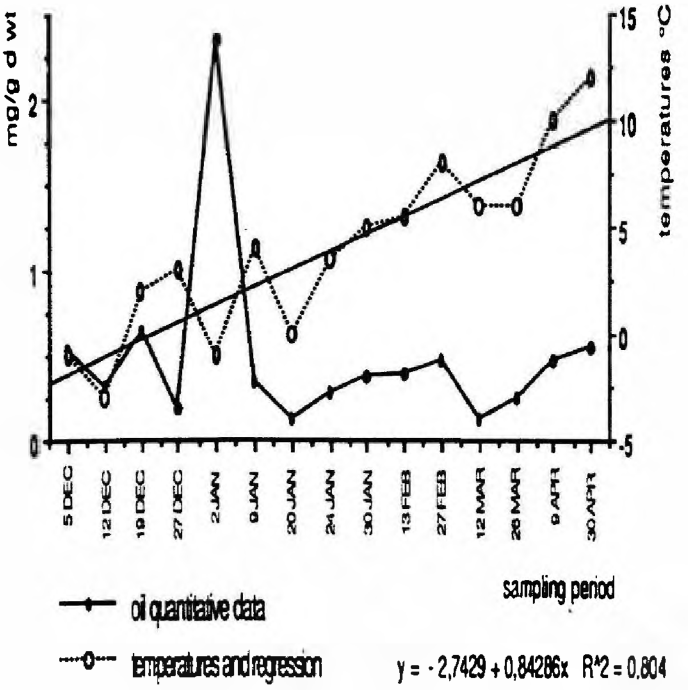

Figure 1

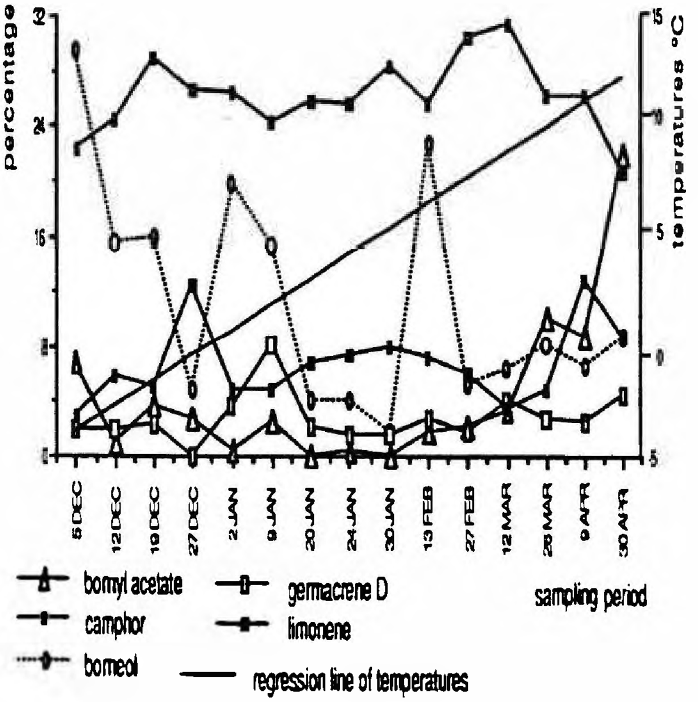

Figure 2

II-P8 Relationship between Canopy Temperature of Peppermint (*Menta piperita* L.), Irrigation Requirements, Some Physiological and Phenological Data

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In Bulgaria peppermint (*Menta piperita* L.) must be grown under irrigation if high yields of oil and leaves are to be realised. It was found that the highest yield was obtained when drip surface irrigation or drip subsurface irrigation was used. Both of these irrigation practices were found to conserve water to a greater extent than the sprinkler irrigation. Field experiments were conducted at Kazanlak during 1989-1990 to investigate the usefulness of canopy temperatures in determining water use. Measurements were made by hand-held infrared thermometer Raynger II daily between 1330h and 1400h. Three treatments were investigated as follows:

1. Drip surface, the soil moisture was maintained at 100% of field capacity.
2. Drip surface, the soil moisture was maintained at 70% of field capacity.
3. Non irrigated - under water stress.

Diurnal measurements of canopy and air temperatures indicated that the greatest differences between canopy and air temperatures occurred near solar noon. Canopy temperature differences between water stress and fully irrigated crops up to 8.5°C were measured. Seasonal water use was estimated *via* a soil water budget approach. Global radiation, net radiation, air temperature, relative humidity and wind speed were measured simultaneously. The soil temperatures at 0730h, 1430h and 2130h every day on the surface and 5cm into the soil were recorded.

II-P9 Effects of Different Growing Conditions on Fennel and Peppermint Essential Oil Composition and Biological Activity

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Several essential oils from aromatic plants showed an inhibitory action against bacteria and fungi [1,2]. Biological activity of the oils is often due to a synergistic action among the components [3]. Growing and environmental conditions and ontogenetic developments influence the biosynthetic pathway of the oil constituents [4].

In this research the essential oils obtained by steam distillation from three varieties of fennel (*Foeniculum vulgare* Mill.) harvested at different ontogenetic stages and from Italian peppermint (*Mentha* x *piperita* L.) grown on different fertilisation conditions and collected from two planting times (Autumn and Spring) were tested against several micro-organisms and for their anti-oxidative properties.

Quali-quantitative composition of oils was determined by GC/MS. The fennel oils showed relevant differences in the quantitative composition and their antibacterial activity was more effective against *Brocothrix thermosphacta, Staphylococcus aureus* and *Alcaligenes faecalis* while against *Enterobacter aerogenes, Micrococcus luteus* and *Moraxella sp.* was not exhibited.

Peppermint oils obtained from Autumn planting showed higher biological activity than those from Spring.

All peppermint oils had great effects against *Aspergillus niger* (inhibition from 86 to 99%).

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II-P10 Effect of Mechanical and Chemical Weed Control on the Growth, Development and Productivity of *Mentha* Grown for Planting Material

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Three regimes for weed control were tested in field experiments: mechanical weed control (three tillages); chemical weed control (Sinbar NP80 at 200 g/dha); and combined weed control (Sinbar NP80 at 200 g/dha and one hoeing). The investigation was carried out with two species - clone N1 from *Mentha piperita* Huds and cv.Mentolna-14 from *Mentha arvensis* L.

It was established that in both species the composition and chatacter of essential oil was not significantly affected by the method of weed control. Mechanical weed control provides the highest growth indices and the longest rhizomes.

II-P11 Comparison between Three Methods of Propagation of the Mint and their Effect on the Yield of Fresh Material and Essential Oil

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Three methods were compared for the propagation of the mint: with rooted cuttings in the autumn; with rhizomes in the autumn; and with rhizomes in the summer. For the test material we used clone N1, cv. Tundzha and cv. Zephir of *Mentha piperita* Huds; cv. Mentolna-14 and cv. Mentolna-18 of *Mentha arvensis*.

The tested cultivars produce the highest yield of fresh material when propagated *via* rooted cuttings, except for clone N101. The highest yields of essential oil are obtained from cv. Zephir, Mentolna-14 and Mentolna-18 following propagation from rooted cuttings; from clone N1 and clone N101 with rhizome propagation in the autumn; and from cv. Tundzha - with rhizome propagation in the summer.

II-P12 Effect of the Time and the Density of Planting of Rooted Mint Cuttings on the Yield of Fresh Material and Essential Oil

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The optimum values were established for the factors time and density of planting under a new method of propagation for mint, using rooted cuttings taken in the autumn. The experimental materials used were clone N1 and cv. Zephir from *Mentha piperita* Huds. and cv. Mentolna from *Mentha arvensis* L.

It was established that the tested cultivars respond extremely well to propagation *via* rooted cuttings in the autumn and at a planting density of only 3 plants/m^2 they provide normal density for the plantation during the following year. The yields of fresh material and essential oils are enhanced by increasing the density of planting and when the planting is done at the start of September.

II-P13 Productivity of *Mentha* Grown with Increasing Levels of Fertiliser

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In field experiments we have studied the vegetative behaviour and productivity of *Mentha*, fertilised with levels of fertiliser previously calculated to give variation of yields of essential oil. As test material we have used cv. Tundzha, cv. Zephir and clone N1 of *Mentha piperita* Huds and cv. Mentolna-18 of *Mentha arvensis* L. The growth parameters investigated include plant height, number of branches, percentage of foliage, and the content, yield and chemical composition of the essential oil. With increasing rates of fertiliser, the yield of essential oil is increased by 16 to 119% relative to the control plants. The highest yield of essential oil has been obtained from the cv. Zephir.

III-P14 Essential Oil Composition of Two Endemic Madeira Island *Teucrium* Species: *Teucrium abutiloides* L'Hér. and *Teucrium betonicum* L'Hér

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The genus *Teucrium* comprises more than 300 species, 49 of which are found in Europe, mostly in the Mediterranean basin [1,2]. Many species are currently used in folk medicine. A great number of clerodane-type diterpenoids have been isolated from *Teucrium* species, some showing an insect antifeedant activity [3,4]. Many flavonoids have also been isolated from *Teucrium* species, and some of them have proved to be useful taxonomic markers [5]. Studies concerning the volatile constituents of *Teucrium* species are scarce [2,6,7]. In this paper we report on the composition of the volatile oils isolated from aerial parts of *Teucrium abutiloides* and of *Teucrium betonicum*, respectively.

The essential oils of these two species were obtained in almost similar yields (<0.5%). The oils were complex mixtures consisting mainly of octen-3-ol (20% in the oil of *T. abutiloides* and 24% in that of *T. betonicum*). The monoterpene fraction was relatively poor in both oils: it represented 6% of the total oil of *T. betonicum* and contained merely linalol; in the oil of *T. abutiloides* all monoterpenes appeared in a concentration less than 1%, linalol being the most important one (0.7%). The sesquiterpene hydrocarbons formed the main fraction in both oils, amounting to 45% in the oil of *T. betonicum* and to 55% in that of *T. abutiloides*. However, the major component of these fractions differed: ß-caryophyllene was the most abundant sesquiterpene in the oil of *T. betonicum* (12%), whereas germacrene-D (13%) and δ-cadinene (11%) were the dominant sesquiterpenes in the oil of *T. abutiloides*. The oxygen-containing sesquiterpenes represented also an important fraction in the oil of *T. abutiloides* (13%), whereas in that of *T. betonicum* they represented 5% only. The most characteristic feature of the composition of the essential oils of the *Teucrium* species we analysed is the high amount of octen-3-ol. This compound has only been found in traces in the essential oils of other *Teucrium* species [2].

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III-P15 Chemotaxonomy of Commercial Buchu Species (*Agathosma betulina* and *Agathosma crenulata*)

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Two species of Buchu, *Agathosma betulina* and *A. crenulata*, which are endemic to the Western Cape region of South Africa, are commercially exploited. The Buchu oil is used for both perfumery and flavouring, with various users showing marked preferences for specific oil types. Although the exact usages for the different qualities of Buchu oil are unclear it is generally understood that the diosphenol component is used to develop mint-like notes, whereas the complex of thiols (8-Mercapto-*p*-menthan-3-one and 8-Acetylthio-*p*-menthan-3-one) are used for the "catty" note in flavouring.

Extensive hybridisation occurs between these two species thus complicating the already difficult identification of the species. Previous work has shown that the chemical composition may be used to separate these species and their hybrids.

From the analysis of 50 Buchu specimens randomly collected from widely dispersed communities it was found that:

1. Individual plants having high 8-Mercapto-*p*-menthan-3-one and 8-Acetylthio-*p*-menthan-3-one were identified.

2. There is very little correlation between chemical composition and geographical location of the taxa. Though there are some generalisations: The low diosphenol plants are found in the central localities, and the high 8-Mercapto-*p*-menthan-3-one plants in the south.

3. Extensive hybridisation appears to occur between *A. betulina* and *A. crenulata* with the hybrids possessing morphological and chemical attributes of both species.

4. The three taxa can be clearly separated on the basis of the percentage content of pulegone, and interspecific differences on the basis of diosphenol, menthone, isomenthone and 8-Acetylthio-*p*-menthan-3-noe are less clear. It can be concluded that *A. crenulata* does not contain diosphenol and *A. betulina* contains little pulegone.

5. The specimens studied were classified into 5 distinct chemotypes. There are three *A. betulina* chemotypes, based on the percentage of diosphenol. The hybrids have two chemotypes based on high diosphenol, high menthone plus isomenthone.

III-P16 Chiral Constituents of Essential Oils Analysis by Enantioselective Gas Chromatography using Cyclodextrin Derivatives

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Essential oils are known to contain numerous chiral constituents. Owing to chirality the contribution of the enantiomers to odour and taste may be different. Fragrances and flavours are perceived through a direct interaction of chemical constituents with specific chiral receptors.

Previous investigations have already shown that the enantiomeric composition of chiral constituents of essential oils may vary considerably depending on origin and processing. It is also well known that essential oils may be adulterated by addition of other natural or synthetic compounds. The determination of enantiomeric composition has become possible with enantioselective gas chromatography.

After the introduction of hydrophobic cyclodextrin derivatives in gas-liquid chromatography in 1988 [1], now, only four years later, it is possible to investigate the enantiomers of almost the complete spectrum of substances of olfactorial importance [2,3].

We have developed a number of selectively substituted cyclodextrins with unique properties for special purposes:

- Monoterpene hydrocarbons are the most volatile constituents of essential oils. All important chiral and non-chiral natural monoterpene hydrocarbons, such as α-thujene, α- and ß-pinene, δ-3-carene, sabinene, camphene, α- and ß-phellandrene and limonene can be resolved with a dual column system applying two complementary cyclodextrin stationary phases heptakis(6-O-methyl-2,3-di-O-pentyl)-ß- and octakis(6-O-methyl-2,3-di-O-pentyl)-gamma-cyclodextrin [4].

The proportions of monoterpene hydrocarbons in some selected essential oils were investigated and were found to be very similar in oils of different origin.

- Many terpene alcohols can be investigated using modified cyclodextrins: Lavandulol, linalool, borneol, isoborneol, hotrienol, nerolidol α-terpineol, terpinen-4-ol etc [5]. (-)-α-Bisabolol is a major constituent of chamomile oil. With recently developed cyclodextrin derivatives (heptakis(2,6-di-O-methyl-3-O-pentyl)-ß- and octakis(2,6-di-O-methyl-3-O-pentyl)-gamma-cyclodextrin) all four stereoisomers can be separated. Even muscone (3-methyl cyclopentadecanone), a rather bulky ketone contained in musk as the (-)-enantiomer [6] is separated with one of these new phases for the first time.

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III-P17 Chemical Composition of the Essential Oil from the Rhizomes of *Pavonia odorata* Willd.

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Pavonia odorata Willd., belonging to family Malvaceae, is a pubescent herb found in open woods, waste places and gardens in many parts of India. The plant is reported as a cure for rheumatism in Indian system of medicine. The yellow coloured essential oil obtained from the dried crushed rhizomes of *P. odorata* in a yield of 0.2%, has been analysed using chemical, chromatographic and spectroscopic methods. Thirty-nine components viz. α-pinene, camphene, ß-pinene, sabinene, myrcene, δ^3-carene, α-terpinene, p-cymene, limonene, ocimene, gamma-terpinene, α-terpinolene, gamma-terpinolene, linalool, α-terpineol, -terpineol, l-borneol, decanol, methyl heptenone, fenchone, furfural, isovaleraldehyde, isovaleric acid, n-caproic acid, linalyl acetate, bornyl acetate, ß-acryophyllene, cedrene, ß-salinene, aromadendrene, ß-fernesene, α-bisabolene, gamma-murrolene, α-murrolene, δ-cadinene, α-elemene, cedrol, ferneseol and eudesmol could be identified in the oil.

Thirty-one new constituents have been identified which were not reported in the earlier investigations. Many minor constituents consisting 12.60% in the oil, however, remained uncharacterised. The oil has been found to be a rich source of isovaleric acid (34.66%).

III-P18 Volatile Constituents of the Wood-Rotting Fungus *Inonotus obliquus*

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The wood-rotting fungus *Inonotus obliquus* (Pers. ex Fr. Pilat) (*Basidiomycetes*) is encountered on living trees as fruitbodies and as sterile conks which have a hard woody to almost stony texture. According to our earlier investigations the fungal mycelium *in vitro* was able to produce the same lanosterol type triterpenes as the natural fungus. Volatile constituents, however, can have a very different pattern [1]. The aim of this study has been to compare the occurrence of certain volatiles between the natural and cultivated fungus.

The fungus was collected from the trunk of birch (*Betula*) and the fungal mycelia were cultivated in liquid cultures. The volatile oils were isolated by hydrodistillation and the constituents were studied by GC and GC-MS analysis. Identification of the constituents was based on the library spectra and MS literature data.

The results showed that the main identified volatiles in the natural *I. obliquus* were benzaldehyde, *Z*-α- and *E*-ß- bergamotenes, linalool, α- and ß-santalenes, ß-selinene, eudesmol and farnesene isomers, α-bisabolol and free fatty acids. Contrary to the lanosterol type triterpenes of natural fungus, the composition of the volatiles of cultivated mycelium were quite different. The oil from cultivated mycelium was characterised by some specific compounds such as *ar*-turmerone and bisabololoxide-B and the compounds such as p-cymene, α-cedrene and *ar*-curcumene. However, both oils contained farnesene isomers and free fatty acids (C_{14}-C_{18}). The main fatty acids were palmitic and linoleic acid. These results are biosynthetically interesting since some ingredients in cultures can stimulate or inhibit the formation of volatiles. This study also confirms that fungal cultures can produce a great variety of volatile terpenoids which are not present in the natural fungus.

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III-P19 Constituents of the Essential Oil from *Satureja cilicica* P.H. Davis

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Plants belonging to the *Satureja* genus of the Lamiaceae family are mainly distributed in Eurasia. Some species of these plants are well known as a culinary herb, and also used medicinally. In chemotaxonomic studies of numerous species, several chemotypes are frequently noted [1,2].

Fourteen *Satureja* species are growing wild in Anatolia. *Satureja cilicia* P.H. Davis is an endemic species which is native to south Anatolia. The composition of the oil from *Satureja cilicica* has not been investigated so far. In this study the chemical composition of the essential oil of *Satureja cilicica*, collected in August 1991 near Içel (southern Turkey), was examined. The amount of the oil was determined on hydrodistillation and its composition was investigated by combined LSC and GLC (on capillary columns) [3].

The content of the essential oil was 1.6%. Carvacrol was the main component (63.71%) of the oil.

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III-P20 Comparative Studies on the Composition of the Essential Oils of Two Subspecies of *Skimmia japonica* Thunb.

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Skimmia species which originate in East Asia and the Himalayas are the most important hardy, woody *Rutaceae* cultivated for ornament in Europe. The most common *Skimmia japonica* Thunb. is to be found in many parks and botanical gardens, where it is valued as a flowering evergreen.

In the course of our studies on plants belonging to *Rutaceae* we have investigated the aerial parts of *Skimmia japonica* ssp. *japonica* and *S. japonica* ssp. *reevesiana* with respect to its essential oils by CC, GC and GC-MS.

In contrast to the ssp. *reevesiana* which yields an oil rich in monoterpene hydrocarbons (up to 80%), oils from the ssp. *japonica* contain appoximately 70% oxygenated monoterpenoids, mainly alcohols and the respective esters.

In leaves and stems of the ssp. *reevesiana* we found the oil to contain ß-phellandrene (52%), α-pinene (13.9%), myrcene (5.8%) and limonene (5.95%) as main hydrocarbons. Oxygenated monoterpenoids which are prominent constituents of the oil of the ssp. *japonica* are only detectable in small amounts: linalool (1.9%), linalyl acetate (1.7%), α-terpineol (0.9%), neryl acetate (1.1%), geranyl acetate (2.7%).

The essential oil of *Skimmia japonica* ssp. *japonica* contains mainly oxygenated monoterpenoids above all linalool (33%) and linalyl acetate (27%). Additionally α-terpineol (4.9%), nerol (1.5%), geraniol (4.2%) and their acetates (α-terpinyl acetate (1.2%), neryl acetate (1.8%), geranyl acetate (4.3%)) have been identified. Within the hydrocarbons only ß-phellandrene appears in higher amounts (16%) followed by α-pinene (5.5%), myrcene (4.9%) and limonene (2.5%).

III-P21 Comparison of the Essential Oil Composition of Two Subspecies of *Satureja montana*

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Satureja montana L. ssp. *montana* L. and *Satureja montana* ssp. *variegata* (Host) P.W. Ball (Wettst.) are two of three subspecies of *Satureja montana* L. found in the Dinaridic Karst, from 150m to 1200m above sea level. Both plants are used in folk medicine as an anthelmintic, diuretic, nervinum and stimulant. The aim of this work was to compare the essential oil composition of these subspecies.

The aerial parts of the plants were subjected to hydrodistillation for 3 hours in a Clevenger-type apparatus. The obtained essential oils were investigated by means of capillary gas chromatography.

Table 1. Yield of some oil samples and their main components.

Plant Species	Oil Yield (% v/w)	Major components (% of total oil)	
Satureja montana ssp. *variegata*		Thymol	Carvacrol
a) Sinj [a]	0.66 - 0.90	0.58, 2.84	83.84, 84.19
b) Livno [b]	0.90 - 1.00	1.03, 2.31	63.54, 5.58
Satureja montana ssp. *montana*			
a) Ljubuski [b]	1.00 - 1.20	2.56	57.24
b) Vrgorac [b]	0.66 - 0.95	1.05	60.93
c) "Dalmacijabilje"	1.47	13.61	30.83
Commercial sample	-	33.84	24.88

a = before flowering, b = flowering plant
Interpretation of complete chromatograms obtained during this study will also be discussed.

III-P22 The Essential Oils of Two Iberian *Teucrium* Species

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The volatile oils from two *Teucrium* species, *Teucrium valentinum* and *Teucrium gnaphalodes*, endemic to Spain have been investigated here from the first time by means of GC/MS.

Both species were submitted to steam distillation and were collected at two different times (6 and 12 hours). The four samples obtained were separately analysed.

The percentage of sesquiterpenes increased with distillation time. In *Teucrium valentinum* the amount of monterpenes was always greater than those of sesquiterpenes (83.85 -16.15% and 56.81 - 43.19%). Conversely, *Teucrium gnaphalodes* produces a larger percentage of monoterpenes at 6 hours (68.39 - 31.64%) and more sesquiterpenes at 12 hours (27.58 -72.43%).

The essential oil of *Teucrium valentinum* was shown to contain α-pinene (15.8 - 4.6%), ß-pinene (11.7 - 2.1%), sabinene (7.2 - 1.5%), terpinen-4-ol (4.5 -2.3%), thujanol (4.3 - 2.9%), p-cymene (4.3 - 1.8%), limonene (3.4 - 0.9%) and linalool (2.7 - 0.7%) as main monterpenes constituents and ß-bisabolene (2.5 - 4.9%), elemene (1.1 - 3.1%) and δ-cadinene (0.4 - 1.1%) among the sesquiterpenes.

The volatile oil of *Teucrium gnaphalodes* was characterised by a high percentage of sesquiterpenes, mainly trans-ß-caryophyllene (12.1 - 25.8%), δ-cadinene (4.12 - 6.0%), caryophyllene epoxyde (3.5 - 7.2%), α-humulene (3.4 - 6.7%) and humulene epoxyde (0.8 - 1.3%). The major monoterpene compounds were thujanol isomer II (11.6 - 2.4%), sabinene (8.1 - 0.7%), ß-pinene (7.1 - 2.3%), myrtenal (6.7 - 4.6%), terpinen-4-ol (5.0 - 1.6%), thujanol isomer I (4.3 - 2.0%) and limonene (3.1 - 0.8%).

III-P23 Comparative Study of Volatile Components of Eleven *Aframomum* Species from Cameroon

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In continuation of our research program on the screening of the essential oils from aromatic plants of tropical Africa, we have undertaken a systematic study of the different species of the genus *Aframomum* growing in Cameroon.

The essential oils from seeds (S) of the eleven species of *Aframomum* have been extracted and analysed; for most of the species, the leaf (L) oil has also been studied. The species examined so far are: *A. albovialaceum* (S and L), *A. chlamydantum* (S and L), *A. citratum* (S), *A. daniellii* (S and L), *A. kayserianum* (S and L), *A. melegueta* (S), *A. polyanthum* (S and L), *A. pruinosum* (S and L), *A. sanguineum* (L), *A. subsericeum* (S and L) and *A. sulcatum* (S).

All the oils were analysed by capillary gas chromatography and GC/MS. Concerning the yield in essential oil, it appears that the seeds are much richer (0.8 - 4.8%) in volatile components than the leaves (0.2 - 2.3%).

The compositions of the oils are very different from species to species; within the same species, the leaf and seed oils may also differ.

However, some compounds are present in almost all the samples examined, although in different percentages: for example, α- and ß- pinenes, 1,8-cineole, ß-caryophyllene, α-humulene and their epoxides can be found in all the essential oils.

These oils can be roughly arranged in 3 classes: those rich in *monoterpenoids* (8 oils), those rich in *sesquiterpenoids* (7 oils) and those containing comparable amounts of both (3 oils).

In most cases, there is no predominant compound in the oils. We have, however, found 4 oils which seem to us particularly interesting in that they contain very large amounts of a single component:

- *A. albovialaceum* (L)	: 1,8-cineole (72.8%)
- *A. citratum* (S)	: geraniol (68.9%)
- *A. polyanthum* (S)	: α- and ß- selinenes (77.8%)
- *A. pruinosum* (S)	: (-)t-nerolidol (91.7%)

The detailed chemical compositions of these four oils will be given and their potential economic interest briefly discussed.

III-P24 The Essential Oils of Two Endemic Portuguese Thyme Species: *Thymus capitellatus* Hoffmans. & Link and *T. lotocephalus* G. Lopez & R. Morales

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Thyme is the common name of many species, hybrids, varieties and ecotypes belonging to the genus *Thymus* [1]. The essential oils of different *Thymus* species have been extensively studied, as is clearly shown by a recent review article of Stahl-Biskup [2], and several chemotypes have been defined for thyme oils. *T. capitellatus* and *T. lotocephalus* are two vulnerable endemic Portugese thyme species with special ecological niches. In the first reports on the composition of their essential oils Fermandes Costa [3] found 30% cineole and 30% alcohols for *T. capitellatus*, and 60% cineole and 12% alcohols for *T. lotocephalus*. According to Palhinha [4], the latter species contained linalyl acetate in addition to cineole. Recently, Salgueiro described three chemotypes of Portuguese *T. capitellatus*, two of which containing large amounts of 1,8-cineole [5]. In the oils isolated from a number of plants of this species, the percentage of 1,8-cineole varied from 25% to 59%. In the same paper [5], the essential oil of *T. lotocephalus* was described to consist of large amounts of 1,8-cineole, camphor, linalol, linalyl acetate and α-pinene, but more precise data on this species were not given.

In this study, we analysed the essential oils from flowers of *T. capitellatus* and *T. lotocephalus* using GC and GC-MS. In addition the oils from the leaves collected during the vegetative phase of these species were investigated. The essential oils of *T. capitellatus*, isolated either from flowers or from leaves of the plant during its vegetative phase, showed a more or less similar composition, but the corresponding oils from *T. lotocephalus* showed marked differences. The oils of both species consisted mainly of oxygen-containing monoterpenes (55-69% of the total oils). 1,8-cineole (50-56%) was the main component of the oils of *T. capitellatus*, both in the oil from its flowers and in that from its leaves. The oil isolated from the flowers of *T. lotocephalus* was dominated by linalyl acetate (23%); the other major components were linalol (11%) and 1,8-cineole (10%). However, in the oil from the leaves of this species 1,8-cineole (24%) was again the major component, while linalyl acetate and linalol amounted to 5% and 6% respectively. Sesquiterpenes were more important in the oils of *T. lotocephalus* (15-17%) than in those of *T. capitellatus* (3-4%). The presence of 1,8-cineole, in relatively large amounts, in the oils analysed was in agreement with the earlier finding of this compound as a major component of other Iberian thyme oils. However, the oil isolated from flowers of *T. lotocephalus* must be considered as being characterised by linalyl acetate/linalol instead of by 1,8-cineole.

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III-P25 Identification of Elecampane Essential Oil (*Inula heleniul* L.) Quantization of Bacteriostatic and Fungistatic Activities

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Elecampane (*Inula helenium* L.) is a perennial plant of humid meadows and shaded areas of Central Europe. Roots hydrodistillation give 1 to 3% of essential oil which is solid at room temperature. The content of this essential oil is mainly made of sesquiterpenoid lactones mixture (88%), endesma 5,11 (13) -diene-8ß, 12-olide being the main isomer.

No analysis of the total essential oil has been published up to now. Previous works give the structure of the main constituents (7 alantolactones isomers).

Our study discloses 92% of volatile compounds: 7 other constituents have been identified for the first time in this essential oil.

Investigation on the use of natural original molecules in field of stabilizers, antiseptics or pesticides brought us to carry out microbiological tests on the global essential oils as well as on resinoid.

Microbiological tests are done on Petri boxes (solid medium) and microplates (liquid medium) on 17 animal and vegetable (bacteria, yeast, mould) pathogen strains. The activity levels (minimum inhibitory concentration) are found between 62.5 and 4000ppm according to the strains and are comparable to oils or constituents known to be active like *Eucalyptus globulus*, *Thymus vulgaris*, Eugenol, Citral, etc.

The different contents of sesquiterpenoid lactones of the extracts will be correlated to bio-static variations.

III-P26 A Chemotaxonomic Hybrid of *Thymus* from Portugal

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Thymus x viciosi (Pau) Morales (= *Th. bracteatus* fma. *viciosoi* Pau; *Th. variabilis* Hoffmanss. & Link) is an hybrid of *Th. pulegioides* L. and *Th. zygis* L. subsp. *zygis*, which was not known from Portugal yet and was collected in the NE mountainous region (Serra de Nogueira).

It has morphological intermediate features between the parents such as hairy stems only on the angles and globular glands on the leaves, which are typical features of *Th. pulegioides*, or leaves revolute, calyx 3-4mm long with the upper teeth up to 1mm, not ciliate, and the calyx-tube 1.5-2mm long which are *Th. zygis* subsp. *zygis* features.

Its essential oil has also an intermediate composition between the parent ones. In that region, the *Th. pulegioides* essential oil is of the thymol chemotype and the *Th. zygis* subsp. *zygis* one is of the carvacrol chemotype. The essential oil of the hybrid is a thymol-carvacrol chemotype.

III-P27 Composition of the Essential Oil of *Origanum minutiflorum* O. Schwarz, P.H. Davis

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Many species of the Lamiaceae are aromatic, and are often used as herbs, spices, folk medicines and as a source of fragrance. One of them is Origanum species and it has been used as seasoning and medicine since antiquity, mainly because of their content of essential oil.

21 species of Origanum grow wild in Turkey. One of them is *Origanum minutiflorum* O. Schwarz, P.H. Davis, which is endemic in southern Turkey [1]. The plant material was collected on July 1991 from Kemer (Antalya) at 1500m. The essential oil was obtained by hydrodistillation, 3 h, in a Clevenger apparatus. The yield was 5.3% based on dry material.

The analysis of the essential oils were done by using LSC and GLC techniques. The oil was submitted to LSC over silica using n-pentane and ethyl acetate as eluents, yielding the hydrocarbons and the oxygenated components respectively [2]. These fractions were analysed by GLC with capillary columns.

The essential oil is characterised by the high content of carvacrol (95.77%).

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[1] Ietswaart, J.H., A taxonomic revision of the genus Origanum (Labiatae), The Hague/Boston/London 1980, Leiden University Press.
[2] Sarer, E., Kökdil, G., Planta Medica, 57 (1), 89-90, 1991.

III-P28 The Essential Oil of *Levisticum officinale* Koch

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Levisticum officinale Koch (Apiaceae = Umbelliferae) cultivated plants from the Botanical Gardens of the Komarov Botanical Institute. Fresh leaves were steam distilled to produce an oil in 0.16% (1) and 0.26% (2) yield. Two modified receivers of oil were used:

1. Ginzberg's apparatus and 2. receiver with forced cooling.

The oil was found to be a pale yellowish liquid with distinctive spicy odour.

The quantitative amounts of the identified principal components of the leaf oil are as follows: 1. myrcene (1.2%), limonene with 1,8-cineole (9.5%), ß-terpineol (1.0%), terpinene-4-ole (1.7%), α- and gamma-terpineol (3.2%), anethole (2.8%), eugenol with terpinilacetates (70.0%), methyleugenol (0.5%), longifolene (1.5%), apiole (0.9%), and iseolemicine (7.6%).

2. α-pinene (0.6%), ß-pinene with sabinene (1.2%), myrcene (4.2%), α-phellandrene (0.4%), p-cymene with α-terpinene (0.4%), limonene with 1,8-cineole (18.6%), gamma-terpinene (1.4%), terpinolene (0.4%), ß-terpineol with menthone (0.3%), pinocamphone with isomenthone (0.2%), $C_{10}H_{18}O$ (0.4%), terpinene-4-ole (2.8%), α- and gamma-terpineol (2.8%), anethole (1.0%), eugenol with terpinilacetates (60.0%), methyleugenole (0.4%), longifolene (0.8%), caryophyllene (0.6%), apiole (0.8%) and isoelemicine (2.0%).

The results of the identification of the components is based on the GC and GC/MS data in combination with their partition coefficients in the hexane/acetonitrile system.

III-P29 Essential Oil of *Cyclotrichium niveum* (Boiss.) Manden & Scheng

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The essential oil of *Cyclotrichium niveum*, an endemic *Labiatae* species, was analysed by GC and GC/MS. Pulegone (56.11%) and isomenthone (35%) were found to be the major components.

III-P30 Occurrence of Three Chemotypes of *Thymus longicaulis* C. Presl. subsp. *longicaulis* in the Same Population

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GC and GC/MS analysis of the essential oils of three chemotypes of *Thymus longicaulis* subsp. *longicaulis* collected from the same population growing in one square metre area have been carried out. Major components of the three chemotypes were characterised as follows:

a) α-terpinylacetate, α-terpineol (lavender odour).
b) geraniol - geranylacetate (rose odour).
c) thymol - carvacrol (thyme odour).

III-P31 Scanning Electron Microscopic Investigation on Pollen Morphology of Bulgarian Population of *Ocimum basilicum* L., Lamiaceae

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In this article we present data from a scanning electron microscope study of the pollen from two Bulgarian cultivars of *Ocimum basilicum* L. In addition, information is given about the chromosome number (2n=48) of these cultivars. It has been established that palynomorphological features of the two cultivars are virtually identical. The tricellular pollen grains are flattened - spheroidal with 6 furrows and 6-sided colpi. The exine in the mesocolpal and apocolpal regions is of the same type. Parallel cytological and ultrastructural analyses have confirmed the high viability of pollen from Bulgarian cultivars of *Ocimum basilicum*.

IV-P32 Sekoms, an Effective Database to Analysing Essential Oils and Aroma Compounds

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Gas chromatography coupled with low resolution mass spectrometry (GC-MS) is a most efficient method to analyse complex essential oils and flavour of foods which contribute to the number of natural products with approximately 10,000 substances [1]. Computer aided GC-MS is recognised as one of the best methods to realise a fast and reliable identification of known substances including isomers. Researchers working in this field usually build up their own databases suitable to their own requirements regarding compounds and search specificity which are not always fulfilled by commercially available standard databases [2]. Thus, we saw a need for a comprehensive and effective database covering as many volatile flavour compounds as possible.

During our work on the essential oil of *Cedronella canariensis* (Lamiaceae) we created a database, SEKOMS (SEARCH KOVATS-INDICES AND MASS SPECTRA), which now includes several thousand compounds with their Kovats-indices on two standard columns (polar, eg. DBWax$^{(R)}$, and apolar, eg. DB1$^{(R)}$) and of mass spectral data.

The SEKOMS database presents a compromise of a maximum of information (obtained from the literature including bibliographic data) together with a high working speed (indexed database - dBase$^{(R)}$). New compounds can rapidly be included which makes updating fast and simple. Key of differentiation is a Kovats-index system extended by MS data. The use as a stand-alone Kovats-database, however, is possible. Application examples of different search procedures are presented. The use and future possibilities are discussed.

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[2] McLafferty, F.W. and Stauffer, D.B., 1989. The Wiley/NBS Registry of Mass Spec tral Data. John Wiley & Sons Inc., Sci/Tech Division, New York.

IV-P33 Influence of a Plant Essential Oil (*Thymus vulgaris*) on the Nerve Elements in Ageing Rats (An Electromicroscopic Study)

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The purpose of the recent study was to determine the changes in the number and distribution of some neuropeptide-containing nerve elements in ageing rats. There is almost no information on the age-related changes in gastrointestinal neuropeptides which may have some influence on the functional alterations of motility as well as absorptive processes during ageing. The present study is based on the comparison of three groups of ageing rats, ie. old control (24-month-old); old treated (for four months) with an essential oil, *Thymus vulgaris*, and the third one was treated (16 months) with 2-mercaptoethanol (2-ME).

With the use of different antisera and Avidin-Biotin Peroxidase technique, several regulatory neuropeptides (vasoactive intestinal polypeptide, VIP; substance P, SP; neuropeptide Y, NPY) and some transmitter (dopamine-hydroxylase, DBH; and serotonin, 5-HT) were studied in the wall of small intestine with the aid of light and electronmicroscopy. A large amount of immunoreactive (IR) neuronal elements of NPY, SP, VIP were observed in all layers of small intestine and their quantity was the most numerous.

After the thyme treatment the quantity of the IR nerve processes were the most apparent of the old controls. Some of the DBH and 5-HT IR neuronal processes were also found in all layers and they were mainly located around the blood vessels. Their number was increased considerably in comparison to the untreated old rats. A few IR nerve perikarya were also observed in the myenteric and submucous plexuses. After 2-ME treatment only very slight changes were found in the number of the different neuronal elements. Electronmicroscopically, the IR nerve processes contained a large amount of vesicles, most of them were large granulated vesicles of 80-120nm in diameter and some of them were small, round vesicles of 40-50nm in diameter. These IR nerve fibres were found in a very close situation to the smooth muscle cells and to the epithelial lining of the villi, and sometimes in association to the vessels. It is concluded that these neuropeptide containing nerve fibres may influence the blood flow, may regulate the motility and the absorptive processes as well. The thyme treatment seems to be a beneficial effect on the quantity of the different neuronal alements of the small intestine during ageing.

IV-P34 Mode of Action of Methyl Jasmonate in Barley Cotyledon Chlorosis

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Methyl jasmonate is a bisubstituted cyclopentanone originally isolated from the essential oil of *Jasminium grandiflorium*. The compound is now thought to be ubiquitous in higher plants and potent biological activities of growth inhibition, senescence promotion, tuberization promotion and induction of specific proteins have indicated that it may be a novel plant growth regulator. Although these effects are now well reported there has been little research on the mode of action. Methyl jasmonate is slightly volatile at room temperature and this has raised the possibility that gaseous signalling may be involved in its mode of action. The effect of the vapour on barley cotyledon chlorosis with reference to pigment destruction, lipoxygenase activity, lipid peroxidation and electrolyte leakage has been examined.

On exposure to the vapour barley cotyledons rapidly undergo chlorosis in light and darkness. Chlorophyll *a* and *b* are rapidly lost, but the protective cartenoids and xanthophylls are lost at a slower rate. Lipoxygenase is an essential enzyme for methyl jasmonate biosynthesis and the secondary reactions of the enzyme also have a chlorophyll degrading action due to the formation of singlet oxygen and lipid peroxide radicals. Activity is raised rapidly within hours on exposure to the vapour and then declines steadily after one day. Untreated tissue shows a slower gradual rise in activity with no decline. Lipid peroxidation assays showed no differences between treated and control tissue and no overall increase from the initial levels. Electrolyte leakage assays again indicated no differences after one and three days. It appears that the vapour does not damage lipids to the extent that membrane integrity is lost.

This study indicates that the mode of action of methyl jasmonate may be *via* the gaseous phase in a similar manner to ethylene. Barley cotyledons respond to the vapour with a rapid but transient increase in lipoxygenase activity leading to rapid chlorosis. However the damage to membrane lipids does not appear to be extensive and may suggest that the effect is limited to chloroplast membranes. The activation of lipoxygenase may be direct with methyl jasmonate being a positive effector for its own synthesis, thus leading to further chlorosis. Specific induction of lipoxygenase synthesis could also account for the increase in activity but the loss of activity after one day seems to discount this idea. Alternatively the mode of action may involve the activation of a membrane hydrolase to release a pool of free fatty acids to serve as substrates for lipoxygenase. These explanations for the mode of action are currently under investigation.

IV-P35 Effect of *Matricaria chamomilla* and *Rosmarinus officinalis* on Bleomycin-Induced Lung Fibrosis in Rat

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Bleomycin (BLM) is antitumor antibiotic was found by Umezawa in 1974. BLM is a group of (antitumor) glycopeptides isolated from *Streptomyces verticullus*. BLM causes single and double-stard breaks in DNA. Biochemical interaction between BLM and oxygen have shown that BLM facilitates the production of reactive oxygen species especially, superoxide anion, generated by BLM living cells [1,2].

The aim of this project is to use fractions of *Rosmarinus officinalis* (RMY) as antioxidant and *Matricaria chamomilla*, (MCL) as anti-inflammatory agent.

Male rats weighing 155-175g were assigned to specific groups. Under ketamin (50mg/kg ip) anaesthesia, rats in each group received intratracheally either 50μl sterile isotonic saline or 1 unit of BLM sulphate. Each animal received a daily injection of RMY and MCL. The controls were similarly treated with 0.5ml of saline. Rats were sacrificed on 21 days after treatment. The thoracic cavity was opened. Each rat's lung was homogenised for measuring OH-proline, protein and DNA. Total OH-proline lung values in BLM group were significantly elevated over control by 113% in all BLM treated animals. A similar effect was observed in protein data (56%). The same data obtained from using RMY, 105% and 60%, for protein and OH-proline respectively.

The values for the animals treated with CML were 12% and 43% respectively compared to the control.

It was concluded from the present study that the administration of CML caused a significant reduction in OH-proline, protein DNA and body weight but RMY had no apparent effect on lung level of collagen, DNA and protein.

References:
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[2] Wang, Q., Shri, S.N. and Javadi, I., 1990. Bioch. Toxicol. Vol 15, 3: 13-21.

IV-P36 Transformed Roots of Aromatic and Medicinal Plants: A Novel Source of Biologically Active Secondary Metabolites

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As part of an investigation into biologically active secondary metabolites produced by aromatic and medicinal plants, transformed or 'hairy' roots were produced from a number of species. Stem explants of *Artemisia absinthium* (wormwood) and *Phytolacca americana* (pokeroot/pokeweed) were transformed using *Agrobacterium rhizogenes* strain LBA 9402, carrying plasmid pRi 1855. The resultant hairy roots were cultured in B 5 0 medium in flasks [1]. Root lines from both species were stable, and exhibited very rapid growth rates. Confirmation of transformation was by agropine detection [2]. The roots were subsequently analysed for secondary metabolites of interest.

Wormwood (Asteraceae) is a shrubby perennial which can grow up to 130cm in height and is native to warm Mediterranean countries and the British Isles. The dried leaf material produces a very bitter, toxic volatile oil, with thujone as the main component (40-70%). Transformed roots were cultivated in a one litre fermenter vessel which yielded 155g fresh weight of root after 28 days growth. Normal root material was also harvested from a field grown plant and both types of root steam distilled to yield volatile oils. Normal roots gave an oil yield of 1.4% (v/w) while transformed roots gave only 0.7% (v/w). GC, GC-MS and NMR analysis revealed the major components in each oil. The normal root oil contained chiefly α-fenchene (53%), while the major component of the transformed root oil was identified as neryl isovalerate (47%). Differences in the oil profiles were thought to be due to the lack of maturity in the transformed root, and the absence of translocation effects [3].

Pokeroot or pokeweed (Phytolaccaceae) is a herbacious perennial, up to 3m in height, with a thick, taprooted rhizome. The leaves of the plant have been found to contain strong antiviral agents called Pokeweed Antiviral Proteins (PAPs), which can inhibit protein synthesis on ribosomes of various plants and also prevent the transmission of viruses to plants. PAP I was extracted from transformed and normal roots by the method described in [5], and estimation of PAP levels was by an indirect ELISA procedure [6]. The untransformed root material was found to contain a very high level of PAP I (2.5mg/g), while the transformed roots contained only 13.3μg/g PAP I.

References:

[1] Hamill *et al*, 1987. Biotechnol, 5: 800-804.
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[6] Barbara and Clark, 1982. J. Gen. Virology, 58: 315-322.

IV-P37 Application of Liquid Carbon Dioxide to the Extraction of Essential Oil of Coriander (*Coriandrum sativum* L.) Fruits

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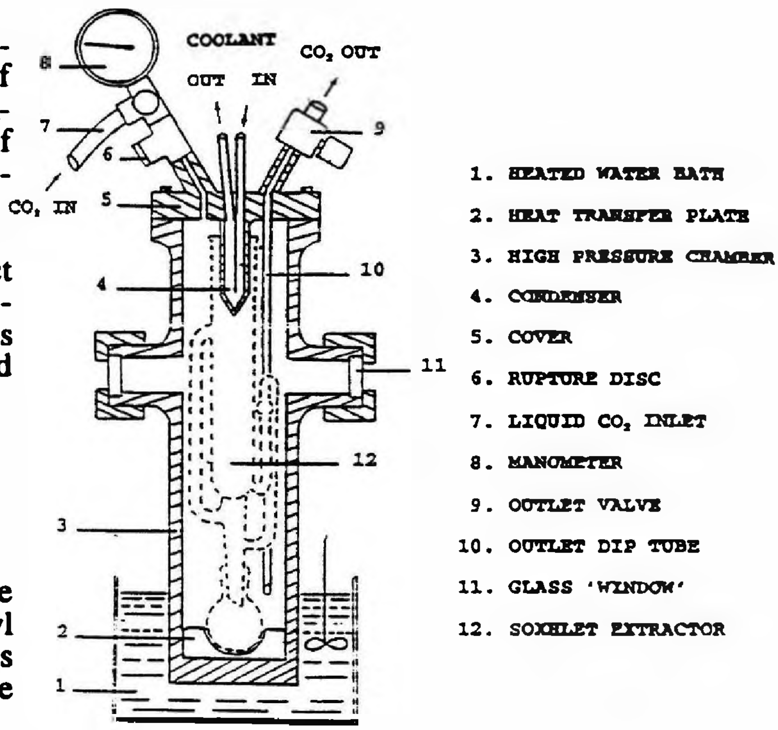
In essential oil extraction either elevated temperatures or various solvents and solvent mixtures are commonly used and in some cases both are needed. The composition of the extracts obtained differ from the original composition of essential oil in fresh plant material due to artefact formation or the loss of most volatile compounds during isolation or concentration procedures. An essential oil, which is free from solvent and with a better resemblance to the oil genuinely present in botanical material, can be achieved using liquid or supercritical carbon dioxide as extractant. We investigated the suitability of liquid carbon dioxide for essential oil extraction of coriander fruits in laboratory scale.

The J&W High Pressure Extraction Apparatus was modified by providing means of visual control of the extraction. The apparatus could be filled with optimum amount of extractant without disassembling the extractor.



The modification made it possible to select the Soxhlet unit to function either continuously or in siphon cycles. The extractor was operated under subcritical pressures and relative low pressures:

at 4.7 MPa (680 psi) and 25°C
at 5.4 MPa (780 psi) and 25°C
and finally 6.9 MPa (1000 psi) and 45°C.

The liquid carbon dioxide extracts were compared with hydrodistillates and diethyl ether-pentane extracts using capillary gas chromatography. Some 40 compounds were identified by mass spectrometry.

No significant difference was found in the total yield of isolated extract between hydrodistillation and carbon dioxide extraction: 0.72g and 0.70g/100g fresh weight, respectively. Isolation was performed as triplicates and extracts were analysed three times. We determined the coefficient of variation for each compound in order to compare the reproducibility of the extraction procedures. In both hydrodistillation and solvent extraction the coefficients varied from 3 to 15%. In the carbon dioxide extractions the coefficients of variation were significantly larger, with 25% as average. The variation was explained by inaccuracy of visual adjustment of the amount of CO_2 in the apparatus and insufficient control of pressure during extraction. The total amount of monoterpenes was lower in CO_2-extracts (about 13.5%) than either in hydrodistillate (20%) or solvent extract (24.5%). Oxygenated monoterpenes constituted the largest group of compounds: in hydrodistillates about 79%, the solvent extracts 72% and CO_2-extracts from 68 to 81%. Linalool, α-pinene, γ-terpinene, camphor, geraniol, D-limonene, geranyl acetate and borneol were major compounds in all extracts.

IV-P38 Study on the Rooting of Kazanlak Rose *In Vitro*

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The world famous attar of roses is obtained from the Kazanlak rose. The necessity of high-volume production of planting material for Kazanlak roses, forced the beginning of investigations for its propagation *in vitro*. After the introduction of explants into a sterile culture and a period of multiplication, a series of experiments have been carried out for finding out the optimal nutrient medium and conditions for cultivation that enable the achievement of a higher rate of rooted plants with vital power and normally developed root system. The effect of several factors on the process of rooting has been an object of study, ie. the presence of NAA at a concentration of 0.05 and 0.1 mg/l, the existence of phloroglucinol in the nutrient medium and the variability of the quantity of mineral composition (1/4 and 1/8 MS).

At the same time, the conditions for the growth of micro-plants were an object of study; A - growing in a chamber at light intensity of 2500 luxes, temperature - 20°C and photoperiod - 16 hours; B - initially growing without any light (for 20 days) at the temperature of 2°C, followed by introduction into a chamber with the same condition as A. The best results are obtained in a nutrient medium with 1/4 MS salts, 0.1 mg/l of NAA and including phloroglucinol, in the presence of light.

As a third stage of the present experiment, the changes in the rhizoid processes have been studied, when the plants have been grown in a two-phase nutrient medium - solid containing the above-mentioned nutrient media plus 2 MS salts without growth regulators. The microplants have been grown at regimes A and C. The optimum results concerning the percentage of rooting and the ability of adaptation under *in vitro* conditions, have been obtained in the two-phase nutrient medium at the presence of light with an inessential difference between the prevailing number of the experimental media.

IV-P39 Data of the Plant Antioxidants Used for Gerontological Research

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In this presentation, a short demonstration of a part of the joint research programme is given. It involves the technology and phytochemical analysis of some essential oils and other plant extracts used as antioxidants for animal experiments in gerontology.

Qualitative and quantitative gas chromatographic analyses were carried out on the following essential oils: Clove, Thyme, Nutmeg, Pepper and Almond oils. The oils were found to be especially rich in aromatic (phenyl propane derivative) compounds.

For the investigations a Jeol JGC 1100 gas chromatograph was used, nitrogen carrier gas, FID - detector, packed column coated by 3% OV-17 and linearic temperature program. The components of each oil were identified by standard addition methods also using the data of earlier essential oil research works [1, 2, 3].

In addition, three aromatic plants were selected (Lavender, Peppermint and Celery) - well known in traditional and folk medicine - and several plant products of three different polarities were prepared: polaric (lyophylizate), semipolaric (tincture) and apolaric (essential oil) extracts.

For the phytochemical evaluation of the plant products the flavonoid, tannin and polyphenol contents of tinctures, lyophylizates and the composition of the essential oils were determined.

On the base of the high flavonoid content, it was established that the peppermint tincture has as expected, the strongest antioxidant effect. Our supposition was verified by the animal experiments.

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IV-P40 Multi-Dimensional Gas Chromatography as a Tool in the Determination of Enantio-Purities of the Monoterpene Hydrocarbons in a Galbanum Oil

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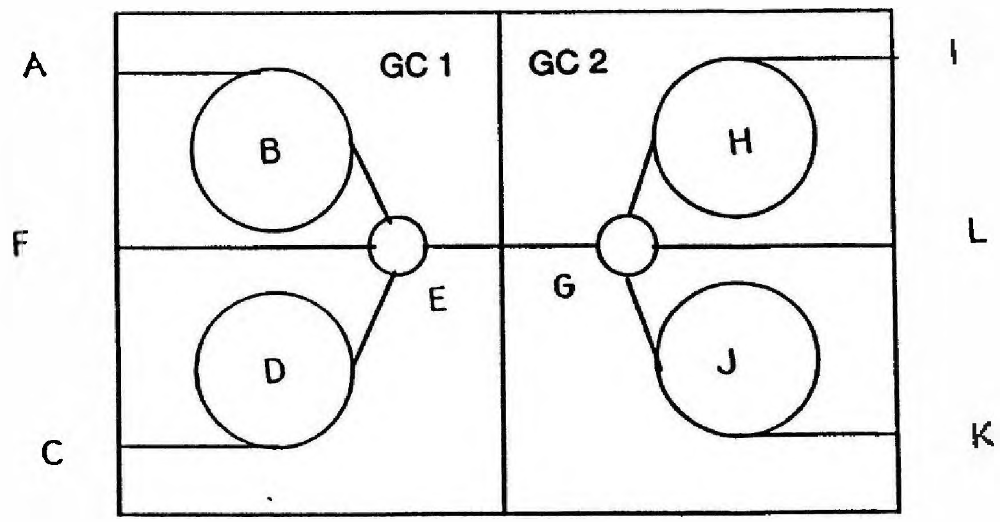
A multi-dimensional GC system which allows the simultaneous use of two different enantio-selective capillary columns as well as the possibility to use, without any column changing, a polar or an apolar column for the pre-separation, is presented together with the results from an analysis of the monoterpene hydrocarbons in a Galbanum oil.

The difficulty to find reference compounds for the determination of enantiomeric compositions will be discussed.



A,C,L: Injectors; **B**: Apolar column; **E,G**: Valco micro valves; **F,I,K**: Flame Ionisation Detectors; **H**: Enantio-selective column 1; **J**: Enantio-selective column 2.

IV-P41 Comparative Accumulation of Indole and Non-Indole 'Mustard Oil' Glucosinolates by Cell Cultures of Three Species of Cruciferae

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Hydrolysis of 'Mustard oil' glucosinolates yields pungent, volatile flavour components. These are important in the food industry, but also have a range of biological activities, as well as plant-insect interactions. There have been a number of investigations into the 'Mustard oil' glucosinolates produced by tissue cultures derived from members of the Cruciferae.

Most workers are either unable to detect them, or reveal lower levels than in the intact plant. We were able to demonstrate the occurrence of series of indole glucosinolates in callus cultures of *Brassica napus var. napobrassica* and *B. oleracea var. botrotys* by GC of trimethylsilyl derivatives. Neither callus nor suspension cultures of *Hirschfeldia incana* accumulated any of the glucosinolates present in mature plant. However feeding of 2-propenyl glucosinolate to suspensions of the latter was shown to lead to production of the isothiocyanate. The results obtained show total degradation of exogenous glucosinolate over the 21 day period after feeding. Previous workers detected the presence of myrosinase enzyme, which is responsible for metabolism of glucosinolate to volatile derivatives, in cultures of Cruciferae. Co-production of both 'Mustard oil' glucosinolate and enzyme responsible for its breakdown in near proximity *in vitro* could explain reported low levels of the flavour precursors.

IV-P42 Antifungal Properties of the Essential Oil of Summer Savory [*Satureja hortensis*] as Determined by a Radio-isotope Assay for Chitin Synthesis in *Aspergillus niger*

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An investigation was conducted into the effect of Summer Savory [*Satureja hortensis* L.] essential oil on the spoilage fungus *Aspergillus niger* using two seperate techniques. The first being a simple dry weight determination using the whole oil and five of its major constituents on the fungi. The second technique involved a radio-isotope assay to investigate the effect of the whole oil on chitin synthesis within the fungi.

The dry weight determination indicated that the whole oil can cause inhibition of the fungi at concentrations as low as 5 $\mu l ml^{-1}$ YES broth, while the five major constituents showed greater antifungal effect by causing inhibition at concentrations as low as 0.5 $\mu l ml^{-1}$. The radio-isotope assay emphasised the dry weight determination results by indicating that chitin synthesis was indeed affected by the presence of the oil.

References and Acknowledgements:
SAC receives financial support from the Scottish Office Agriculture and Fisheries Department. M.M. gratefully acknowledges financial support from the Perry Foundation. The authors wish to thank Elizabeth Eaglesham for her technical assistance and Frank Fox [Canberra Packard], Kevin Conner [Hannah Research Institute] and Anne Humphreys [University of Liverpool] for their advice.

[1] Binks *et al.*, 1991. J. Gen. Micro., 137: 615-620.
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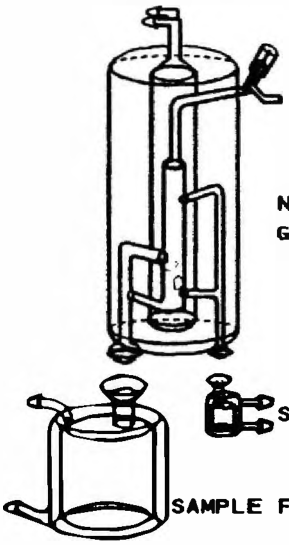
IV-P43

SIMULTANEOUS DISTILLATION-EXTRACTION UNDER STATIC VACUUM: AN ISOLATION OF VOLATILE COMPOUNDS AT ROOM TEMPERATURE.

L. MAIGNIAL - NESTEC RESEARCH CENTER, LAUSANNE SWITZERLAND.

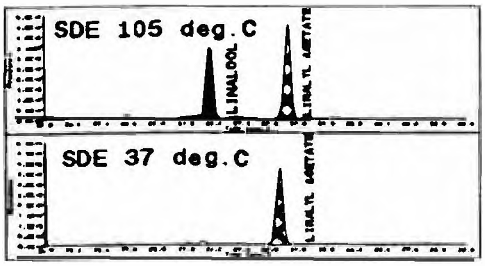


■ VARIOUS SOLVENTS CAN BE USED ALLOWING TO EXTRACT UNDER THE FOLLOWING TEMPERATURES:

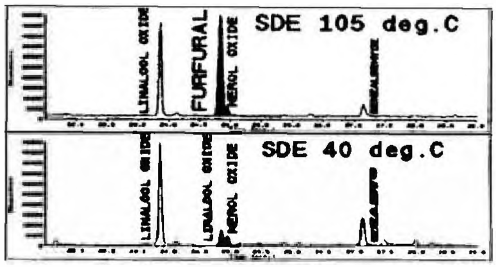
	SAMPLE T deg.C	SOLVENT T deg.C
n-OCTANE	19	26
2-PENTANONE	25	17
TOLUENE	30	20
HEPTANE	30	17
ISOOCTANE	37	20
HEXANE	50	22

■ COMPARISON BETWEEN STANDARD AND STATIC VACUUM S.D.E.:

Distillation-extraction of LINALYL-ACETATE



Distillation-extraction of MEXICAN HONEY



Recovery yields of various molecules (%) (Internal standard quantitation).

COMPOUND	ATM. SDE	VACUUM SDE
ETHYL BUTYRATE	118 / 13	92 / 13
LIMONENE	109 / 2	103 / 2
2-E-HEXENAL	91 / 3	76 / 4
2-ACETYL PYRAZINE	55 / 2	44 / 2
E-ANETHOL	102 / 2	92 / 2
DODECANOL	97 / 2	92 / 2

■ CONCLUSION :

- Selective extraction of volatile compounds in a single step;
- Thermal artefacts discarded;
- Choice among several solvents allows to optimize each application;
- One sample can be analysed with several techniques (GC / FID, MS, FTIR, Sniffing);
- Efficient extraction yields;
- Accurate quantitative measurements (ISTD).

IV-P44 Biotransformation of (+)-Aromadendrene and (-)-Globulol in Rat

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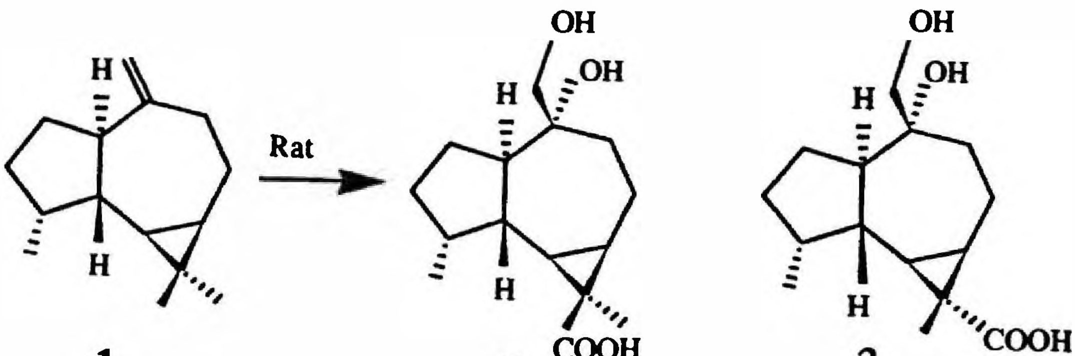
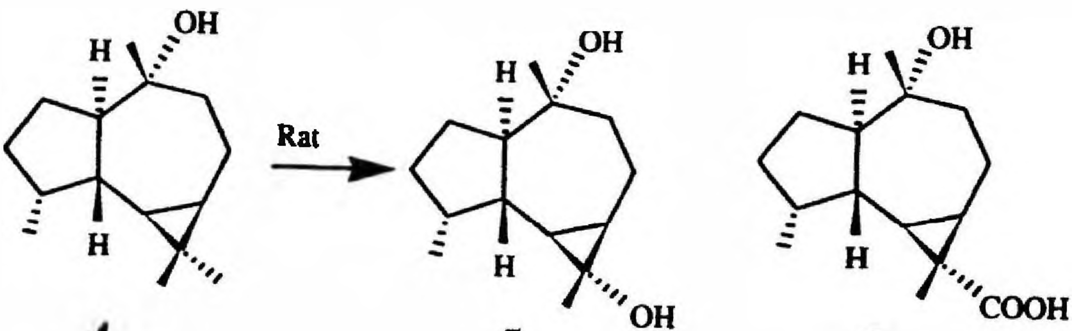
The biotransformation of (+)-aromadendrene (1) and (-)-globulol (4) in Rat were investigated. From (1), two major acidic metabolites were purified by chromatographic methods, to give aromadendrene-10,14-diol-12-carboxilic acid (2) and aromadendrene-10,14-diol-13-carboxylic acid (3). On the other hand, (4) was trnsformed to 13-hydroxyglobulol (5), globulol-13-carboxylic acid (6) and $C_{15}H_{26}O_2$.



1 2 3



4 5 6

IV-P45 Biological Activity of Several Aromatic Plants Grown in the Mediterranean Area

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In recent years an increasing interest in the use of natural substances instead of synthetic chemicals has promoted more deep studies on plant resources. Aromatic plants and their essential oils, used since antiquity in folk medicine and in preserving food, are a source of secondary metabolic products having biological activity such as antibacterial, antifungal and antioxidant actions [1,2].

The effectiveness of essential oils depends on their composition which is greatly influenced both by the genotype and by the geographical origin [3]. In this research we studied the antimicrobial and the antioxidant properties of eleven essential oils obtained by steam distillation of aromatic plants grown in the Mediterranean area. The tested plants were:*Lavandula angustifolia* Mill. x *L. latifolia* Med., *Thymus vulgaris* L., *Satureja montana* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Mentha* x *piperita* L. (Lamiaceae family), *Chamomilla recutita* (L.) Raush, *Anthemis nobilis* L., *Artemisia dracunculus* L. (Asteraceae family), *Foeniculum vulgare* Mill. var. *vulgare* and var. *dulce* (Apiaceae family).

Biological assays were performed against twenty-five bacteria including food poisoning organisms and animal and plant pathogens.

All the oils showed antibacterial activity with a wide range of effectiveness degree. Those from *T. vulgaris* and *S. montana* possessed the higher activity probably due to their high content of phenols. Oils from *A. nobilis* and *L. hibryda* had the higher antioxidant effects.

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IV-P47 Black Truffle Essence: A Potential Industrial Flavouring Recovered from Processing of Flavoured Atmosphere of Cold Storage

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Black Truffles (*Tuber melanosporum Vitt.*) are the fruiting bodies of hypogeous fungi which grow in symbiosis with certain trees, especially oaks. The production areas are limited to several regions of southern Europe, particularly France, Italy and Spain. The harvesting is exclusively carried out with animals during winter. Due to its typical flavour, black truffles are very much appreciated by gourmets and traditionally used by the French food industry to flavour its products.

Later to be sold on professional production markets, truffles are cold stored for several days before being industrially processed, generally by heat treatment. During this period of storage, truffles lose a part of their typical aroma and in the same time the cold storage atmosphere becomes rich in such volatiles, but the associated fannel device continually eliminate these aromatic compounds.

First, isolations of volatiles from the odorous atmosphere of a $15m^3$ cold storage for black truffles are performed by using a portable vacuum pump connected to stainless steel traps (active charcoal and TENAX GC) for analytical purposes. The chromatographic analysis of the traps are carried out with a D.C.I. device (dynamic headspace injector) connected on-line to a gas chromatograph. Dual detections are carried with both a FID detector and a sniffing port, in order to instrumentally and sensorially evaluate the traps. In combination with the comparison of the finger prints, a qualitative sensory analysis (definition of the aromatic notes) and a quantitative ones (titration of the aromatic intensity of each chromatographic peak by using a notation scale with 5 levels) are performed.

Then, large scale experiments are carried out with a specially designed adsorption concentration pilot unit allowing the use of 130g of active charcoal. The ethanol elution of the trap is then performed with a Soxlhet device prior to concentration with a Kuderna-Danish concentrator. According to both GC (FID and sniffing detections) analyses and sensory evaluation carried out by a panel of experts, the organoleptic qualities of the extracts obtained allow us to propose a potential new industrial aromatic base, "a truffle essence", for industrial uses.

IV-P46 Natural Antioxidants from *Thymus vulgaris* (Thyme) Volatile Oil: The Beneficial Effects Upon Mammalian Lipid Metabolism

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Biologically active secondary metabolites have been recognised in numerous aromatic and medicinal plants. In the food industry, oxidative rancidity is the most important type of fat spoilage. Antioxidants act primarily as hydrogen donors or free radical acceptors. Antioxidants from natural sources have been receiving increased attention over the last decade due, at least in part, to the possible toxicity of the synthetic chemicals used as antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

Preliminary screening of plant volatile oils showed *Thymus vulgaris* (Thyme) oil and a number of its constituent compounds to possess strong antioxidative properties. Table 1, shows the five most active constituents to be linalool, thujone, camphene, carvacrol and thymol. To avoid synergistic effects, whole thyme oil was used in the mice feeding trials. 720μg of thyme oil were administered every alternate day orally to groups of young (6 month) and ageing (22 month) mice.

Following the feeding regime, the animals were sacrificed and the polyunsaturated fatty acids (PUFAs) in several organs were analysed. Table 2, shows that oil of thyme had a dramatic effect upon the liver PUFA levels in the mice. Animals fed the diet supplemented with thyme oil showed significantly higher levels of PUFAs with arachidonic and docosohexænoic acids having particularly elevated levels. In addition, levels of the enzyme glutathione peroxidase, a key enzyme in lipid metabolism, were elevated in animals fed thyme oil. Both the very young and the very old have a high dependence on PUFAs; in both cases of particular importance is the maintenance of adequate levels of arachidonic acid and docosohexænoic acid.

It is known that during the ageing process, levels of PUFAs decline with concomitant loss of cellular integrity and function. It is therefore of prime importance, particularly in tissues such as cell membranes having a high PUFA content and consequently a potentially high free radical and oxygen content, to have an adequate antioxidant supply. Clearly, plant volatile oils capable of reversing this trend have an important role to play in human health. Disease conditions such as senile dementia and acute memory loss are now seen as manifestations of inadequacies in the cellular levels of PUFAs.

Table 1: Antioxidative screening of the major components of Thyme volatile oil.

Compound	Zone Diameter[mm]
Whole Oil	25.0
Linalool	20.6 ☼
Thujone	19.3 ☼
Camphene	16.2 ☼
Thymol	13.2 ☼
Carvacrol	13.2 ☼
τ - Terpinene	12.3
ß - Caryophyllene	12.3
Borneol	11.4
Myrcene	10.8

Table 2: Liver phospholipid profile in mice fed oil of thyme over a five month period (% total lipid).

	Young Control	Aged Control	Thyme Oil
Linoleic Acid [18:2]	20.16±1.58	16.20±1.63	16.89±0.90
Linolenic Acid [18:3]	0.72±0.28	0.61±0.09	0.36±0.11
Arachidonic Acid [20:4]	13.47±2.69	7.26±0.36	**16.47±2.62**
Docosohexænoic Acid [22:6]	3.60±1.90	2.35±0.62	**6.77±0.25**
Total PUFAs	37.95	26.42	**40.49**

References and acknowledgements:

SAC receives financial support from the Scottish Office Agriculture and Fisheries Department. E.S. gratefully acknowledges financial support from the Perry Foundation. The authors wish to thank Elizabeth Eaglesham and Ruth McCartney for their technical assistance.

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[2] Deans *et al*, 1992. Proceedings of First World Congress on Medicinal and Aromatic Plants for Human Welfare (1992) Maastricht, The Netherlands.
[3] Deans *et al*, 1992. Age. In Press.

IV-P48 Investigation of Some New Fungicides for the Control of Rust (*Puccinia Menthae* Pers.) on Mint

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During the period 1989-90, in the laboratory and field experiments, we studied the effects of some new systematic fungicides on mint rust (*Puccinia menthae* Pers.), within two species: *Mentha piperita* Huds and *Mentha arvensis* L. The influence of the fungicides on the quantity and quality of the essential oil was examined too.

The best results were achieved by using the fungicides Folicur-plus and Bicor.

B-P49 Inhibition of Fungus Germination and Growth by Essential Oil Components

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Several essential oil components were studied in order to determine their fungistatic and fungicidal activity against fruit and vegetable post-harvest pathogens.

In vitro trials were carried out to study the inhibition activity of fungal spore germination and mycelium growth.

The essential oil components studied included Anethole, Anisaldehyde, Carvacrol, Carvone, 1-8 Cineole, Limonene, Myrcene, α-Phellandrene and α-Pinene at concentrations ranging from 31 to 1000 ppm.

The inhibition activity of these components was examined against *Botrytis cinerea* Pers., *Monilinia laxa* (Aderh. et Ruhl.)Honey, *Mucor piriformis* Fischer, *Penicillium digitatum* (Pers.)Sacc., *Penicillium italicum* Wehm, *Penicillium expansum* Lk. and *Rhizopus stolonifer* (Ehremb.)Lind.

Best results were obtained with Carvacrol. At a concentration of 125 ppm the growth of all pathogens mycelium tested was completely stopped, while the inhibition of spore germination was considerable especially against *M. laxa, M. piriformis* and *R. stolonifer*. At 62 ppm, the control of spore germination and mycelium growth was not completely achieved (except for *M. piriformis* spore germination).

Anisaldehyde and Carvone at 250 ppm exhibited a fungistatic activity, while Anethole was seen to be active only against certain parasites at doses higher than 500 ppm.

All remaining essential oil components tested at concentrations of 1000 ppm, showed limited or no fungistatic activity.

B-P50 Essential Oil From Roots of Edelweiss, *Leontopodium alpinum*

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Leotopodium alpinum Cass. (Compositae; Inuleae) is a protected plant indigenous to the alpine regions of Europe and commonly known as Edelweiss. Although the plant has touristic associations with alpine countries and some suggested medicinal properties [1,2], few researchers have examined for the presence of various secondary metabolites. Compounds which have been identified in aerial parts include hydrocarbons [3], sterols [4], flavonoids [5] and hydroxycinnamic acid esters [4]. Although these latter esters were found to occur in small amounts in the roots [4], the paucity of information associated with these plant parts led us to examine them for the possible presence of an essential oil.

Plants were derived from commercially available seed material and cultivated under garden conditions in a peat-based compost. Roots were obtained by separation from aerial parts, washed and dried. Powdered material was subjected to steam distillation for five hours using an Apparatus for the Determination of Essential Oils in Vegetable Drugs [6]. A percentage (v/w) was recorded and the separated oil was used for chemical examination. GC Analysis used a column packed with Carbowax 20M and operated at 120-200°C (2°C/min rise), with a Nitrogen flow of 30ml/min. GC-MS were recorded on a quadropole instrument fitted with an SE 54 column and operated at a temperature range of 50-280°C. Separation of individual compounds was carried out on a Si gel - a silver nitrate column, gradient eluted with pet.ether : ethyl acetate or chloroform : methanol mixtures.

The roots were found to produce ca 2.0% of an essential oil. GC analysis of this indicated the presence of 15 compounds, two of which were present in major concentrations (35 and 24%). GC-MS showed the majority of compounds to be various sesquiterpene hydrocarbons and oxygenated derivatives. This is in keeping with literature reports of essential oils from other members of the Inuleae [7]. Examination of oil yields and composition over a 12 month period failed to indicate any major seasonal trends. Identification of individual constituents has proved difficult because of (1) chemical instability and (2) small amounts of available essential oil through insufficient natural root material. To overcome (2) fast-growing, transformed (ie. "hairy") roots of L *alpinum* have been developed by infecting sterile plants with a strain of *Agrobacterium rhizogenes*. These roots have been found to produce an essential oil identical in composition to the natural root oil. It is hoped that they can be used as an alternative source for the oil and so facilitate the identification of individual constituents.

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B-P51 MAP: Production of Novel Extracts from *Achillea millefolium*

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The essential oil of *Achillea millefolium* obtained by steam distillation is well-known for its deep blue colour resulting from the presence of chamazulene as one of its constituents. The olfactive properties of the oil however, are less interesting; consequently the oil is of little value to the perfumers. Chamazulene itself is produced via a hydrolytic reaction of a lactone that occurs during the steam distillation process.

A novel extract was obtained using the microwave-assisted process (MAP) on *Achillea millefolium* grown in the Province of Québec (Canada), without the addition of any water. The MAP extract is produced in a matter of seconds and it offers an expanded range of fragrances. Furthermore, the so-treated plant material can be subsequently extracted a second time using conventional steam distillation and still yield a valuable deep blue extract. The contents of both type of extracts have been compared and characterised by gas chromatography/mass spectrometry work.

B-P52 The Essential Oils of *Thymus praecox* Subspecies Growing in the Alpine Region of Austria

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Our investigation of *Thymus x citriodorus* prompted us to analyse the essential oils of other *Thymus* species with a distinct lemon flavour. Studies at the Herbarium Hamburgense and contacts with scientists working in this field revealed that the analysis of the Alpine *Thymus* species would be worthwhile on this matter.

In July 1991 the plants were collected at ten different places of the Alpine region of Austria (Otztaler Alpen and Karwendelgebirge). They were classified as *Thymus praecox* s.1. The hydrodistilled oils from fresh plant material analysed by GC and GC-MS gave some remarkable results concerning the main constituents.

In some oils geranyl acetate was the main compound (up to 65%). In such oils the corresponding alcohol geraniol ranged from 1% to 14%. Other oils contained geraniol as the main compound, accompanied by the other monoterpene alcohols nerol and citronellol. All these essential oils showed considerable portions of neral and geranial both causing the typical lemon smell like that of *T. x citriodorus*. Besides those lemon smelling types, plants with the well known spicy odour, known from *T. vulgaris*, could be found. They contained thymol together with carvacrol, -terpinene and p-cymene. In addition, some plants had α-terpeniol rich oils, others showed oil compositions of mono- and sesquiterpenes, as previously described for some chemotypes of the northern *T. praecox* ssp. *arcticus*.

As a result the essential oil polymorphism, a widespread phenomenon within the genus *Thymus* [1], can also be postulated for our Alpine thyme plants. The variability of the essential oil composition will be shown together with the locality and some morphological data in detail.

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B-P53 Effect of Sucrose on Essential Oil Formation in Caraway Fruits of Detached Umbels

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Caraway (*Carum carvi* L.) is mainly used as a spice. Biological activity of the essential oil found in the oil ducts in the fruits pericarp has been reported, such as inhibition of fungal growth, insect repellence and inhibition of sprouting of potatoes. Caraway essential oils consists for 95 to 99% of two monoterpenes, (+)-limonene and (+)-carvone in a ratio of approximately 1:1.

To increase the economic feasibility of industrial applications of caraway essential oil a higher and more stable production of seed and essential oil with a high carvone content will be necessary. This paper describes a study on the regulation of the accumulation of the components of the essential oil in developing seeds.

To eliminate influences of the intact plant we developed two *in-vitro* systems. In the first system an umbellet was grown on liquid medium, containing sucrose and nutrients according to Murashige and Skoog (1962) [1]. In the second system an entire umbel was used, the sterilised stem was put in a tube while the umbel itself remained outside. In this way transpiration is assured which may ease transport of components from the medium. Both systems were used to study the effects of sucrose in the medium on seed growth and essential oil production.

In-vitro culture on liquid media with sucrose concentrations varying from 0 to 8%, showed that fruit growth and the rate of essential oil production increased by sucrose concentrations up to 4%. Above 4% seed growth and essential oil formation were not stimulated any further. Several combinations of sucrose and mannitol were applied to the medium to study whether part of the effect of sucrose on fruit growth and essential oil formation was an osmotic effect. Results of these experiments are presented.

From earlier experiments with intact plants, it appeared that limonene and carvone accumulation depended on the developmental stage of the fruits. *In-vitro*, essential oil accumulation was strongest in young fruits and declined in older fruits. Accumulation of limonene occurred earlier during seed development than accumulation of carvone. Formation of both components was stimulated by sucrose.

The *in-vitro* systems described here proved to be suitable and will be used for further research. We intend to investigate the regulation of the biosynthetic pathway of caraway essential oil by using labelled (^{13}C or ^{14}C) sugars, phytohormones and specific inhibitors of its biosynthesis.

References:
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B-P54 The Essential Oil of *Smyrnium olusatrum* (L.)

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Smyrnium olusatrum is a biennal umbellifer growing in South and West Europe, especially in coastal areas of the British Isles, the Mediterranean region extending to the caucasus and the Canary Islands. In ancient times all parts of the plant were used as a remedy. Additionally its young sprouts, stems and roots were cultivated as a vegetable until the Middle Ages.

A survey of the literature revealed that there are several papers dealing with the isolation of individual components belonging mainly to the furanosesquiterpenes and the sesquiterpenic lactones whereas the composition of the essential oil has not been the subject of any investigation before. We have therefore analysed the essential fruit oil obtained by hydrodistillation by means of chromatographic and spectroscopic methods (LC, GC, GC-MS, IR).

The oil is characterised by a high content of hydrocarbons (77%), most of them monoterpenes (75%) with ß-phellandrene and α-pinene as major components (40% and 21%, respectively). The remaining 23% are predominantly oxygenated terpenoids which mainly consist of furanosesquiterpenes.

B-P55 Antimicrobial Activity of Volatile Phenylpropanoids and Related Aromatic Compounds.

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Essential oils are generally mixtures of mono- and sesquiterpenoids but a smaller number, eg. some oils from the Apiaceae contain phenylpropane derivatives in more or less high amounts. Especially this group of natural substances is found to be resposible for the pronounced pharmacological, antimicrobial and toxic effects of the respective oils.

In contrast to terpenoids and entire essential oils up till now, a comprehensive treatment of the antimicrobial features of individual phenylpropane derivatives does not exist. We have, therefore, examined most of the phenylpropanoids occuring as constituents of essential oils including related aromatic compounds and determined their antimicrobial properties.

By use of a modified serial dilution technique (1,2) the minimal inhibitory concentration as well as the minimal microbicidal concentration of each sample were estabished against *Candida albicans* (1), *Staphylococcus aureus* (2), *Listeria monocytogenes* (3), *Bacillus subtilis* (4), *Klebsiella pneumoniae* (5), *Pseudomonas aeruginosa* (6), and two strains of *Eschericia coli* (ATCC 25922) (7) and N.N. (8). The results were compared to data using eugenol and phenol as reference (Table 1).

Table 1. Minimal inhibitory concentration (MIC) and minimal microbiocidal concentration (MMC) of eugenol (a) and phenol (b).

Microorganism		1	2	3	4	5	6	7	8
MIC	(a)	200	750	550	300	800	1000	550	600
	(b)	2000	1250	2500	1750	1500	1750	1750	2500
MMC	(a)	400	950	550	450	800	2500	700	800
	(b)	3500	2000	3500	1750	2000	3500	2500	2500

In general, phenolic derivatives of phenylpropanes exhibit a higher antimicrobial activity than the related ethers and are microbiocidal in a concentration range from 250 to 2500 μg/ml depending on the substitution pattern of the aromatic ring. A significant higher antimicrobial activity was found in some cases among phenolics with a partially (aldehydic) or completely degradated side chain, exhibiting minimal inhibitory concentrations from 25 and 10 μg/ml, respectively.

B-P56 The Effect of Different Growing Conditions on Quantitative and Qualitative Composition of the Essential Oil of Chamomile (*Chamomilla recutita* (L.) Rauschert)

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The chamomile is one of the most important medicinal plants in the world. Pharmaceutically useful are primarily its flower anthodia - *Flos chamomillae*. The healing power of chamomile drug is due to the presence of the essential oil constituents. Among them, the most valuable are [-]-α-bisabolol and chamazulene as estimated on the basis of their pharmacodynamic properties.

The effect of various edaphic and climatic conditions on the essential oil content or quality was followed. Three-year field experiment (1988, 1989 and 1990) was performed with our diploid variety "BONA" in the three various localities: Bracovce - a warm climatic region of the Lowlands of East Slovakia; Kosice - a warm climate of the Basin of Kosice with prevailing North winds; Nova Lubovna - a mountain region with a moderately warm climate.

Essential oil was isolated by steam distillation and its weight was determined gravimetrically. Gas chromatography was used to characterize the main components of the chamomile essential oil.

Different conditions of localities, where the chamomile plants were grown, influenced the yield of essential oil and its main components but had no effect on the composition of the essential oil.

B-P57 The Composition of the Essential Root Oils From Several Species Belonging to the Genus Geum

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The genus *Geum*, belonging to the Rosaceae family consists of 59 species, which are subdivided in 11 subgenera (Gajewski, 1957). These species mainly grow in the holarctic circle, but also in the mountaineous regions of South America and in Australia. The roots of one of these species, *Geum urbanum* L., are of particular interest since they are used in folk medicine and homeopathy. Further more, the roots are used in industry for flavouring liqueurs. The latter application is based on the essential oil (approximately 0.02 - 0.1%) which consists predominantly of eugenol, accompanied by several minor compounds mainly belonging to bicyclic monoterpenes (eg. cis/trans-myrtanal, myrtenal, cis/trans-myrtanol and myrtenol).

Since we found that the commercial drug "Herb Bennet" ("Wood Avens") does often *not* derive from *Geum urbanum* L. but from other *Geum* species we investigated the essential root oil of several further *Geum* species (*Geum x hybridum*, *Geum rivale*, *Geum macrophyllum*, *Geum fauriei*, *Geum rhodopeum* and *Geum montanum*, *Geum reptans* and *Geum bulgaricum*) in order to find out to which degree a characterisation of these different *Geum* roots is possible.

It could be shown, that the root oils of the species *Geum rivale*, *Geum fauriei*, *Geum rhodopeum* and *Geum bulgaricum* are very similar in composition to that of *Geum urbanum* and that these roots cannot be distinguished by means of their volatile oils. In contrast, the oils of *Geum x hybridum* (characterised by a group of monoterpenoids) and *Geum montanum* (with small amounts of eugenol) exhibited a different composition. The same holds true for the root oil of *Geum reptans* which differs significantly from the before mentioned oils.

B-P58 GC/MS - Investigations on the Composition of Commercial Samples of Parsley Oil

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The commercially available essential oils of Parsley are obtained from different parts of *Petroselinum crispum* (Miller) A.W.Hill (= *P. hortense* auct., *P. sativum* Hoffm.), native to the Mediterranean region and now widely cultivated around the world. The oils are offered under the names "Parsley seed oil" (correctley a fruit oil), "P. herb and P. leaf oil" and sometimes without any specification as "Parsley oil". They are used primarily in the food industry for flowering meat, sauces, canned food, seasonings and to a much lesser extent in perfumery and pharmacy.

We have analysed 27 samples of commercial oills, in particular 12 "seed oils", 12 "herb oils", 1 "leaf oil" and 2 "parsley oils" by means of chromatographic and spectroscopic methods to elucidate their qualitative and quantitative composition.

The characteristic compounds of 8 commercial "seed oils" are the phenyl propanoids apiole and myristicin, however remarkable differences concerning the percentages of these compounds can be observed. In one of the oils the dominating compound was found to be apoile with 66%, while the content of myristicin was considerably lower (13%). 5 oils were rich in both apiole (37-47%) and myristicin (17-28%). In 2 further samples myristicin was the dominating substance (33% and 42% respectively), whereas the apiole content was lower, amounting to 23% in one case and even unusually small in the second oil (3%). Tetramethoxy-(2-propenyl)-benzene (<9%) was also detected in some of the 8 before mentioned seed oils. The remaining 4 seed oil samples were rich in monoterpene hydrocarbons with α-pinene (43-46%) or p-mentha-1,3,8-triene (22%) as dominating compounds, while the phenylpropanoids were of minor importance (below 15%).

In contrast to the commercial fruit oils, all investigated herb oils showed monoterpene hydrocarbons as principal constituents: in 3 oils α-pinene (22-43%) was dominating, in one oil each limonene (32%) and myrcene (27%), respectively, and in 7 oils p-mentha-1,3,8-triene. The latter occurred in all parsley herb oils and must be regarded as the most characteristic component of all these oils. Apart from that, all investigated samples with one exception, exhibited low concentrations of phenylpropanoids with myristicin (0.3-17%) as the main compound. In several herb oils noticeable amounts of tetramethoxy-(2-propenyl)-benzene (about 1.5%) were found, a compound which is generally typical for parsley fruit oils.

P59 A Study of the Agrochemical and Pharmacological Properties of the Essential Oils of *Helichrysum oderatisimum* (Compositae) and *Salvia stenophylla* (Labiatiae)

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Both the plants are indigenous to South Africa and widely used as folk medicine for a variety of ailments. *Salvia stenophylla* (Labiatiae) is also burnt indoors to disinfect rooms after sickness, also the leaves are sometimes mixed with tobacco for smoking. *Helichrysum oderatisimum* (Compositae) found in Kidd's Bay is used for the treatment of coughs, colds, stomach ache etc., similarly to that of *Salvia stenophylla*.

The antimicrobial potential was screened against 25 (Gram-positive and Gram-negative) microorganisms using seeded agar plates with wells. 10 μl of extract was introduced and inhibition of zones was measured after 48 hours. Antifungal assessment was against *Aspergillus niger*, *A. ochraceus* and *Fusarium culmorum* in flasks of YES broth. Antioxidant activity of the essential oils was assessed using the bleaching of ß-carotene in agar (Araujo and Pratt).

The essential oils were screened for pharmacological action using the isolated field-stimulated guinea pig ileum, rat stomach and *Taenia coeci* preparations. The smooth muscle preparations were mounted in 30ml organ baths containing Krebs bicarbonate buffer maintained at 34°C and gassed with 95% O_2 in CO_2. A tension of 1g was applied and changes in tension were recorded with an isometric transducer attached to a pen recorder. Field stimulation was applied by two parallel platinum electrodes placed either side of the tissue; these were attached to a stimulator (0.5 ms pulse, 0.1 Hz, 70 V).

The results for bacterial inhibition are shown in Table 1. The essential oil of *H. oderatisimum* was effective against all Gram positive and Gram negative organisms except for *Lactobacillus plantarum* and *Streptococcus faecalis* ; *S. stenophylla* essential oil was effective on all but *Beneckea natriegens*, *Erwinia carotovora*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Streptococcus faecalis*. Both *Helychrysum* and *Salvia* essential oils were effective antifungal agents, against all three fungi tested. Both essential oils were potent antioxidants, but colour retention in each case was low.

The essential oil diluted in hexane produced a contraction in the electrically stimulated guinea-pig ileum. This was a long lasting effect and was reversed by washout. There was a comcomitant inhibition of the ES contractions; this effect lasted well after washout.

When the essential oil was diluted in methanol at the same concentration, there was an increase in tone which was maintained until washout and again a rapidly developing inhibition of ES which persisted until washout. Returning to normal took a considerable time. The results using the two solvents were virtually identical and other tissues showed that an increase in tone without a contraction was possible using the hexane diluted extracts.

There appears to be two components responsible for the dual action of the essential oil. The first is a contractory effect on smooth muscle and the second an inhibitory effect to ES contractions. Although the mechanism of action has not been fully studied, the inhibitory either as an astringent or smooth muscle relaxant. The hexane diluted essential oil of *Salvia stenophylla* caused an increase in tone of the simulated guinea-pig ileum. There was a definite inhibition of response to electrical stimulation (ES) and a considerable delay in recovery after washout. The essential oil diluted in methanol again produced the same effect. This confirmed that the solvent has no effect on the tissue. A ten-fold concentrated sample showed a profound contraction - a similar response was also obtained using the hexane extract. Recovery of the tissue was extremely long.

The results indicate that more than one component is responsible for the pharmacological action: one component causes a contraction, whilst the other causes an inhibition of the ES induced contraction, which is long lasting and resistant to washout.

The site of action of the second component is likely to be post-synaptic and probably due to the relaxation of the tissue. This view is supported by the results in preparations of *Taenia coeci*. There is now some pharmacological evidence for the folk-medicinal usage of this plant. There seems to be some scope for the further development of the plant as a tobacco substitute as it is already mixed with tobacco for smoking in Southern Africa.

Table 1: Measurement of bacterial inhibition (zone in mm)

	Plant Essential Oil	
Organism	*Helichrysum*	*Salvia*
Acinetobacter calcoacetica	9.4	26.0
Aeromonas hydrophila	9.0	27.0
Alcaligenas faecalis	17.2	23.0
Bacillus subtilis	11.8	8.7
Beneckea natriegens	8.0	4.0
Brevibacterium linens	0.5	28.2
Brocothrix thermosphacta	8.8	16.0
Citrobacter freundii	9.6	15.0
Clostridium sporogenes	18.5	31.0
Enterobacter aerogenes	8.2	8.8
Erwinia carotovora	8.0	4.0
Escherichia coli	8.2	2.0
Flavobacterium suaveolens	9.9	17.0
Klebsiella pneumoniae	6.5	4.0
Lactobacillus plantarum	4.0	13.5
Leuconostoc cremoris	10.5	20.0
Micrococcus luteus	8.3	17.4
Moraxella sp	10.8	8.3
Proteus vulgaris	8.6	4.0
Pseudomonas aeruginosa	7.0	4.0
Salmonella pullorum	7.0	7.4
Serratia marcescens	6.0	8.3
Staphylococcus aureus	8.8	13.4
Streptococcus faecalis	4.0	4.0
Yersinia enterocolitica	6.9	10.2

P60 A Study of the Agrochemical and Pharmacological Properties of the Essential Oils of *Tagetes minuta* (*Asteraceae*), *Lippia javanica* (*Verbenaceae*) and *Barosmia cremulata* (*Rutaceae*)

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Tagetes minuta, Lippia javanica and *Agathosma betulina* are widely used in South Africa in different ways. *Tagetes minuta* is used as a fly and vermin repellant (largely due to its disagreeable odour). It is marketed in a flea-repellant dog shampoo in South Africa. The plants, grown as a manure crop, have a marked insecticidal action on the rootknot eelworm; houseflies and blowflies are repelled by the oils; larvacidal action is shown by the oil when mixed with lanoline and a preservative (Gunther). Unfortunately some allergic photosensitivity has been shown in man when the concentrated oil has been applied to the skin.

L. javanica is used as a folk medicinal herbal tea for coughs, colds and influenza. *A. betulina* is again a folk medicinal plant of long standing, used in a brandy or vinegar tincture for kidney and urinary tract diseases and also externally for bruises, rheumatic pains. A tea is taken to stimulate perspiration in rheumatism and gout and for bladder disease.

The antimicrobial potential was screened against 25 (Gram positive and Gram negative) microorganisms using seeded agar plates with wells. 10μl of extract was introduced and inhibition zones measured after 48 hours. Antifungal assessment was against *Aspergillus niger*, *A. ochraceus* and *Fusarium culmorum* in flasks of YES broth. Antioxidant activity of the essential oils was assessed using the bleaching of ß-carotene in agar (Araujo and Pratt).

Cabbage leaves were sprayed on the surface with the individual essential oils (ie non-systemically). 50 female whitefly or thrips were introduced onto each leaf in separate sealed containers. Recordings of the white flies settled on the leaf were made after 24 hours and 5 days.

The essential oils were screened for pharmacological action using the isolated field-stimulated guinea-pig ileum, rat stomach and *Taenia coeci* preparations. The smooth muscle preparations were mounted in 30ml organ baths containing Krebs bicarbonate buffer maintained at 34°C and gassed with 95% O_2 in CO_2. A tension of 1g was applied and changes in tension were recorded with an isometric transducer attached to a pen recorder. Field stimulation was applied by two parallel platinum electrodes placed either side of the tissue ; these were attached to a stimulator (0.5 ms pulse, 0.1 Hz, 70 V).

Agathosma was the most effective antimicrobial essential oil while *Lippia* was the least effective. Overall there was no bias towards either Gram positive or Gram negative bacteria. The antifungal action indicates that *Lippia* essential oil is very effective as an antimycotic agent , high levels of inhibition being recorded at even lower concentrations. *Tagetes* was also effective but at a higher concentration. *Agathosma* was not studied.

There was considerable antioxidant action by *Tagetes* and *Agathosma* essential oils, colour retention was however low. *Lippia* was ineffective altogether. There was no significant insect repellent action of the essential oils, against whitefly and thrips, suggesting that the insecticidal use may be specific and restricted to South African species in the case of *T. minuta*.

In the case of the pharmacological action of *T. minuta* there was a rapid contraction with increase in tone which was maintained until washout plus an inhibition of the electrically stimulated contractions (ES). In the guinea-pig ileum the inhibition persisted for a long period after washout. The trace showed that inhibition lasted more than an hour and included a 30 minute period where the ES was discontinued and was still recovering after ES was resumed. When the hexane extract was diluted in methanol it produced an increase in tone in the ES guinea-pig ileum with a rapid reduction in response to ES. There was an irregular recovery on washout . This shows that there is virtually no solvent effect on the tissue. The hexane diluted extracts of *L. javanica* caused an increase in tone of the ES guinea-pig ileum; on larger doses a contraction was obtained. There was also a concomitant inhibition of the ES induced contraction. The inhibition persisted after washout.

When the essential oil was diluted in methanol there was a similar effect. The trace showed that the inhibition of the ileum contractions lasted for over an hour as there was a break in recording for 30 minutes when the ES was stopped, and when ES was resumed there was a gradual return to normal. Hexane extracts caused a relaxation of the rat stomach strip, following an initial contraction. There was a definite reduction in tone and loss of spontaneous activity. When the essential oil was diluted in methanol a relaxation followed and the return to normal took a long time even after two washes.

A. betulina produces a profound contraction which is shown for both the unstimulated and ES guinea-pig ileum. This response is dose related. The response is also unassociated with the solvent dilution, as shown for both the hexane and methanol diluted essential oil. There was also an inhibition of ES contractions which was not long lasting after washout. The contraction was resistant to atropine, whilst acetyl choline was obliterated and histamine was reduced to half. The results suggest the main effect of the essential oil of *A. betulina* on smooth muscle is contractile. There is also some evidence for secondary effect - that of inhibition of ES induced contractions. This effect is probably post-synaptic. The contractions are not blocked by atropine but present results do not indicate the exact mode of action.

In conclusion the essential oils of *T. minuta*, *L. javanica* and *A. betulina* have at least two components. One component causing an increase in tone or a contraction, whilst the other causes an inhibition of ES induced contractions on the guinea-pig ileum. The effect on rat stomach was similar. The site of action of the second components is likely to be post-synaptic and is probably due to the relaxation of the tissue. This view is supported by the studies on rat stomach *Taenia coeci*. The relaxatory effect on smooth muscle may explain the folk medicinal usage of *Lippia* in the treatment of coughs.

Table 1: Measurement of bacterial inhibition (zone in mm)

Organism	Plant Essential Oil		
	Buchu	*Lippia*	*Tagetes*
Acinetobacter calcoacetica	6.9	4.0	8.7
Aeromonas hydrophila	5.8	7.9	10.5
Alcaligenas faecalis	5.6	4.0	9.7
Bacillus subtilis	6.2	14.4	10.5
Beneckea natriegens	10.9	4.0	7.5
Brevibacterium linens	11.1	4.0	23.0
Brocothrix thermosphacta	9.5	7.8	10.6
Citrobacter freundii	16.0	4.0	4.0
Clostridium sporogenes	9.4	4.0	4.0
Enterobacter aerogenes	9.4	4.0	4.0
Erwinia carotovora	4.0	4.0	6.1
Escherichia coli	7.5	4.0	6.3
Flavobacterium suaveolens	8.0	8.4	14.4
Klebsiella pneumoniae	4.0	4.0	4.0
Lactobacillus plantarum	9.2	4.0	10.1
Leuconostoc cremoris	10.9	4.0	11.3
Micrococcus luteus	8.0	4.0	9.0
Moraxella sp	4.0	4.0	4.0
Proteus vulgaris	10.8	4.0	8.5
Pseudomonas aeruginosa	4.0	4.0	11.0
Salmonella pullorum	12.2	4.0	4.0
Serratia marcescens	19.0	5.8	4.0
Staphylococcus aureus	8.2	4.0	24.0
Streptococcus faecalis	8.2	4.0	4.0
Yersinia enterocolitica	8.3	4.0	8.0

P61 Flower Volatiles: Scent Cacophony or Adapted Blends?

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A literature survey of publications reporting the chemical composition of floral scents collected by head-space methods was made. From 174 studied plant genera a total of over 700 scent compounds are reported. Most of the flowers share some "typical" volatiles. The most common compounds are isoprenoids and benzenoids.

Based on these data we suggest a basic floral scent to be composed of monoterpenes (eg limonene, myrcene, linalool) and benzenoids (eg methyl 2-hydroxybenzoate, benzaldehyde, benzyl alcohol). Such basic scent can be a general signal for the presence of a flower. Within the different pollination syndromes scent chemistry is expected to be modified and adapted to certain groups of pollinators. The basic scent as well as specialised scent blends may be of mutual advantage to both plants and pollinators.

Multivariate data analysis is used to recognise patterns of variation in floral scent chemistry. Variation among individuals, populations and taxa is considered. Two dimensional plots make good overviews of chemical relatedness of individual plants. In a study of two species of moth-pollinated *Platanthera* (Orchidaceae) species, scent composition varied more within than between species. In the morphologically generalistic *Angelica* (Apiaceae) scent profiles suggest selection for specific scent chemistry in the different taxa. These two sets of data show patterns of variation which complicate interpretations of floral scent specialisation. We want to emphasize the importance of replicate sampling for biological interpretations of floral scent data.

P62a APPLICATION OF MUTIVARIATE ANALYSIS IN STUDYING THE BIOGENESIS OF MONOTERPENE ENANTIOMERS IN PINE NEEDLE OIL

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Finnish Scots pine populations (*Pinus sylvestris L.*) are characterized by high and low 3-carene chemotypes with varying amounts of α-pinene (1). A considerable enantiomeric variation of α-pinene was recently found in these chemotypes (2). Biosynthetic studies on sage have shown the occurrence of two stereospecific enzymes pinene cyclase I and II, which produce the (+)R-and(-)S-series of monoterpene enantiomers, respectively (3). A multivariate analyses was applied in order to study the enantiomeric groups of monoterpenes and their biogenesis in the needle oil of Scots pine.

The materials consisted of parental trees of high (N-6) and low 3-carene (N-3, 3-carene below 4%) chemotypes and their crosses (N-11, partly resiprocal). A total of 147 GC analyses of monoterpene hydrocarbon fractions were made using both heptakis-β-cyclodextrin and achiral NB-351 columns.

Table 1. Propoportions (%) of the enantiomers in the monoterpene fraction of pine needle oil.

Compounds	Low 3-carene chemotypes (n-63)						High 3-carene chemotypes (n-84)					
	R(+)-series			S(-)-series			R(+)-series			S(-)-series		
	Mean	Range	Sd	Mean	Range	Sd	Mean	Range	Sd	Mean	Range	Sd
-Pinene	55.2	11.6-73.2	12.9	23.3	10.4-62.3	9.9	39.4	8.6-69.3	16.5	14.5	4.6-57.0	9.2
Camphene	1.2	0.7- 1.9	0.3	6.5	3.2-12.9	2.4	1.8	0.6- 2.9	0.5	5.4	0.7-12.5	2.5
-Pinene	0.5	+ - 1.0	0.2	4.2	+ -13.9	3.3	1.1°	+ - 7.8	1.5	0.8°	+ - 6.1	1.2
Limonene	0.6	0.1- 1.4	0.3	0.8	0.1- 2.9	0.5	0.5	+ - 1.1	0.2	0.5	+ - 1.9	0.4

°not completely separated from 3-carene

The results show that the variability of α-pinene enantiomers is rather similar in both chemotypes, the proportion of R(+)-α-pinene being more than twice as that of S)-)-α-pinene, on the average (table 1). Camphene and β-pinene occurred primarily as S(-)-enantiomers.

Table 2. Groups of monoterpenes most often linked with each other (cluster analysis).

Low 3-carene chemotypes		High 3-carene chemotypes	
R: (+)- -pinene	S: (-)-α-pinene	R: (+)-α-pinene	S: (-)-α-pinene
(+)- -pinene	(-)-camphene, tricyclene	3-carene, terpinolene	(-)-camphene, tricyclene
	(-)-β-pinene	(+)-camphene, sabienene α-thujene	

Enantiomeric group of the major monoterpenes could be clearly distinguished by cluster analysis (Table 2). R(+)-α-Pinene often formed a single group. The close linkage between S(-)-α-pinene, tricyclene and (S)(-)-camphene found in both chemotypes supports the occurrence of a common precursor for these compounds (4S-1-p-menthene 8-carbonium ion). The results also suggest that 3-carene and related compounds (Table 2) are more closely related to the R(+) enantiomeric pattern.

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