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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Grower Summary

Headline

This project has identified key factors affecting yield, essential oils, aroma, flavour and shelf life of a range of herbs including basil, chive, coriander, dill and rosemary. It has also highlighted gaps in our knowledge to assist in the planning of future work for the UK herb industry.

Background and expected deliverables

Changes in consumer buying and eating habits have induced a dramatic growth in the fresh herb market over the last decade and herbs now take a considerable share of a UK-grown horticultural market. Multiple retailers are increasing pressure on growers to provide a stable supply of high-quality products with sustained flavour, lush appearance and long shelf-life. Although, from the consumers' point of view, herbs may appear as a unified item, in reality they are a group of species of different botanical and geographical origins, each having its own optimal climatic preferences, with high intra-species variability and even intra-varietal chemical diversity.

Research on herbs remains sparse and non-systematic, full of apparent or real contradictions, published in numerous different sources and very rarely reviewed. Consequently, it is difficult for growers to acquire relevant information and trust the available sources. A review of the literature describing the current state of research on five herbs has been undertaken and areas where future research might benefit growers identified.

Summary of the project and main conclusions

The review begins with a general description of the current research situation on herbs. This is followed by detailed information on five herbs: basil, chives, coriander, dill and rosemary, and a bibliography for mint. Finally, current storage and packaging practice is discussed. In addition, information is provided on biosynthetic pathways and the ways in which the production of oils might be modified.

Basil

Basil is fairly well investigated, although little of this research has been focussed on the environmental conditions found in the UK. Growing conditions need evaluating as do, for example, the development of chill-tolerant varieties, flavour and toxicity. In particular, applied projects targeted to particular regions of the UK are required. We suggest that the following key issues are gaps in our knowledge and need to be investigated further:

- Non-toxic varieties of high total quality, adapted to UK growing conditions.
- Chilling tolerance in basil, which will decrease waste due to poor shipment and storage conditions.
- Optimal growing conditions for high essential oil and biomass yield in UK-grown basil.
- Evaluation of flavour and research on flavour components in basil to find non-toxic substitutes for methyl eugenol and methyl chavicol.

Chive

Chive is virtually unstudied. Since consumption of chives is valuable for human health - it has been shown that the *Allium* species help to prevent tumour formation, cardiovascular diseases and aging, consumption of chive is therefore in line with government guidelines on 'healthy eating'. The key issues to address are as follows:

- Search for the 'best varieties' for the pot and field grown herb industry in the UK.

- Understand what the optimal growing conditions are, especially the effect of light (intensity, quality, photoperiod), fertilisation and irrigation, that are best for production of flavour substances and biomass in chives.
- Evaluate and understand the chemical composition of chives and the components of its flavour.

Investigation of guttation in chive, as it detrimentally affects appearance, is a special consideration, and among the six herbs we have reviewed, only the appearance of chive is affected by guttation. If growers want to decrease guttation in chive, water relations (root water uptake versus transpiration) and the dependency of chive on irrigation, nutrition and light require investigation.

Coriander

As the review shows, coriander as a herb is not well studied. Most research on coriander has been performed with the aim of improving seed yield: these reports are not included in the review, but their total number is probably 5-6 times higher, than that on leaf production. The issue of research is also complicated by the fact, that coriander leaf (cilantro) is only very popular in cooking in a few regions of the world. The UK and the former USSR are more difficult places to grow coriander, in terms of climate, than Latin America and South Asia where the herb is popular and where it grows well in every garden. As a result, there has been little research to investigate cilantro production in Latin America or the Indian subcontinent. The most vigorous investigations were conducted in the former Soviet Union, but there is currently no research programme in that country. It is therefore apparent that British growers will have to be involved in forwarding their own understanding of coriander if they are keen to improve the quality of this crop. .

The key gaps in our knowledge are:

- Understanding which varieties are the best suited for UK pot and field production.
- Understanding of the flavour components/constituents in coriander and the effect of growing conditions on these and the composition of coriander oil.
- How to optimise growing conditions, especially those that increase shelf-life, improve quality and decrease supermarket waste.

Dill

Research has been performed on early and medium varieties, and rarely on late or bush varieties, which are the most suitable for dill herb production. No research has been conducted on irrigation regimes or the effect of mycorrhizas. There are a range of results reported on the requirements for fertilisation and light conditions from a number of countries but much of this is rather controversial. The British climate is very suitable for dill herb production, much more so than in Scandinavia or India where crops are widely grown. With improved quality through research and an increase in Slavic migration dill consumption is likely to rise, as it is a favourite herb in Eastern Europe.
Key areas for future investigation are:

- The evaluation of cultivars suitable for different regions of the UK.
- Understanding the constituents of flavour in dill.
- Evaluation of cultivation practices including the use of mulches to reduce weeds.

Rosemary

There are only a few studies published on rosemary and these have been performed in hot and dry Mediterranean climates and appear to be less relevant to UK growing conditions. However, we think, that it is probably unnecessary to undertake research relevant to the UK climate, since rosemary grows so well in the Mediterranean region. There is also money allocated to make it a profitable African crop, consequently British growers will soon be likely to find it difficult to compete with cheap products from these regions.

A bibliography is provided for **spearmint**, to enable growers to analyse the information available.

Insufficient information currently exists for herbs to describe optimum storage conditions for each species. More research is needed to provide this detailed information and to suggest the best packaging and storing techniques.

Financial Benefits

This review of current available research has been undertaken as a step towards improving the quality of herbs, i.e. shelf-life, appearance, yield and flavour. It should provide growers with an understanding of what is currently known to be the best horticultural handling and storage practices available.

This information also helps to identify gaps in our knowledge so that the herb industry can plan for the future with a research and development strategy to improve herb yields, quality and shelf life.

Action points for growers

Growers need to consider the points raised in this review which is a first step to unravelling the intricacies of future research. There is a need for growers to digest the wealth of information currently available and integrate this with other sources of knowledge, such as personal experiences, to enable realistic research targets into herb production to be set. This review can be now used to highlight gaps and priorities for the coming years.

Science Section

1. Introduction

The herb industry is a relatively young branch of horticulture which has developed, especially in the UK, over the last two decades as a potentially profitable venture. The recent development of herb production does not, however, reduce the pressures for research and development. Herb growers face the same challenges as for other crops - productivity should be high with a long harvesting-window and the product should have a good appearance with an excellent aroma and long shelf-life. The striking difference between the UK herb industry and the production of more traditional crops is that the latter has been the subject of attention from scientists for two centuries or more, and so is a long way ahead of herbs in terms of the choice of varieties best adapted to local climatic conditions. If herb growers want to compete over the long term with traditional crops they should act swiftly to produce quality products that will remain attractive to the market.

Although, from the consumers' point of view, herbs appear as a unified item, in reality they are a group of species of different botanical and geographic origin, each having its own optimal climatic preferences, with high intra-species variability and even intra-varietal chemotypic diversity. Herbs are generally retailed for culinary purposes, both as a fresh product, which may be cut or pot-grown, or dried (this review does not address the issue of production of herbs sold and used as seeds). Here we focus on six key herbs, basil, chives, coriander, dill, mint and rosemary, selected by the industry. For the grower of fresh herbs, the presentation of an attractive product with good culinary properties that encourages repeat buying by the consumer is a critical issue. For the grower of pot-herbs, the demands of transport and display can mean their product has a poorer appearance than when it left the glasshouse. In the cut-herb market, there is an increasing move for retailers to display products in stands with ambient conditions, but currently ambient-stored cut herbs are limited by an extremely short shelf life. Coriander and dill have the shortest shelf life of the key group of herbs.

Flavour perception is an important element in determining both choice and acceptability of food products, but flavour of coriander, dill, mint, basil and many other herbs does not necessarily persist unchanged through the seasons and deteriorates quickly during storage, thus failing to meet consumer expectations. Meteorological conditions, fertilisation and irrigation can all affect flavour and shelf-life. Research on herbs remains sparse and non-systemic, full of apparent or real contradictions, published in numerous different sources and very rarely reviewed.

We begin this review with a general description of the current situation in research on herbs. Then we provide detailed information on five herbs: basil, chives, coriander, dill and rosemary with a bibliography provided for mint. Finally, we discuss storage and packaging. In addition we provide information on biosynthetic pathways and the ways in which the production of oils might be modified.

2 General description of research on herbs

2.1 Can diversity improve herb quality?

Varietal selection has always been key to success for traditional crops and the same applies to herbs. However, for some reason, the choice of the best varieties has been almost ignored by the UK herb industry - and sometimes by researchers, although many varieties, cultivars, wild and cultivated populations are stored in national and international genebanks.

Four of the six herbs on which this report is focussed - basil, coriander, dill and chives - are already recognised worldwide as crops, so their varieties[1] are collected and properly stored.

The biggest collection is of **basil** (156 varieties) is stored in the Vavilov Research Institute of Plant Industry (VIR), Russia (http://www.vir.nw.ru/index.htm). Basil is also collected by the United States Department of Agriculture (USDA; 65 varieties) (http://www.ars-grin.gov/npgs/searchgrin.html) and in the Research Institute of Crop Production (RICP– 46 varieties), Czech Republic (http://genbank.vurv.cz/genetic/resources/).

There is a European collection of **chive** (282 varieties) stored in ECP/GR European Allium Database, Warwick, HRI – see:
(http://www2.warwick.ac.uk/fac/sci/whri/about/staff/dastley/gbrhrigru/ecpallium/).

The largest collection of **coriander** is stored in VIR, Russia (334 varieties); the Institute for Plant Genetics and Crop Plant Research (IPK), Germany (http://www.ipk-gatersleben.de/Internet) has 237 varieties and the USDA, USA has 164 varieties.

There is a large (481 varieties) collection of **dill** in VIR, Russia. The USDA, USA has 87 varieties and in RICP, Czech Republic, 23 varieties are listed.

Spearmint and **rosemary** have only recently achieved the level of production comparable with basil, coriander and dill, and are not yet properly collected and stored. Some spearmint (116 varieties) are stored by USDA, USA. Rosemary is now recognised as an important crop for Africa (http://www.asnapp.org/resources/plantlist.html) and efforts are being made to establish its collection.

It is interesting that sizeable collections of basil, spearmint, coriander, chive and dill are stored in two huge Asian genebanks – CAAS, China and ICRISAT, India. But the former database is indexed in Chinese and the latter one has no on-line search. It is also important to note that many interesting and rare varieties of herbs can be obtained from the Institute of Roses, Aromatic and Medicinal Plants in Bulgaria, upon personal communication. In our report we provide a description of the range of available varieties and also general recommendations of which varieties should be used if high biomass and essential oil production is desirable.

There are few true varieties of any given species of herb, see above and in 3.1, 4.1, 5.1, 6.1, 7.1. They often would not pass the rigorous varietal testing regulations, including distinctness, uniformity and stability, as required by the European Community. Organisations with commercial interests in such high value species as mint, basil, tea tree, thyme and lavender usually perform these studies and the results are often confidential. Anatomy, embryology, palynology, chemistry, morphology and molecular biology (DNA sequencing and PCR-based assays) all contribute to the complex task of establishing a systematic description of those species. New plant breeding technologies, including transgenic techniques are now employed in many aspects of plant breeding of aromatic plants, aiming to achieve higher yields, an optimal composition of the particular essential oil and disease/pest resistance and consequently these are included in the report (Section 11).

2.2 Parameters of growth conditions which should be monitored.

Understanding how growth conditions (soil, temperature, light, water and nutrients) influence crop productivity provides a grower with the ability to predict yield and plan harvests. Manipulation of growth conditions can improve crop performance in unfavourable

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[1] In this review all genotypes taken into cultivation, whether they are ecotypes, breeding lines, or cultivars are called 'varieties', because it is not always possible distinguish their true status from published reports.

weather. However, research on how growth conditions influence herbs is sparse, although such knowledge could bring large gains in yield of green biomass and of essential oils. In this review we report what has been done, and suggest what might be done, for each of the six herbs. Soils preferred by each herb (their composition, nutrient value, texture and pH) are described in popular manuals on herb cultivation and have rarely, to the best of our knowledge, been scientifically investigated. Consequently, we have not included a description of soils into our review. Irrigation has been more widely studied and corresponding sections are included for each herb.

Crop yield is highly dependent upon fertilization. However, nutrient requirements are species-specific and so should be carefully adjusted for each herb. Macronutrients (N, P, S, K, Ca, Mg) and micronutrients (Fe, Mn, Cu, Ni, Mo, B, Cl) are absolutely essential for production of green biomass and essential oil. The form of element delivery to the plant is also very important: N comes in nitrate, ammonium or urea and plants have evolved, enzymatically, to utilise one form or another. The problem for Ca, Mg and P supply is their solubility, so measures should be taken to avoid precipitation. A further complication of adjusting nutrient levels is that plants can accumulate nutrients to excess, which, although not harmful to the plant, may become toxic for humans. Thus all these parameters (the element, its concentration and its form) have to be taken into consideration when optimising nutrient supply for production of biomass and essential oils. Such studies should be performed systematically for herbs although there occurrence is very patchy.

Light acts dually on plants. Firstly, through photosynthesis providing production of dry matter. In poor light condition production of green biomass and appearance are likely to be affected, however, optimal light does not necessarily mean full sunlight as many plants are adapted to shade and full sunlight is a stress for them. Secondly, light acts through three systems of receptors: red/far-red light receptors - phytochromes, blue/ UV-A light receptors – cryptochromes and phototropins, and UV-B light receptors – still unknown. Each photoreceptor systems controls certain steps in plant development and light quality is able to switch off a vegetative period and induce flowering, fruiting, bulbing and dormancy. This control is species-specific. As a result three important parameters need to be monitored when an optimal light regime is investigated: photoperiod (day-length), light intensity and light quality.

There is usually a range of temperatures in which the growth of a given plant species is possible and large deviations from the temperature range in which the plant has evolved are generally unfavourable to growth or can be detrimental. Small variations from normally tolerable temperatures can be obtained by developing chill/heat hardiness. The effects of temperature can be accumulated by plants during a season, so growth conditions are often described in terms of ‘day-degree’. Day-degree is a more useful parameter than temperature itself, especially when plants are grown in a continental climate. Finding the optimal day-degree regime rather than temperature may help save energy in greenhouses and extend season in field conditions.

There are two non-traditional ways to improve herb quality, which are now more and more studied. The first is the application of phytohormones (auxins, cytokinins, gibberellins) and vitamins (e.g. folic acid), which are known to promote plant growth and development. The second is the application of “artificial signals”, such as elicitors or plant stress signals. Elicitors (molecules derived from structural substances in cell walls of a microorganism or fungus) are now used to mimic biological stress (microbial attack), which often induces a plant to produce more essential oil (terpenoids and phenols), as they play a role in defence and have also been shown to promote plant growth. Stress signals are molecules that are synthesised by the plant itself in response to biotic and abiotic stress (methyl jasmonate and methyl salicylate), and which may, when applied artificially, promote essential oil production.

2.3 General information on volatile oils and secretory structures

Since the culinary value of herbs is based on their content of particular suits of chemicals, we summarise here the ways in which plants produce, store and secrete compounds that determine their aroma; compounds which also make major contributions to the flavour of herbs, although other contributors to flavour such as texture and tastes of sweet, bitter, acidic are also present (Bauer *et al*., 1997). The UK Food Standards Agency and the US Food and Drug Administration do not impose any standards for essential oil composition in fresh herbs; however the producer should be aware of the key flavour substances for each herb, and problems associated with variability in these components. This will be important in a grower's choice of seed or propagules, and growing conditions. In the text tables 1-6 we list the major components in the essential oils of five selected herbs. In the review we distinguish between 'herb oils' – those present in leaves and stems – and 'seed oils'. Variations in the chemical composition of essential oils within a given species are referred to as chemotype, e.g. basil oil has methylchavicol or linalool chemotypes (see also 3.3).

2.3.1 Role of secondary metabolites in plants

Plants produce flavour substances through both primary and secondary metabolites (Crozier *et al*., 2006). Primary metabolites are those compounds usually produced during photosynthesis and respiration, and are involved in the fundamental processes of growth, development and regulation. Primary metabolites mainly contribute to taste of plants: sweetness, bitterness, acidity, texture most often come from these chemicals; they also contribute to odour - aromatic compounds of many fruits and leaves, coriander in particular, are produced through pathways of primary metabolism. Plants also synthesise secondary metabolites, including tannins, coumarins, anthraquinones, flavonoids, saponins, steroids, glycosides, alkaloids and terpenes, which contribute to taste and aroma (Hay and Waterman, 1993; Tisserand and Balacs, 1996; Walton and Brown, 1999). Their functions are not completely understood, but it is thought that they attract insects and animals for pollination, deter predators, inhibit growth of fungi and bacteria, heal plant wounds and act as bioactive agents in allelopathy. Many terpenes are toxins, acting as feeding deterrents to herbivores or oviposition deterrents to insects; they have diverse modes of action. The production of essential oils has also been considered to be part of an adaptive strategy of the plant, making it more fit to survive in any particular environment.

In every plant volatile oil is a complex mixture, often with over 100 constituents. Plant tissues can be highly specialized for the synthesis and secretion of secondary metabolites (Svoboda and Svoboda, 2001). Plant secretory structures are divided into two main groups: those that occur on the plant surface, secreting substances directly to the outside (exogenous secretion) and those within the plant, secreting substances into specialized intercellular spaces (endogenous secretion). The simplest endogenous secretory structures are single cells called secretory oil cells (e.g. in bay laurel, lemon grass, cardamom, ginger). Secretory cavities form unconnected reservoirs in the plant tissues, for example they are distinct features in cloves, pimento, myrrh and the frankincense tree, and in all citrus fruits (Weiss, 1997). Secretory ducts form networks of canals, often branching, from the roots through the stem to leaves, flowers and fruit. In fruit, secretory ducts are known as vittae. Oil ducts are common in anise, fennel, dill, parsley, coriander and lovage.

On the surface of plants exist modified epidermal hairs called glandular trichomes – some more detail can be found in Wagner *et al.*(2004). Trichomes are the primary sites of volatile oil production and their density and morphology varies between different species, organs and tissues, and with the ontogeny of the plant (Hornok, 1992). The number of the glands and their distribution are genetically coded and cannot be influenced by external factors; they are stable for any given species. These characteristics are useful for identification in plant taxonomy and drug adulteration. For example, *Melissa officinalis* is a very low yielding species (less than 0.05% oil v/w) with a very low density of glands (10-20 cm^2). In comparison, oregano has an oil yield of 3-5% v/w, and a high gland density (80-150 glands/cm^2).

Basil and mint have glandular trichomes on the epidermis of leaves and stems whereas dill, coriander and caraway have longitudinal ducts forming a network extending from the roots through the stem to the leaves, flowers and fruits.

Essential oils obtained from flowers such as roses, are usually not secreted by glandular trichomes, but by the epidermal cells of the petals. Specialized structures, nectaries and osmophores, produce fragrances and are found mainly in flowers and fruits. The fragrance may vary with flower age, abiotic factors, especially humidity and air turbulence, and even be diurnally controlled by circadian rhythms.

The chemical constituents are not static components of the plants, but are under the influence of active metabolism. Various extrinsic factors also influence the absolute amount of oil present in the plant and the relative composition of individual components. Light can significantly change the oil composition, and has been investigated in thyme, basil (see 3.3.7) and savoury species. Altitude, latitude, and stress factors including high irradiance, lack of nutrients, drought, wind, disease, low temperature all contribute to pronounced changes in the composition of the essential oil. Detailed knowledge of these environmental and ontogenetic trends, and the plant response, is very important for successful growing systems and for the implementation of optimal harvest and post-harvest technologies for the essential oil crops.

The real problem is to balance production of biomass with production of essential oils. As reviewing of basil, dill, spearmint and rosemary show (and as might be expected) optimal growth conditions for biomass production never coincide with optimal conditions for production of essential oils. This is due to competition between primary (growth) and secondary (biosynthesis of terpenes and phenols) metabolism for carbon and energy. Thus for these herbs, either compromised growth conditions should be found, or production of oils should be sacrificed to biomass or *vice versa*. For other herbs – chives and coriander - optimal growth conditions for essential oil production are yet to be investigated, but their components of essential oil and flavour originate mainly from primary metabolism, so it is possible that growth conditions optimal for biomass production will coincide with optimal conditions for high essential oil yield.

3 Basil – *Ocimum basilicum* L.

3.1 Diversity of basil

Basil as a species (*Ocimum basilicum*) is characterised by high diversity, having many sub-species, varieties, cultivars and populations in cultivation. All these forms appeared by cross-pollination by bees of sub-species and, following hybridisation, have produced abnormal and sub-normal types with unstable composition and content of essential oils. Thus an informal mixed chemical-geographical classification of genotypes within the species is commonly used (Vernin and Metzger, 1984; Marotti *et al.*, 1996; Bowes and Zheljazkov, 2004 - see also 3.4).

1. **European type.** Cultivated in Italy, France, Germany, Bulgaria, USA, Israel, Egypt and South Africa as sweet basil - *O. basilicum* and several subspecies: *O.b. var. purpurascens* Benth*, O.b. var. thyrsiflorum* Benth*, O.b. var. album* Benth*, O.b. var. crispum* E.G. Camus. This type mainly contains methyl chavicol and linalool, and no camphor. Essential oil of this type has a high quality and fine aroma.
2. **Asian type** is rich in methyl chavicol and has little linalool and is grown in Japan, China and Taiwan.
3. **Reunion type** originates at Reunion Island, Islands of Comor, Seychelles, Thailand, Vietnam and Madagascar. The botanical classification of these plants is very uncertain. Oil is of low quality, contains mainly methyl chavicol, camphor, and no linalool.

4. **Tropical type** is traded in India, Pakistan, Guatemala. Oil contains methyl cinnamate, along with methyl chavicol and linalool. This type is not used by the food industry.
5. **Eugenol type** is grown in Java, Samoa, Seychelles, North Africa and former USSR. The main component of the oil is eugenol; other components vary greatly. Populations taken into cultivation may originate from *Ocimum basilicum* L. *var. selasih mekah* and *O. b. var selasih besar*, but classification is again uncertain: plants from Java, Samoa, Seychelles may be attributed even to other species of *Ocimum – O. minimum* L. or *O. gratissimum* L.

Thus the basil grown and sold as an herb could be of a variety of types: in Europe at least, it is likely to be mainly the European type. Many varieties of basil are known and sold by seed companies: 'Genovese', 'Thai', 'Liquorice', 'Spicy Globe', 'Nufar', 'Magical Michael', 'Lettuce Leaf', 'Mammoth', 'Red Rubin', Purpurascence', 'Opal', 'Bush', 'Lemon basil' and others. Varieties differ in leaf colour (black, purple, silver and green), leaf form (spoon, lettuce, narrow and crinkled) and size. Varieties of Asian and Eugenol types are less known in Europe than the other types.

3.2 Changes in yield and appearance due to diversity and growth conditions

3.2.1 Varieties

We did not find any systematic research into varietal differences in productivity. Most investigations involve a single variety; in rare instances (as a side study) comparisons of two or more genotypes have been compared. For example sweet basil (*O. basilicum*) produces twice the herb biomass of opal basil (*O. b.* var. *purpurascence*) (Suh *et al.*, 1999). Cultivars 'Mostruoso mammouth' and 'Napoletano a foglia di lattuga' were 60% more productive than 'Genovese profumatissimo' (Sifola and Barbieri, 2006). In another study no difference was found in productivity between 'Mesten' and 'Italian Broadleaf' (Bowes and Zheljazkov, 2004). Different varieties of Eugenol type of basil have been trialled in field experiments in Georgia (former USSR) and several were selected as producers of high biomass (Alborishvilli, 1984). **We recommend an investigation of biomass production by different genotypes of basil as such a study has never been performed in the UK.**

3.2.2 Ontogenesis

Biomass production of basil depends on the stage of plant development, as leaf production slows or stops on any stem which flowers (Szabo and Bernath, 2002). Again, no systematic research has been undertaken to find how to delay flowering by selection of late flowering varieties or changing the growth conditions. It is, therefore logical to recommend pinching off any flowers to keep the plant in production. Picking the leaves off the plant also helps 'promote growth', largely because the plant responds by converting pairs of leaflets next to the uppermost leaves into new stems. Indeed, the highest bulk harvest per hectare was reached in basil when it was repeatedly cut 1.5-2 cm above the root base (for 3 cuts giving total 40000-50000 kg/ha) (Alborishvilli, 1984).

3.2.3 Mulches

Black plastic (over trickle irrigation tubes) is widely used in raised-bed culture of high-value crops. These practices can conserve water, improve soil drainage, reduce the need for weed control and keep soil from splashing onto leaves. Black plastic can also change soil temperature (as black absorbs incoming radiation), so it may be possible to change the biomass production of basil varying the mulch. Black polyethylene and wheat straw were found to increase basil production when compared to bare ground - by 20% and 5%, respectively (Davis, 1994). Hardwood bark, pine bark and mixed wood chips decreased production of basil compared to production on bare ground by 30%, 35% and 20%, respectively. While 75% of plants grown in wheat straw, 61% in black polyethylene and

58% grown in bare ground had symptoms of bacterial soft rot. Surprisingly, only 5% were infected, when grown in pine bark and only 15% in hard wood bark (Davis, 1994). As the summer was very wet, the authors (Davis, 1994) concluded that in such summers, pine and hardwood mulch could effectively save basil from bacterial soft rot infection.

3.2.4 Temperature and photoperiod

As basil originated in the Mediterranean region, it is very sensitive to low temperature and prefers warm conditions. In cold conditions, even seed germination is affected, and it was shown in Israel that germination was optimal at 21-30°C during the day and 16-31°C during the night (Putievsky, 1983). For pot grown basil in a greenhouse in the UK, it was shown that plants grown at 25°C constant temperature were 30% taller and heavier (both fresh and dry weight), than plants grown at 15°C: they also had more and bigger leaves (Chang *et al.*, 2005). Plants grown at 30°C did not differ from those grown at 25°C (Chang *et al.*, 2005).

The effects of temperature can be accumulated by plants during a season, so growing conditions are often described in terms of 'day-degree'. Day-degree is a more useful parameter than temperature, especially when plants are grown in a continental climate. As chill hardening of basil occurs when it is grown at cooler condition (Lange and Cameron, 1997 - see also 4) it is important to understand how day-degree affects yield. It was shown for pot-grown basil in a greenhouse in the UK that when it was grown for 1 week at 15°C and for 2 weeks at 25-30°C, taller plants were obtained than in a reversed treatment (2 week of warm plus 1 week of cool conditions), although these plants had higher dry weight (Chang *et al.*, 2005).

It was also shown in Israel for basil growing in hydroponics, that temperature and photoperiod had an additive effect on production of biomass. Yield at low temperature (18°C day/12°C night) was very low both in short and long days; medium yield was achieved at high temperature (30°C day/12°C night) and short day (photoperiod 10 h) and the highest yield was obtained at the same temperature, but long-day (photoperiod 16 h) (Putievsky, 1983).

In the field, the effect of temperature and photoperiod was also found additive. The length of the growth period for basil was shown to change with season: plants sown in March (cool short days) reach a saleable state twice as slowly as those sown in June (hot long days) or September (hot short days) (for Georgia, former USSR) (Alborishvilli, 1984).

The effect of photoperiod alone was studied in Michigan, USA for pot grown basil. Here, long days (15-18 h photoperiod) resulted in higher production (25% increase) than in short days (a photoperiod of 9 h). In the 18 h photoperiod plants also reached a harvestable stage quicker than in the 9 h photoperiod, as inflorescence developed more rapidly (Skrubis and Markakis, 1976). ***Thus we can conclude that basil production is at its best in long warm days, although it should be noted that flowering also starts quickly under these conditions, so if flowering is to be avoided, the photoperiod should be shortened. Temperature is a major factor in producing basil; cool conditions reduce yield and this effect cannot be overcome by photoperiod.***

3.2.5 Light of different quality and quantity

Although it is recommended in popular books to grow basil in a sunny location, we did not find any research on the comparison of biomass production in sun and shade. The effect of light quality on herb production is also poorly documented, but it has been shown that biomass production is definitely sensitive to light quality. Use of plastic mulches with different surface colours (white, black, green, yellow, blue and red) has been trialled. The colour of the mulch reflects the light of respective wavelength; thus a red mulch reflects light in red-far red region (630-755 nm), a blue mulch reflects blue light (at 420-500 nm), green mulch reflects green light (at 480-530 nm) and yellow mulch reflects light at 520-580 nm.

With the use of particular mulches, the spectrum of light which a plant absorbs becomes enriched in regions, sensed by plant photoreceptors, thereby controlling its growth and development, (see 2). White mulch reflects all light, so overall light quantity is increased and black mulch absorbs all light and heat with a consequence that the temperature at the root level is increased. Trials were performed on the basil cultivar 'Italian sweet' in the field in Norfolk (UK) and in South Carolina (USA) giving the same results. It was shown that leaf area and fresh weight were significantly increased by growing the plants over red mulch and white mulch, but decreased by black and blue mulch (Loughrin and Kasperbauer, 2001). Svoboda also (pers. comm., 2006) observed significant changes in plant morphology of five selected herbs, using various type of coloured mulches. Coloured mulches and LEDs are particularly recommended, as they do not decrease light intensity as do photo-selective covers.
Basil is sensitive to light of different quality and quantity, so we recommend performing detailed research into how basil growth is promoted by light of different quality and quantity, as morphogenetically active light can increase biomass production and also postpone flowering.

3.2.6 Density

The effect of planting density in hydroponics was studied in the UK (var. Genovese). Increasing plant density up to 8 plants in a small pot (≤0.5 l) led to an increase in the yield of basil per pot. This increase was accompanied by a decrease in plant height, leaf area and number of branches. Increasing the density to 16 plants per pot did not change the yield. At this density the plant population was found to be more varied in size with a few large plants effectively shading and out-competing smaller plants for light (Smith *et al.*, 1997). ***We therefore recommend an investigation on the effect of planting density on yield of field and pot-grown basil.***

3.2.7 Nutrition

Basil is, in general, very sensitive to fertilization. Without fertilization plant growth is characterized by diffuse yellowing of lower leaves. In Italy the effect of fertilisation was studied on pot-grown basil where NPK fertilizer (20:20:20 kg NPK/ha) was found to increase green colour of leaves, plant height (by 500%), fresh weight (by 100%) and leaf area (by 500%) when compared with unfertilised plants. The NPK fertiliser was found to be optimal at a relatively low dose (100 kg/ha) with further increases (200-500 kg/ha) causing a decrease in height, fresh weight and leaf area leading eventually to the necrosis and chlorosis of the young leaves (Tesi *et al.*, 1995). The optimal N concentration found in this study of pot-grown plants was less, than required in the field (see below).

Complex fertilizer was found to increase plant biomass and height more than nitrogen fertilization alone (either as nitrate or ammonium). When different ratios of $N:P_2O_5:K_2O$ were trialled, the optimal ratio was 1:1:2, in comparison with 1:1:1 and 1:0.25:1.5, so K was found to be a limiting macro element for basil growth (Tesi *et al.*, 1995).

The effects of different sources of N – inorganic versus organic fertilizers or their combination - were studied in the field in Egypt. It was shown that combining inorganic and organic N resulted in significantly greater plant height, fresh and dry weight, compared to organic or inorganic fertilization alone (Kandeel *et al.*, 2002). In field trials in Italy, similar results were obtained on three commercial cultivars of basil ('Mostruoso mammouth', 'Genovese profumatissimo', 'Napoletano a foglia di lattuga'). The combination of inorganic and organic N (ammonium nitrate) fertilization improved yield of above-ground fresh biomass, number of leaves per plant and leaf fresh biomass, compared to organic or inorganic fertilization; a statistically insignificant increase in plant height was also observed (Sifola and Barbieri, 2006). Studies on the cultivar 'Vikas Sudha' in India also showed that a combination of inorganic and organic N is the best for growing basil; plants had the highest growth, biomass and dry matter when grown on the combination of Vermicompost at 5 t per ha + NPK at 50:25:25 kg per ha (Anwar *et al.*, 2005).

An important result from research in Israel showed that a sharp increase in yield of field-grown basil was obtained when N fertiliser (KNO_3) was applied several times in the growing season (after each cutting) as compared to once or twice in the season (Putievsky and Basker, 1977).

Basil grown in hydroponics appears to react differently to nutrition from field- and pot-grown basil. The effect of solutions derived from organic fertiliser and conventional salt-based nutrients was studied in a hydroponic system based on Rockwool slabs, Perlite frames and sphagnum peat/perlite/compost medium in Colorado, USA. There were very few differences between the nutrient sources. Basil plants grown in Perlite on organic fertiliser were as productive as those grown in sphagnum/perlite/compost and Rockwool on conventional nutrients, although there were some different effects in different years. Taste-test panellists could discern differences between samples from organically and conventionally grown basil, yet no preferences were shown (Succop and Newman, 2004). Contradictory results have, however, been obtained in the UK. Basil was grown in fine, medium and mixed Perlite; fine and coarse peat, and coir. Plants in fine peat or coir failed to thrive, showing symptoms of water logging and there was appreciable plant death. Plants grown in coarse peat looked healthier, but were shorter than plants grown in Perlite. Between Perlite treatments, Perlite mix was the best substrate (Smith *et al.*, 1997). It is important to mention that humic substances (water extracts from peat) were found to affect the uptake of micronutrients (Fe, Mn, Zn and Cu) in basil in hydroponics. The affect was more pronounced at a low pH than at a high pH. At a low pH the addition of humic substances also decreased plant fresh weight, but at high pH the effect was very small. Chlorosis showed the opposite tendency with humic substances increasing chlorosis at a high pH but not changing the plant appearance at a low pH (De Kreij and Basar, 1995). The authors supposed that at different pH values, different microelements were bounded by a compound in humic substrate (probably phenolic acid), with consequent effects on growth and chlorophyll production. K, Ca and Mg content was not affected by humic substances (De Kreij and Basar, 1995).

A comparative study on the interaction between nitrogen and phosphorus on the growth of basil (var. 'Genovese') in hydroponics was performed in the UK. An increase of N concentration (mixture of ammonium and nitrate 40:160 mg/l,) up to 300 mg/l increased fresh and dry weight and leaf area, but had no effect on plant height and frequency of branching. Increasing the concentration of phosphorus in the nutrient solution up to 100 mg/l increased the yield of basil, largely due to an increase in leaf area: neither branching nor plant height were changed (Smith *et al.*, 1997). A more detailed analysis of the role of anion variations (NO_3^-, $H_2PO_4^-$, SO_4^{2-}) on basil biomass and appearance in hydroponics was carried out in Japan. The combination of NO_3^-, $H_2PO_4^-$, SO_4^{2-} = 40:20:40 and 40:40:20 in 15 meq/l* nutrient solution produced 15% increase in leaf number and branch number compared to 20:40:40 combination. Thus NO_3^- was found to be a limiting anion. Fresh biomass production had its peak in the 40:20:40 combination and dry weight in the 20:40:40 combination (Takano and Yamamoto, 1996). Thus, depending on which parameter of productivity is desirable (appearance, fresh biomass or dry weight), the composition of the nutrient solution should be adjusted.

K and Ca relations in terms of herb biomass production in hydroponics has also been studied in Japan. It was shown that leaf and stem weight increased with increasing concentration of nutrition solution from 20 to 40 meq/l. In 40 meq/l nutrient solution, leaf and stem weight increased by 30% when K:Ca ratio was 25:75 and by 15% when the K:Ca ratio was 50:50, compared to ratios of 87.5:12.5 and 75:25; thus Ca is a limiting cation (Takano, 1993). The variety may also be important in the reaction to changes in nutrient solution. It was shown for hydroponically grown sweet basil (*O. basilicum*) and opal basil (*O. basilicum purpurascens*) that the growth (height, leaf area, fresh weight) of sweet basil was best in the half strength nutrient solution, while that of opal basil was best in a full strength solution (Suh *et al.*, 1999).

***((meq/L) milliequivalents per litre** - meq/L is a method of expressing concentration, when the analytes are dissolved and disassociated in solution. meq/L is also equal to millimoles of charge per litre (mM+/L or mM-/L depending on valence).

Therefore in the field, basil is more productive when N is applied as organic and inorganic mixture with several applications in the season. In hydroponics, the form of N is not important, but the substrate is crucial and Perlite of mixed sizes is the best. Phosphate in the field should be applied at the same dose as N, but in hydroponics less phosphate might be applied and sulphate could partially replace phosphate. K was found to be a limiting element for field grown basil, but in hydroponics Ca is more important than K. The role(s) of other elements (Mg and microelements) and of pH were not studied and are therefore not clear either for soil or for hydroponically grown basil.

It is very important to note that the reaction of basil to changing nutrition depends not only on growing conditions, but also on the variety of the plant used, so for each variety the nutrient balance might need to be adjusted.

3.2.8 Mycorrhizas

The effect of arbuscular mycorrhizal fungi on growth of sweet basil (var. 'Genovese') in pot culture was investigated in Italy: fungal lines used were *Glomus mosseae* BEG12, *Gigaspore margarita* BEG 34 and *Gi. rosea* BEG 9. Two fungi were found to colonise basil roots differently: *Gl. mossea* quickly produced abundant intercellular hyphae, but relatively few arbuscules, which often appeared with their density decreasing with time; *Gi. rosea* colonised the roots more effectively than *Gl. mossea* and arbuscules were abundant and increased with time. According to the colonization pattern, the two fungi increased basil growth at different stages. *Gl. mossea* promoted basil growth over a short time: plants harvested on 21st day had increased leaf number, leaf fresh weight, leaf area, fresh weight compared to control and other fungal treatments: the increase was between 10% and 60%, depending on parameter. *Gi. rosea* had a long-term effect on basil growth. Infected plants increased their growth parameters by 15-120% when harvested at 63rd day. The effect was already pronounced in a harvest after 42 days (Copetta *et al.*, 2006).

Mycorrhizas can promote the growth of basil and different fungi could be used to increase harvest of basil in the short or long term.

3.2.9 Irrigation

No data was found.

Popular books claim that basil is sensitive both to excessive or limited irrigation, thus it is highly recommended to study the effects of water supply on the productivity of basil.

3.2.10 Growth regulators

Effect of three phytohormones (gibberellin - GA3; auxin – IAA; cytokinin – kinetin) were studied on sweet basil grown in the field in Egypt. All three hormones increased plant height, number of branches, number of leaves, fresh and dry weight. Plants were tallest and produced highest fresh and dry biomass after application of GA_3 (100% increase compared to control in height, 200% increase in fresh weight, 150% in dry weight). Kinetin increased height by 35%, fresh weight by 130% and dry weight by 80%. IAA increased height by 30%, fresh weight by 50% and dry weight by 15%. Kinetin and GA_3 among the hormones produced the highest number of branches (increase by 100%), and also increased the number of leaves per plant by 80%. IAA produced just a small increase in these parameters (Mahmoud, 1996).

The effect of gibberellin combined with nitrogen fertilisation was found to be additive, as shown for pot-grown basil in Florida, USA. In all combinations there was an increase in growth as the concentration of GA_3 increased up to a concentration of 100 ppm after which it plateaus with any increase in GA_3 not increasing growth. The application of N in the form of ammonium sulphate was optimal at 0.4 g N/l, in comparison to 0.2 g N/l and 0.6 g N/l (Santos *et al.*, 1998).

Evaluating the effects of hormones on basil production in the UK can be recommended. As basil reacts differently to N under different growing condition and sources of N, we recommended adjusting N and hormone concentration depending on the circumstances.

3.2.11 Elicitors and stress signals

Elicitors can promote the growth of basil, as was shown for pot-grown basil in South Carolina, USA. Optimal concentration of chitosan was reached when plants were sprayed with 0.1% concentration dissolved in lactic acid. Plant height and weight increased by 17% and 12%, respectively (Kim *et al.*, 2005). Stress signals, such as methyl jasmonate and methyl salicylate did not change the appearance of basil (Deschamps and Simon, 2006).

3.3 Changes in essential oil and of aroma due to diversity and growth conditions

3.3.1 Components of essential oil

The origin of seeds and propagules, ecological factors (light, temperature, water, altitude, specific growing location) and stress can influence the oil composition (Hornok, 1992; Hay and Waterman, 1993; Kyle, 2004). Table 1 demonstrates the wide range of possible chemical constituents of basil oil.

Table 1. Composition of herb oil from *Ocimum basilicum*

Major components	Composition (% of total)	Comments
Caryophyllenes	1.0-8.0	*
1,8-Cineole	Trace-14.0	*
Eugenol	Trace-11.0	*
Linalol	0.2-75.0	*
Methyl chavicol	0.3-89.0	*
Methyl cinnamate	Trace-16.0	*
α-Terpineol	1.0-3.0	

* components that contribute to the flavour and fragrance of basil

3.3.2 Toxicity

Basil, like fennel and tarragon, contains methyl chavicol and methyl eugenol. Both were found to have a carcinogenic toxicity (Tisserand and Balacs, 1995). An increase in certain types of cancer was reported for methyl eugenol: the National Toxicology Program in USA, following a recent two-year study, reported clear evidence of increased incidence of liver neoplasms and neuroendocrine tumours due to methyl eugenol (eugenol did not show any genotoxic or carcinogenic activity). Several studies have shown that methyl chavicol (=estragole) is also an inducer of hepatocarcinogenicity in mice and rat (Anon, 1998). The rodent experiments indicate that it would take 100–1000 times the normal anticipated exposure to become a cancer risk[2]. Human effects of methyl chavicol are currently unstudied due to its genotoxicity, so that the existence of a threshold cannot be assumed and the European Commission on Health and Consumer Protection could not establish a

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[2] EMEA (2004-03-03). Position Paper on the use of HMP containing estragole (PDF) 5. Retrieved on 2006-11-17. “In particular, rodent studies show that these events are minimal probably in the dose range of 1-10 mg/kg body weight, which is approximately 100-1000 times the anticipated human exposure to this substance”

safe exposure limit. Consequently, reductions in exposure and restrictions in use levels of methyl chavicol are indicated in the report[3].

It is therefore quite possible that, in the near future, the food industry will need to decrease the concentration of estragole and methyl eugenol in herbs. The way forward is to breed the varieties, which are low in both substances. This should be done in parallel with sensory evaluation, firstly to understand role of these substances in flavour, and secondly to find possible substitutes.

3.3.3 Aroma components

Research on oil extraction and, in parallel, sensory evaluation in herbs is rare; one very detailed report originating from Taiwan. Unfortunately, it was not on the type of basil common in Europe (see above), but on the Reunion type, grown widely in Asia (see 3.1, above). In Reunion basil, 40 compounds were detected in essential oil by gas chromatography and the major compound was found to be methyl chavicol (69% - 87%). Then a study was performed to assess if all of the components in real Reunion oil are perceived by humans. Eleven compounds were found to be perceived (at the 5% significance level): methyl chavicol, trans-α-bergamotene, α-terpineol, α-muurolene, cardina-1,4-diene, trans-anethole, eugenol, T-cadinol, β-elemene, ethyl linolenoate. Most of them (except methyl chavicol) were in a very low proportion (0.01%-0.9%) in oil, but were perceived by humans. Interestingly, substances such as 1,8-cineole, β-ocimene, methyl eugenol although present in higher proportions (2%-6%), were not perceived by humans. Among 11 perceived substances only methyl chavicol showed a positive correlation with aroma (= the richer the oil was in methylchavicol, the more preferable was the odour of oil). Another 10 compounds had negative correlations, which was an unexpected finding: of the individual substances, 8 of them had fragrant and fine aromas and only 2 were 'grassy'. The explanation was that in real basil leaf, these 8 substances are co-synthesised with 1-hexanol, cis-3-hexenol, 3-octanol, 1-octen-3-o1. These substances (1-hexanol, cis-3-hexenol, 3-octanol, 1-octen-3-o1) had 'grassy', green and sour aroma, and the effect of the unacceptable aromas on sensory score was greater than that of the preferable ones (Sheen *et al.*, 1991).

This complex and detailed work highlights the difficulties that exist in finding varieties with low concentration of toxic methyl chavicol and methyl eugenol: they are (at least methyl chavicol) the components of pleasant basil aroma and to find substitutes will be challenging. As in the real leaf unpleasant substances are often co-produced with pleasant ones and can over-compete with them.

This work also shows that in a plant, the concentration of any given substance does not necessarily correlate with its perception by humans; substances produced in higher concentrations are not always perceived, while those produced in trace concentration are sensed. Thus all work on selection of appropriate varieties of basil should be accompanied by flavour or at least odour evaluation.

3.3.4 Varieties

There are traded varieties of basil that are naturally low in methyl chavicol and methyl eugenol. For example, Lachowicz *et al.* (1997) evaluated the suitability of different varieties for the Australian market. The range of varieties included 'Cinnamon', 'Dark Opal', 'Bush', 'Reunion' and 'Anise'. Aroma perception was also evaluated. The researchers came to the conclusion that methyl chavicol and methyl eugenol are not the only two determinants of basil aroma, and other substances, if produced in high amount, give a very pleasant and fragrant odour to basil leaves. For example, 'Bush' and 'Dark Opal' had an almost identical composition of oil, mainly linalool, which also had most impact on aroma, described as

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[3] EUROPEAN COMMISSION HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL Directorate C - Scientific Opinions; SCF/CS/FLAV/FLAVOUR/6 ADD2 FINAL 26 September 2001 Opinion of the Scientific Committee on Food on Estragole (1-Allyl-4-methoxybenzene)

"fragrant, sweet, fresh, woody". 'Bush' had a very low amount of methyl chavicol. On the other hand, 'Reunion' oil consisted mainly of methyl chavicol. In between were 'Cinnamon' and 'Anise', the former containing mainly linalool and methyl cinnamate and some methylchavicol and the latter mainly linalool and methyl chavicol and some methyl cinnamate.

In Italy the problem with the toxicity of basil is a high priority, as the variety 'Genovese Gigante' is widely used for production of typical Italian 'pesto'. Unfortunately, 'Genovese Gigante' has the methyl eugenol chemotype, and during 'pesto' consumption the resulting intake of methyl eugenol could reach 250 μg/kg/meal in adults (Miele *et al.*, 2001), close to the carcinogenic threshold determined for rodents, see above. Sifola and Barbieri (2006) evaluated the chemical composition of other varieties currently used in Italy. Cultivar 'Napoletano a foglia di lattuga' was the richest in methyl chavicol, and also contained some linalool and limonene. 'Mostruoso mammouth' and 'Genovese profumatissimo' both contained linalool, limonene, eugenol, but very little methyl chavicol (more than 10 times lower, comparing to the first variety). None of these varieties contain methyl-eugenol (Sifola and Barbieri, 2006).

Turkish scientists also evaluated the chemical composition of wild populations of basil grown in Turkey in order to select only varieties which are low in methyl chavicol and methyl eugenol for industrial cultivation. Only chemotypes having high linalool, methyl cinnamate or citral content, and also a mixture of these, were found to be suitable for the food industry (Telci *et al.*, 2006).
We recommend that similar research should be done in the UK to assess varieties of basil suitable for the British food market – with good growth characteristics and flavour, but low in carcinogenic substances. In basil, as in other plant species, composition and content of essential oil changes (see below) in different growth conditions. This should be taken into consideration, as conditions promoting growth and yield often affect production of oil and vice versa.

3.3.5 Ontogenesis

Oil content and composition changes in basil depending on its developmental stage. It was shown for the variety 'Genovese Gigante' grown in the field that composition correlated with plant height rather than plant age: methyleugenol was predominant in plants up to 10 cm in height, but decreased almost 10 times while plants grew up to 16 cm. The opposite picture was observed with eugenol, which was prevalent in taller plants (Miele *et al.*, 2001). On the other hand, Lemberkovics et al. (Lemberkovics *et al.*, 1995) showed that the oil content in leaf increased up to the full flowering stage, but the oil composition exhibited only minor fluctuations. At the late flowering stage, the composition of oil also started to change with linalool decreasing to 25–30% (at early stage was 40–60%), but total sesquiterpenes increasing to 60–70% (constituted only 5–20% at early stage).

A more precise recommendation was produced by Szabo and Bernath (2002), which included a diversity component. They developed a 'flowering index' that could be utilized to define the phase when the essential oil content reached its maximum level. The flowering index is a formula, which utilises stages of flower development (green bud; white bud; opening of flower; fully opened flower; fading of flower; white seed; brown seed; black/ripe seed) and their assigned mathematical weights. The value of the flowering index varied between –1 and +1: if the whole inflorescence was at the bud stage, the flowering index was –1 and increased continuously as flowering progressed up to +1 at ripe-seed stage. Eleven varieties of basil were investigated in Hungary for correlation of flowering index to maximum oil content. 'Lengyel' produced maximum oil at the stage of early flowering, when the index was - 0.56. In 'A-1' and 'Genoveser', the essential oil reached its maximum value at the end of flowering period, when the flowering index was + 0.9. In 'Rit-Sat', 'Opal'and 'Keskenylevelu' the essential oil reached its maximum value at about full flowering, when the flowering index = 0. 'Piroslevelu', 'OBRS', 'Fodros' and 'Nemet' produced the highest amount of essential oil at the stage of flower fading, when the index = + 0.5. Thus for a particular variety, once the relationship between flowering index and oil accumulation is known, then the index can be used as the basis for harvesting to obtain maximal accumulation of essential oil.

Changes induced in oil during plant development seem to be unique to a variety and should be carefully studied. Thus the very general and rough recommendation, which is usually given - to collect oil at 50% seed set stage (Gupta, 1996) - may not be valid, and should not be used by the herb industry.

3.3.6 Temperature and photoperiod

Oil yield and composition depends on temperature and photoperiod in basil. The effect of temperature on essential oil content of basil was investigated for pot-grown basil in the UK (cv. 'Sweet Genovese'). Plants grown in warm constant temperature produced the highest oil content: after 3 weeks at 25°C and 30°C plants contained three times more oil than those at 15°C. There were no significant differences in the relative content of two main components (1,8-cineole and linalool), but the content of eugenol, α-terpinene, γ-terpinene, ocimene and sabinene was enhanced under warm conditions, while camphor, β-farnesene and α-bergamotene were enhanced in cool conditions. The relationship with day-degree was similar with plants grown for 1 week at 15°C and 2 weeks at 25-30°C resulting in highest oil content compared to the reverse treatment (2 week of warm plus 1 week of cool conditions) or interrupted treatment (1 week warm, 1 week cool, 1 week warm). Eugenol showed the same tendency as total oil as the higher the temperature the higher was its content. In contrast to plants grown at constant temperature, day-degrees changed the concentration of linalool and 1,8-cineol in the oil as a treatment with 2 week of warm plus 1 week of cool conditions enhanced relative content of linalool, but decreased content of 1,8-cineol (Chang *et al.*, 2005).

Field-grown basil composition and yield of oil was also shown to change with temperature. The variety 'Genovese Gigante', which has a methyl eugenol/eugenol/linalool chemotype, when grown in mild climate in Italy produced more eugenol, than methyl eugenol and linalool. Plants grown in northern localities were rich in methyl eugenol (Miele *et al.*, 2001).

Photoperiod was shown to change composition of essential oil in pot-grown basil in Michigan, USA. It was shown that α-pinene, β-pinene, linalool and β-myrcene were produced at a higher concentration and a shorter photoperiod (9-15 h), but decreased at 18 h or longer. β-phellandrene was synthesized at higher concentrations at short photoperiod (9 h) (Skrubis and Markakis, 1976).

All changes in essential oil due to temperature and photoperiod should be taken into account, and a balance should be found between biomass and essential oil production. It is also highly recommended to do this in parallel with flavour evaluation, as these treatments change oil composition.

3.3.7 Light of different quality and quantity

The effects of light intensity on oil production in basil have not been well studied, although there is good reason for such studies; terpenes in general (linalool, 1,8-cineol, α-bergamotene, α-pinene, β-pinene, β-ocimene in basil in particular) are very expensive for a plant to produce in terms of energy equivalents, and production of terpenoids depends on intensity of photosynthesis (Sangwan *et al.*, 2001).

The effect of light quality has been better studied. Plastic mulches with different surface colours (white, black, green, yellow, blue and red) have been trialled on field-grown 'Italian sweet' basil. For most of the monoterpenoids, the highest levels of production were obtained from leaves grown over yellow, green and black mulch, whereas those grown over white and blue emitted the lowest concentrations. The emission of the phenylpropanoid eugenol also followed this trend (Loughrin and Kasperbauer, 2001).

UV-B light was found to increase total oil content and improve the composition of essential oil. Johnson *et al*. (1999) noted that even before analysis, the difference in aroma between plants treated with UV-B and controls was immediately obvious to the nose. They have shown that the effect of UV-B on the linalool chemotype of basil depends on growth stage. At the two-leaf stage, UV-B has a negative effect on the major component, linalool, but virtually no effect on the other terpenoid compounds and a positive effect on methyl eugenol. At the three-leaf stage, methyl eugenol and eugenol levels strongly increase, but so also did the terpenoid β-ocimene. By the five-leaf stage, all phenyl-propanoids and terpenoids (including linalool) were strongly enhanced, with the largest effect (a more than fivefold stimulation) being found in eugenol and three- and four-fold enhancement in others. When experiments with UV-B supplemented light were conducted in the very different climatic conditions of the UK and Greece similar results were obtained. Again, when a linalool-rich basil was used, UV-B treatment led to increased total essential oils content in both developing and mature leaves, but this was more significant in mature leaves in which the volatile content more than doubled as a result of UV-B treatment; the composition was not much changed by UV-B. (Ioannidis *et al.*, 2002). Even more encouraging results were obtained in Germany when 'Bageco' with the chemotype methyl eugenol/eugenol was used. UV-B light increased total oil by 50%; 1,8 cineol, linalool and eugenol accumulated in above average amounts, which resulted in an enhanced fresh aromatic flavour note. However the best finding was that methyl eugenol decreased by 50% (Nitz and Schnitzler, 2004).
UV-B light can be used to increase total oil yield in basil; in phenylpropanoid chemotypes UV-B light can be used to diminish unwanted components.

The effect of UV-B light was explained when glandular trichomes were studied. UV-B did not change the number of glandular trichomes, either of the smaller capitate glands nor of the larger peltate glands, but drastic changes were observed in the fullness of the glands. Peltate glands in basil plants grown without supplementary UV-B were characterized by only partially filled oil sacs. By the second or third day of UV-B treatment (1 h each day, before dawn), the sac membranes had become distended and smooth and entirely filled with oils. After the fourth or fifth day of the 15 d treatment no further differences were observed. Even before analysis, the difference in aroma between plants treated with UV-B and controls was immediately obvious to the nose. The possibility that part of the increase in odour was due to the rupture of glands, leading to release of volatiles into the air, was examined. Inspection of a large number of leaf samples for broken oil sacs did indeed reveal a significantly increased number in UV-B-treated plants. From an initial rate of about 17%, the number rose to around 40±45% (Ioannidis *et al.*, 2002).
Thus UV-B also increases the number of broken glands in basil, which subsequently increases its aroma more than could be predicted from an increase in oil production alone. We recommend further experiments to establish the correct type of light and regime.

3.3.8 Density

The effect of planting density was studied in hydroponics in the UK (var. 'Genovese'). An increase in plant density of up to 8-16 plants per pot led to a decrease in total oil yield. Oil composition was also sensitive to plant density, so the proportion of linalool increased and methyl cinnamate decreased at high densities (Smith *et al.*, 1997).
Depending on the task (to increase oil yields or to improve composition of oil) planting density should be carefully adjusted for basil.

3.3.9 Nutrition

In the field, contradictory results on effect of type of N (organic versus inorganic) on basil oil production have been obtained. In Egypt, it was reported that application of inorganic N resulted in significantly greater oil yield; the mixture of inorganic and organic N affected the chemical composition of essential oil since it decreased linalool and increased methyl chavicol concentrations (Kandeel *et al.*, 2002). But in Italy, a combination of inorganic and organic N (ammonium nitrate) resulted in increased essential oil in three commercial cultivars ('Mostruoso mammoth', 'Genovese profumatissimo', 'Napoletano a foglia di lattuga') (Sifola and Barbieri, 2006). Similar results were obtained in India, where a combination of manure (vermicompost) and fertilizer (NPK) was shown to increase methyl chavicol and linalool and total oil yield in cv. 'Vikas Sidha' (Anwar *et al.*, 2005).

In the UK effect of different sources of N on oil yield and composition in field-grown basil should be evaluated. It is not absolutely clear how other macro- and micro-nutrients affect oil production and composition in basil.

In hydroponics, oil production in basil grown in different combinations of nitrogen and phosphorus has been studied the UK (var. 'Genovese'). An increase of N concentration (mixture of ammonium and nitrate 40:160) up to 300 mg/l did not change total oil yield and composition. Oil yield tended to decrease with increasing P concentration up to 100 mg/l (Smith *et al.*, 1997).

The role of anion variations (NO_3^-, $H_2PO_4^-$, SO_4^{2-}) on content and composition of basil oil in hydroponics was studied in Japan. Sulphate was shown to decrease essential oil production, whilst phosphate and in particular nitrate promoted production. Production of essential oil increased by 150% in the combination 40:20:20 or 40:40:20 compared to 20:40:40. Composition of essential oil also changes with high sulphate concentration decreasing β-caryophyllene and citral. Phosphate and nitrate increased production of eugenol, linalool and citral (Takano and Yamamoto, 1996).

The concentrations of K and Ca in the nutrient solution can also affect essential oil accumulation. It was shown in a study in Japan that total essential oil production was higher when the nutrient solution was of higher concentration (40 meq/l compared to 20 meq/l). At 40 meq/l there was almost no difference in total oil production at any K:Ca ratio (87.5:12.5, 75:25, 50:50, 25:75). The K/Ca ratio in this solution changed the composition of essential oil where optimal production of 1,8-cineol and linalool was at 75:25, 50:50, but the optimum for methyl chavicol and eugenol production was at a ratio of 87.5:12.5, 25:75 (Takano, 1993).

In hydroponics, the effect of nutrients on essential oil production in basil is relatively well studied: nitrate and phosphate increase total oil yield and production of linalool, eugenol and citral. When N is applied as a mixture of organic and inorganic, it does not affect oil production. Sulphate could affect oil production and composition, while the K/Ca ratio changes the composition of essential oil, but not the yield. Yet again, the effect of other important elements (Mg and microelements) and the role of pH has not been studied for hydroponically grown basil.

3.4 Can growth conditions and diversity improve shelf-life of pot and field grown basil?

Basil is, compared to other herbs, extremely sensitive to chilling. For example, it was shown that at 15°C, shelf life of freshly harvested greenhouse-grown basil was about 12 days. But at 0°C or 5°C chilling injury symptoms (appearance of darkened pitted lesions on the leaves, followed by decay) were severe and shelf life was found to be only 1 and 3 days, respectively. Moderate chilling injury was noted at 7.5°C and 10°C (Lange and Cameron, 1994). As herbs are usually transported and stored in cool temperatures, basil needs special arrangements, which are not convenient and are expensive.

The main improvement in basil shelf-life would be to improve its chill tolerance.

3.4.1 Varieties

We found only one report, from Israel, on different resistance to chilling between basil varieties. Two cultivars, cv. '79' (Sunset, USA) and cv. '10' (Botanical gardens, Swiss cultivar no. 676), were found to differ in their susceptibility to chilling. Cv. '10' was less sensitive with its chill injury index, following 8 days of storage at 4°C , being much lower than that of the more sensitive cultivar cv. '79'. It was found that 12°C was the critical temperature for cv. '79' below which chill injury symptoms occurred. This critical temperature is typical for basil and was shown in previous studies (Cantwell and Reid, 1993; Lange and Cameron, 1994). Critical temperature for cv. '10' was lower - +10°C (Meir *et al.*, 1997).

Systemic selection of basil cultivars and development of chill tolerant varieties has never been performed for basil, which should be done, as it has for many other crops.

3.4.2 Chill hardening

Chill hardening is the exposure of a crop to low temperatures, as this sometimes improves chill tolerance at later stages. It was shown for pot-grown basil, that when plants were chill-hardened at 10°C for 4 hours daily (2 h at the end of the light period followed by 2 h at the beginning of the dark period) for 2 days before harvesting and packaging then shelf-life increased from 3-4 days at 5°C to 6-7 days (Lange and Cameron, 1997).

We therefore can recommend testing the effects of chill hardening to pot-grown basil to improve its shelf-life.

3.4.3 Growth regulators

Triazole, a plant growth regulator that inhibits gibberellin biosynthesis, has been found to protect herbaceous plants from chill injury. In Israel, chill injury to basil decreased when mature basil plants were treated in soil trenches with 200 ml of a 16 mg/l solution of triazole 4 days before harvest. The chilling-alleviating effect of triazole was manifested significantly both following 8 days of storage at 4°C and after the post-chilling period at 12°C during which the visual rating of chill injury was higher (Meir *et al.*, 1997).

The effect of triazole should be studied for varieties used in the UK and applied to basil in the field and in pots to improve its chill tolerance.

3.4.4 Time of harvest

Time of harvest was found to be important for the shelf life of basil, due to the fact that greater sugar contents are present in the afternoon, which contribute to maintaining metabolic activity. Harvesting sweet basil later in the day (at 1800 h or 2200 h) increased shelf-life by almost 100% when harvested shoots were held at 10°, 15°, and 20°C, compared to harvesting at 0200 or 0600 h. However, the time of harvest did not alter the development of visual chilling injury symptoms or improve shelf life at 0°C or 5°C (Lange and Cameron, 1994). The influence of harvesting hour on post-harvest oil content was investigated in Brasil. During 9 days of storage, shoots presented a linear decrease in the oil content to about 65% of initial value. The harvesting hour did not affect essential oil content/composition (da Silva *et al.*, 2005).

Time of harvest should be taken into consideration to achieve a longer shelf-life for basil at ambient temperatures.

3.5 Recommendations for research to be performed on basil

Basil is rather well investigated, although little of this research has been focussed on UK conditions. So growth conditions need evaluating as does, for example, the development of chill-tolerant varieties, flavour and toxicity. What is required are applied projects targeted to particular regions of the UK. We think that the key issues which should be addressed in basil are: (not in order of preference)

- evaluation of flavour and research on flavour components in basil to find non-toxic substitutes for methyl eugenol and methyl chavicol.
- finding of non-toxic varieties of high total quality, adapted to UK growing conditions.
- research on chilling tolerance in basil, which will decrease waste due to poor shipment and storage conditions.
- research on optimal growing conditions for high essential oil and biomass yield in UK-grown basil.

4 Chives – *Allium schoenoprasum* L.

4.1 Diversity of chives

There are two subspecies of chives in the European flora, *Allium schoenoprasum schoenoprasum* and *Allium schoenoprasum sibiricum* – see http://www.pgrforum.org/Documents/Conference_posters/Allium casestudy.pdf.
A. s. schoenoprasum is the subspecies used presently in most greenhouses in Europe. Many varieties of *A. s. schoenoprasum* are known: e.g. 'Grolau', 'Wilau', 'Medonos', 'Khibiny', 'Prague', 'Bogemia', 'Finbladet'and 'Triumf'.
A. s. sibiricum (= subsp. *sibiricum* = var. *alpinum* = *A. sibiricum* http://agroatlas.spb.ru/related/Allium_schoenoprasum_en.htm) is an arctic-montane taxon, which is sometimes used in Norway and Russia for leaf production (Kwiatkowski, 1999), and is gradually becoming known to gardeners and industry around the world: for example, it is listed in Promising Plant Profiles of the Herb Society of America.

4.2 Changes in yield and appearance due to diversity and growth conditions

4.2.1 Varieties

The yield of chives depends on the variety used. For example, in Finland varieties 'Tavallinen', 'Hankoniemi' and 'Grolau' produced higher yields each year (5-30%) than varieties 'Finbladet', 'Triumf' and 'Wilau'. On the other hand, in terms of saleable proportion of total yield 'Triumf' and 'Finbladet' were usually of better quality than the other four varieties (Suojala, 2003). Thus it is very important to find and use a variety that is high in yield and good in appearance. It was shown for onion (and may be possibly extended to chives) that leaf production ends when onion reaches a certain critical dry weight, or number of initiated leaves. This critical size can vary with cultivar and an 8-fold larger critical size is the most striking difference between bolting-resistant cultivars suitable for autumn sowing and bolting-susceptible varieties suitable only for spring sowing (Shishido and Saito, 1975).

Biomass productivity is much higher in *A. s. sibiricum* than in *A. s. schoenoprasum* with leaves of *A. s. sibiricum* being twice as long and thicker than those of *A. s. schoenoprasum* (Kwiatkowski, 1999).
We recommend, selecting and using 'leafy' varieties of A. s. var. schoenoprasum in the herb industry. For high biomass production and thick healthy leaf appearance we recommend a trial of A. s. var. sibiricum.

4.2.2 Ontogenesis

The age of the population and forcing[4] conditions influence biomass productivity in chives. Although there are anecdotal recommendations that chives can grow in the same plot for several consecutive years (as it grows in nature), it proved to be disadvantageous in Finland. Six varieties ('Tavallinen', 'Hankoniemi', 'Finbladet', 'Triumf', 'Grolau', 'Wilau') were grown on the same plot for three consecutive years and 10 harvests were obtained in total (Suojala, 2003). Quality was 76-94% saleable with the percentage, depending on variety, starting to decrease after the 5th harvest (middle of second season), and reaching a dramatic 45-59% at the 9th-10th harvest. The quality was impaired by dry or yellow leaf tips, light leaf colour and withered leaves.
It could be recommended to grow chives for only two seasons in one plot and then rotate the crop (Suojala, 2003).

There are two ways to obtain foliage from chives - from seed and from bulbs. Little or no work has been published on what should be done to improve growth from seed. Chives

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[4] breaking dormancy so that bulbs produce green biomass

produced from seed develops slower than from bulbs and the leaves are thinner. In the case of production from bulbs, successful growth depends on a ‘rest’ period and the forcing conditions (the pre-treatment). The rest period in chives is induced by short days at a medium temperature (Krug and Folster, 1976) and has three phases: the "early rest" is characterized by decreasing slow leaf growth and few leaves and starts at the middle to the end of September (for Germany). The translocation of reserve materials from leaves to bulbs has begun but is not yet completed during this period. The ‘rest’ ends between the end of September and the middle of October, depending on weather conditions. This ‘early rest’ is followed by a period with no or very low growth ability of leaves and was called "middle rest", or ‘partial rest period’, as roots continue to grow even during this period. The ‘after-rest’ is characterized by increasing growth ability of leaves. It starts in the middle of November and lasts to about December.

During the "early rest" period the forcing-ability was very low. Forcing during the period of the ‘middle rest’ proved to be promotional for green biomass production. The ‘middle rest’ can be broken by cold or, more effectively, by a heat treatment. Best results were obtained with the technique of starting with warm water at 40°C and cooling over a period of 16 hours to 25°C. This has given an increase in biomass of 400-1200%. A cold air treatment for 3 days at -5°C in a growth chamber had a significant rest-breaking effect, but did not have as great an effect on biomass (just 50-250 % increase) as the warm water treatment. During the ‘after-rest’ the relative effect of a rest-breaking treatment decreases, but the ability of the bulb to produce leaves is naturally high. It should be specially noted that as many roots as possible should be harvested and should be kept healthy during the rest-breaking treatment, as they are an important source of reserve materials for leaf growth and their damage results in a poor future leaf harvest (Folster and Krug, 1977).
The use of these treatments to break dormancy in chives should decrease the length of controlled storage of bulbs, therefore increasing the efficiency of chive production from bulbs.

4.2.3 Mulches

That the use of mulch is profitable in chives, both for yield and weed control, has been shown in Finland for field-grown varieties ‘Tavallinen’, ‘Hankoniemi’, ‘Finbladet’, ‘Triumf’, ‘Grolau’, ‘Wilau’. The use of a black plastic mulch gave, in addition to non-chemical weed control, from 0 to 63% increase in yield, depending on variety and year. Although it should be noted, that in the first year of planting, when roots of seedlings were very shallow and air temperature high, the roots suffered from overheating under the black plastic and yield was 15% lower in comparison with bare soil (Suojala, 2003). This could be avoided if chives were propagated not by seed, but by bulbs.
The use of mulches should be scrutinized in the UK.

4.2.4 Temperature and photoperiod

Chive is a plant species which is very chill tolerant, surviving winters of Norway and Siberia. However, successful growth of green biomass requires much warmer temperatures.

The influence of the temperature and day length was tested in Germany for chives grown from bulbs where forcing was performed in growth chambers with different (constant) temperatures. The highest yields (in growth chambers) after 20 days were obtained with a temperature range of 24-30°C. At lower temperatures, the growth rate was diminished, particularly by short-day conditions. The maximum growth rate per temperature unit was the highest at 24°C, irrespective of day length, and at 18°C with long days. As the latter conditions were found more economical, they were recommended for chive production in a greenhouse in Germany (Folster and Krug, 1977).
The effect of temperature and photoperiod has not been studied in chives grown from seed. It would probably also be relevant to investigate effect of day-degree, as it could conserve much energy in the greenhouse when nights are warm.

4.2.5 Light of different quality and quantity.

Photomorphogenesis in chives has not been studied much, but in the report described above (Folster and Krug, 1977), and also studies on onion, shows that productivity of chives depends on photoperiod and probably phytochrome as a photoreceptor. Therefore irradiation with additional light of red/far-red region could change the morphogenetic program in chives - for example, it could postpone bulbing and thereby improve productivity. Indeed, it was shown for onion, that interaction of bulbing and foliage growth could be manipulated so that in short days bulbing could occur if the ratio of red/far-red light were high and in long days growth of foliage could be achieved if the ratio of red/far-red were low (Brewster, 1997). A low ratio of red/far-red light could be achieved in natural (but not artificial) shade or if supplemented far-red light were provided.

We recommend investigating - if production of chives (foliage) is increased in summer (when day is longest), when the herb is grown in either natural shade, under supplemental far-red light or under far-red selective films. These treatments could stop bulbing and increase yield of foliage. In spring-autumn no shading is needed, as days are naturally short.

The yield of leaves can also be affected by flowering, which should be avoided in chives grown for foliage. It has been shown for onion, that bulbing and flowering compete with each other, rather than with foliage growth, as both occur in long days (Brewster, 1997).

The effects of photoperiod and light quality should be investigated for chives, as they will explain its physiology, competition between foliage production, bulbing and flowering and methods of growth should be adjusted to increase productivity. The effect of light intensity should be also investigated, as optimal light intensity for high productivity has not been studied and is not clear. On the one hand there are anecdotal recommendations that chives prefer full or partial sun or shading could be promotional for biomass production in some circumstances (see above).

4.2.6 Density

There is, as far as we are aware, no scientific data on the effect of planting density on the yield of leaves of chives.

This should be studied, as natural shade in long days could possibly increase productivity.

4.2.7 Nutrition

Fertilisation is necessary to secure high yields in chives. It was shown in Finland, with the field-grown variety ‘Grolau’ that nitrogen is most necessary, and its addition increases yields, even if basic fertilisation (60-24-102 kg NPK/ha) were applied before planting. Addition of 20 kg N/ha gave an almost three times increase in yield; a further increase - by 3.5 times - could be achieved by adding 40 kg N/ha. Addition of NPK in single dose (20-3.5-15 kg/ha) or a double dose (40-7-30 kg/ha) gave the same effect as single or double N fertilisation, so increase of P and K is not needed (Suojala, 2003).

Chives have proven to be suitable for hydroponics. In Italy, chives were grown using a floating system, giving good yields considering the short cycle that often lasted no more than 30 days (bulk harvest about 0.9 kg/m^2). In order to decrease leaf nitrate at harvest, nutrient solution was substituted with water for 2 or 3 days before harvest, resulting in a reduction of 28%, but with a different pattern in two growing cycles (Elia *et al.*, 2001). In Japan, the need for P in hydroponics in gravel culture was investigated. The growth of chives was greatly inhibited in the absence of phosphorus supply, but did not show symptoms of phosphorus deficiency. The optimum P concentration was estimated to be 4 to 8 meq/l (Shinohara *et al.*, 1992).

Mg proved to be essential for high quality chives. It was shown in Poland with pot-grown chives that Mg fertilization increased the contents of sugars and chlorophyll, independently of P contents of the soil (two levels of P were trialled). The highest contents of chlorophyll and carbohydrates were obtained with the application of Mg at a rate of 80 mg MgO/dm^3. Mg fertilization also stimulated the synthesis of vitamin C in leaves of both onion and chives, however, optimal rates for the contents of the ascorbic acid were not equal to those for chlorophyll and sugars (Gurgul *et al.*, 1998).

The high biomass production in chives depends mainly on N, but P and K are also essential. Excess of nitrate could be successfully removed from leaves before harvest, as studies in hydroponics have shown. Mg is essential and was shown to improve quality of leaves in chives. But there is still much to be done in terms of nutrient adjustment for chives. Best sources of N have not been selected, although there are anecdotal recommendations, that organic N is best after harvest for successive growth of next leaves; during growth ammonium nitrate is recommended (anecdotal) which could therefore be studied. The role of Ca, micronutrients and soil pH has not been studied.

4.2.8 Mycorrhyzas

The formation of mycorrhizal connections proved to promote the growth of chives. In Israel, four field experiments were conducted to examine the effect of soil solarization and a pesticide 'Dazomet' on production of chives following application of arbuscular mycorrhiza fungi. The experiments were carried out in four different farm conditions and solarization treatment varied between four to eight weeks starting at different dates during the summer. Reduction in the indigenous arbuscular mycorrhiza population was evident in all sites following solarization and reduction in plant growth was observed at early stages of development, up to the third harvest. Inoculation of chive with *Glomus intraradices* reduced the incidents of growth retardation and resulted in plant growth suitable for export grade at the first and second harvests. A minimum of 2.5% (v/v) of inoculum was required to obtain this result. The authors suggest that the growth retardation induced by soil solarization and Dazomet pre-treatment to soil could be abolished by pre-colonizing chive plants with mycorrhiza before introducing into the field (Wininger *et al.*, 2003).

Thus we recommend investigating using arbuscular mycorrhization to increase yield in chives.

4.2.9 Irrigation

Chive is morphologically well adapted to dry habitats (tubiform leaves with a small transpiring surface, geophytic mode of life with a bulb as water reservoir). Indeed it was proven to be very drought tolerant (Egert and Tevini, 2002), the authors reporting that chives could stand 9 days without water supply. During this period of drought, leaf and soil water status changed significantly: the soil had lost more than 90% of its water content, leaf water content decreased by 3.6%, relative water content dropped almost 25%, leaf transpiration was no longer detectable and the osmolarity of the leaf sap had increased by 19%. But all these differences completely disappeared in one week after re-watering.

In popular books it is recommended that chives should be grown in moist to mesic conditions, because 1-3 days of drought led to coarse leaves high in cellulose and low in sugars. We therefore recommend an investigation into the effects of irrigation in chive crops in terms of leaf quality, rather than yield.

4.2.10 Growth regulators

No scientific data.

These are of interest because in other herbs growth regulators have been shown to promote growth and improve appearance.

4.2.11 Elicitors and stress signals

No scientific data.

The effects of elicitors should be studied, as these substances have been found to increase biomass production in other herbs - but they could also lead to coarse leaves in chives due to the induction of defence mechanisms.

4.3 Changes in essential oil and of aroma due to diversity and growth conditions

4.3.1 Composition of flavour components

The range of secondary metabolites in *Allium* has not been investigated fully, however, the antioxidative properties in this species are due to a high concentration of flavonoids, carotenoids and chlorophylls.

Table 2 Composition of volatile oil from *Allium schoenoprasum*

Major components	Composition (% of total)	Comments
Diallyl disulphide	Data does not currently exist for any of these components.	
Dipropyl disulphide	"	*
Methyl allyl disulphide	"	
Methyl propyl disulphide	"	*
cis/trans-propenyl propenyl disulphide	"	*
Propenyl cysteine sulphoxide	"	
Propenyl sulphonic acid	"	Formed in leaves when crushed
Sulphur	"	
Thiosulphinates	"	
Pyruvic acid	"	

* main components responsible for flavour (% is not described)

4.3.2 Biosynthesis of components

Allium crops synthesise sulphur-containing compounds that are characteristic for their flavour and aroma, and produced by a specific biosynthetic pathway. The alliin-allinase system in this genus has probably evolved as a herbivore defence mechanism. Within the plant cell, the non-volatile substrates (alkenyl – substituted cysteine sulphoxides [ACSOs]) are spatially separated from an enzyme which, after tissue disruption, catalyses substrate conversion to an unstable product that is immediately transformed, by several enzymatic steps, into an array of volatile chemicals that have important sensory qualities. The most prominent is isoalliin (1-propenyl-L-cysteine-sulphoxide). The ACSOs are located in the cytoplasm, while allinase is located within the vacuole. A number of forms of this enzyme have been discovered and chemically described, with genes cloned from various *Allium* species. There are many derivatives of alliin with different biological activities, some of these with beneficial effects, such as anti-inflammatory, anticancer and antihypertension properties.

4.3.3 Flavour components

Flavour components have not been investigated for chives in detail. Using knowledge from garlic and onion, we can suppose that the characteristic flavour is caused after the enzyme alliinase hydrolyses the cysteine sulfoxides to form pyruvate, ammonia and Sulphur containing volatiles. The flavour and pungency is determined by decomposition products like propanethiol, propionaldehyde, di-1-propyl disulfide and methyl propenyl disulfide which have the purest and the strongest onion odour. The lachrymatory factor is thiopropanal S-oxide (Bloem *et al.*, 2004).

We should note that the flavour of chives differs from garlic and onion, so it is worth investigating specifically.

4.3.4 Varieties

No scientific data.

4.3.5 Ontogenesis

No scientific data.

See, 4.3. 1 above

There is no research on how growth conditions change flavour and flavour components in chives. We definitely recommend a study of this topic, because growth conditions certainly affect cystein sylphoxides in other species of Allium. To demonstrate this we include some illustrative studies on onion in 'Nutrition' - see 4.3.10.

4.3.6 Temperature and photoperiod

No scientific data.

4.3.7 Light of different quality and quantity

No scientific data.

4.3.8 Density

No scientific data.

4.3.9 Nutrition

Although there is, as far as we are aware, no scientific data on chive, we give here illustrative examples on how nutrition changes cysteine sulfoxides composition and pungency in onion and garlic. Investigation of the influence of S and N fertilisation on propenyl cysteine sulfoxides content in onion and garlic has shown that an increase in S supply (0, 50, and 250 mg S/pot) in greenhouse conditions increased alliin content in leaves and bulbs of both crops. In onion, leaves increased in propenyl cysteine sulfoxides by 80% and in garlic leaves the increase was 35% at 250 mg S/pot (Bloem *et al.*, 2004), which produced onions with a pungent flavour (Randle *et al.*, 1995). At low sulphate availability, S was efficiently metabolised, accumulating mainly as methyl cysteine sulfoxide (Randle *et al.*, 1995) producing an onion with a mild flavour (Randle *et al.*, 1994).

N fertilisation (250, 500, and 1000 mg N/pot) slightly (but not significantly) decreased propenyl cysteine sulfoxides content in onion and garlic leaves and bulbs (Bloem *et al.*, 2004). In another study, it was shown that changing the application of N changed the composition of cysteine sulfoxides causing an increase in methyl cysteine sulfoxide, but a decrease in 1-proponyl-cysteine sulfoxide (so Alliums with mild flavour are produced) (Coolong and Randle, 2003). However, N fertilisation is necessary when high herb yield is desirable, as it stimulates leaf growth instead of bulb growth (Riekels, 1977; Randle, 2000). Also, an interaction was reported between N and S with an increase in N in leaves resulting in a decrease of S and *vice versa* (Freeman and Mossadeghi, 1971; Randle *et al.*, 2002; Bloem *et al.*, 2004).

A careful balance should be found between these two fertilisers to produce good flavoured Allium leaf in high quantity.

Selenium, a potential competitor with sulphur, was found to mimic sulphur deficiency in Allium, hence, favouring methyl cysteine sulfoxide synthesis over 1-propenyl cysteine sulfoxide (Kopsell and M, 1999).

Calcium chloride, when applied as a solution or dry salt, decreased bulb sulphur accumulation and bulb pungency, but improved bulb shelf-life (Randle, 2005).

4.3.10 Irrigation

No scientific data.

4.3.11 Growth regulators

No scientific data.

4.3.12 Elicitors and stress signals

No scientific data.

4.4 Can growth conditions and diversity improve shelf-life of pot and field grown chives?

No scientific data.

4.5 Recommendations for research to be performed on chives

As the review shows chives is virtually unstudied. Consumption of chives is very valuable for human health - it has been shown that Allium species proved to help to prevent tumour promotion, cardiovascular diseases and aging. Therefore we recommend conducting research on chives, as promotion of these herb is in line with government guidelines on "healthy eating". Key issues to address are: (not in order of preference)

- Search for "best varieties" for the pot and field growers in the UK.
- Research on optimal growth conditions, especially effect of light (intensity, quality, photoperiod), fertilisation and irrigation, best for production of flavour substances and biomass in chives with almost no knowledge being presently available.
- Evaluation of chemical composition of chives herb and components of its flavour.

Investigation of guttation in chives, as it affects appearance, it is a special issue, and amongst the 6 herbs reviewed it is only chives whose appearance is affected by guttation. It is not a very well investigated phenomenon, and was never specifically studied for chives. The word itself comes from Latin 'gutta' – a drop. Under conditions of low or no transpiration (dark and high humidity), a positive pressure builds in the xylem forcing xylem sap, containing ions, to exude from leaves. It is the ions, on evaporation of the water, that cause the damage. Guttation in chives results in withering of leaf tops which become brown. Thus if growers want to decrease guttation in chives, they should investigate water relations (root water uptake versus transpiration) and its dependency on irrigation, nutrition and light growth conditions specifically.

5 Coriander – *Coriandrum sativum* L.

5.1 Diversity of coriander

Coriander is an annual herb, which has been cultivated since ancient times and in many countries both for fruits as a spice, and for leaves - as a vegetable (Ivanova and Stoletova, 1990). During the vegetative stage, plants produce from 1 to 15 leaves in a basal rosette. At the onset of flowering, the leaves in the basal rosette are completely developed and shortly after they wither. The dual use of coriander resulted in spontaneous selection of varieties according to their ontogenetic features. So varieties selected for fruit production have few leaves in the rosette (1-4) and flower early (bolt on 43-55 day). These 'seed' varieties originated mainly in the Indian subcontinent, Near East, Mediterranean countries and Africa (Diederichsen, 1996). In Central Asia and the Caucasian region leaves of coriander (cilantro) were used in the cuisine (Ivanova, 1966), and so local varieties are characterised by high production of leaves in a basal rosette (5-15) and late flowering (stem onset on 66-76 days) (Alborishvilli, 1984; Diederichsen, 1996).

5.2 Changes in yield and appearance due to diversity and growth conditions

5.2.1 Varieties

Biomass productivity in coriander depends on the variety used. For example, in the USA, the Caucasian type of coriander (with many aromatic basal leaves and a long period before bolting, see 5.1, above) was advertised as particularly suitable for use as a vegetable, due to increased fresh yield and harvesting period (Simon, 1990). Several promising vegetable varieties were trialled in Georgia (Alborishvilli, 1971; Alborishvilli, 1984). Vegetable varieties showed higher herb yield than seed varieties. The highest herb yield per hectare was reached when vegetable varieties were repeatedly cut 1.5-2 cm above the root base (for 2-4 cuts giving total 15,000-25,000 kg). Amongst the vegetable varieties there was one, which did not bolt at all under field conditions (seeds from this variety could only be obtained by abnormally long days in the greenhouse - Alborishvilli, 1984).
We recommend the use of 'vegetable' varieties for herb production, as they produce high yield, have a long harvesting window and are suitable for continuous production of leaves during the whole summer.

5.2.2 Ontogenesis.

As explained above, the important factor in the development of coriander is bolting - the later the bolting the higher the yield.
We recommend using vegetable varieties in the UK to postpone bolting and increase harvesting window.

5.2.3 Mulches

No scientific data.

5.2.4 Temperature and photoperiod

Coriander is usually described in popular books as chilling sensitive plant. It is not entirely true, however, as there are coriander varieties which are even frost-resistant. Observations in Germany showed that field-grown frost-resistant coriander survives long winter periods with temperatures below –15°C (Diederichsen, 1996).

Autumn sowing is commonly practiced in Russia, North Caucasus and Ukraine; thus breeding and selection of coriander varieties in the former Soviet Union has concentrated on winter hardiness. Sergeeva and Silchenko (1984) showed that roots of young coriander plants are more sensitive to frost than leaves, but could tolerate low temperatures down to –8°C, whereas leaves tolerated temperatures as low as –13°C. The authors found that oscillations of sucrose were smaller in a hybrid, (hybrid F1 B-47xAmber, i.e. between VIR stored ecotypes – B-47 and commercial variety Amber (=Yantar), previously selected in the USSR. The parental variety Amber (=Yantar) is also frost-resistant, but lees so than the hybrid), which was shown to be more frost resistant than parental varieties, which in turn showed depletion of sucrose at low temperature. Thus, the authors concluded that sucrose oscillations could be used as a marker of frost- resistance during breeding and selection of coriander.

Another marker of frost-resistance was shown to be the form of the rosette. According to Silchenko (1981), the formation of a prostrate rosette is monogenic and recessive. Romanenko (1990) showed that plants with a prostrate rosette possess high frost resistance, because if the rosette is flattened, the vegetative cone is beneath the earth, and therefore has better protection against low temperatures (Romanenko *et al.*, 1991). This makes varieties and hybrids with a prostrate rosette suitable for autumn sowing.

Coriander is often described in English popular books as photoperiod-neutral. This again is not true, as already in 1953 Palamar and Chotina (1953) wrote in their manual on coriander agronomy, published in the USSR, that coriander is a long day plant (which means that flowering is induced by long, but not short days). Since 1953, more studies confirmed their observations. In 1963, tetraploid coriander was obtained, which appeared to be supersensitive to photoperiod, it bolted quicker than normal diploid coriander in long days (12 - 14 hours photoperiod), but in short days (8 hours photoperiod) did not bolt at all (normal coriander showed some bolting) (Konstantinov and Zhebrak, 1963). Alborishvilli (1971), using 13 different vegetable varieties of coriander showed that short days prolong the vegetative stage. This was confirmed in his next study where the length of the growth period of vegetable varieties changes with season, for example, plants sown in March or September reach their flowering state twice as slowly as those sown in June (for Georgia, Alborishvilli, 1984). The same was recently shown in Italy (Carrubba *et al.*, 2006).

Coriander is definitely a long-day plant, so to prolong leaf production - short days are necessary. It is frost-resistant, and so is suitable for autumn sowing in the UK. This is an advantage, because autumn sowing prolongs the season, and the first harvest could be obtained in March-April. The effects of temperature and of day-degree on leaf production has not, as far as we are aware, been studied scientifically for coriander, but it is stated in popular books that low temperatures prolong the vegetative stage, and high temperatures induce bolting. We therefore recommend investigating effect of temperature and day-degree on coriander, as it could improve yield and conserve energy when under protection.

5.2.5 Light of different quality and quantity

The effect of light quantity and quality on coriander is not clear. In popular books it is stated that coriander requires a lot of sunshine (Hornok, 1992), but usually it is for the production of seed, rather than leaves. In contrast, research in the Philippines found that plants grown under full sunlight had significantly greater height increments and more leaflets, but less leaf area, shoot/root ratio and leaf chlorophyll content, than shaded plants and so full sunlight affected appearance of coriander as a herb (Kunyaporn Kongsar, 1992). It was shown that production of herb biomass and appearance dually depends on N fertilisation and light intensity (Kunyaporn Kongsar, 1992).

Coloured mulches or supplemented monochromatic light have not, to our knowledge, been trialled for coriander, although they could improve appearance and possibly yield.

We recommend investigating the effects of light quality and quantity on coriander performance as a herb.

5.2.6 Density

In the USA, Anderson and Jia (1996) presented studies on greenhouse production of coriander for vegetable use, but it is not clear if they used vegetable or seed varieties. Initial trials demonstrated that lower plant densities may increase the harvested fresh weight of cilantro foliage even though the means were not statistically different. Subsequent experiments demonstrated no statistical differences in the mean fresh weight per plant in each trial.

5.2.7 Nutrition

In Argentina, a field fertilization experiment was used to evaluate the response of coriander to supplemental nitrogen. It was shown that the response to nitrogen was related to genotype of coriander. Relatively high levels of nitrogen fertilizer should be used in production of the Argentinian land-race and relatively moderate levels of nitrogen fertilizer should be used in production of the European land-race coriander (Lenardis *et al.*, 2001).

It was shown in the Philippines that the effect of N fertilisation on herb biomass production and appearance depends on light intensity. Significant reductions were observed in height increments and number of leaflets with high nitrogen treatment and high shade levels. However, under open conditions or in 25% shade, favourable effects of higher N levels were noted especially in terms of number of leaflets and leaf area (Kunyaporn Kongsar, 1992).

In Egypt, three modes of phosphorus application (fully through the soil, fully through foliage, partially through soil and foliage) were conducted. The highest values in herb biomass were recorded when P was given partially through soil and foliage (Shaheen *et al.*, 1985).

Coriander is suitable for growing in hydroponics. The effect of N in nutrition was studied in the USA (Anderson and Jia, 1996), where it was shown that there were no differences in harvested fresh weight of cilantro with increased levels of fertilizer (100 ppm N compared to 50 ppm N). Interestingly, the authors also found the effect of N fertilisation on herb biomass production depended on light intensity, a similar conclusion to that drawn for field-grown coriander (Kunyaporn Kongsar, 1992). The yield of cilantro was increased in the experiment completed in the spring, when plants received a total of 992 mol m^{-2} PAR compared to plants grown in the autumn, which received only 435 mol m^{-2} PAR (Anderson and Jia, 1996).

In Thailand, extensive investigations of the composition of growth solution for coriander production in hydroponics have been conducted. Eight different formulae of culture solution were studied; two solutions characterised by high total nutrient content, high Mg, Ca and NH_4 compared to others, gave the highest yield in coriander (Ounruen *et al.*, 1999)

In summary - in the field the fertiliser application for coriander should be studied and adjusted to local conditions. N and P are essential, but the source of N and optimal levels of its application need to be investigated. The role of such important macronutrients as K, Ca and Mg, micronutrients, and soil pH has not been studied. It is also possible that vegetable varieties will react differently to fertiliser, than the seed varieties used at present in the UK. The nutrition of coriander in hydroponics is rather well studied.

5.2.8 Mycorrhizas

No scientific data.

5.2.9 Irrigation

Optimal irrigation for herb production in coriander needs to be further studied. It has been noted that during the juvenile period, coriander is very sensitive to water shortage (Palamar and Chotina, 1953; Diederichsen, 1996). At later stages coriander is drought tolerant, but the provision of adequate water prolonged the vegetative stage, while a shortage of water induced bolting (Diederichsen, 1996).

Optimal irrigation rates should be found for coriander.

5.2.10 Growth regulators

No scientific data.

5.2.11 Elicitors and stress signals

No scientific data.

5.3 Changes in essential oil and of aroma due to diversity and growth conditions

5.3.1 Composition of essential oil.

The major components of the essential oil in coriander leaf (Table 3) are C_{10}-C_{16} aliphatic aldehydes and alcohols, but the particular components vary between samples of different origin. Potter (1996) listed 59 components in essential oil in cilantro. Eyres (2005) recently found a total of 81 components.

Table 3. Essential oils composition of coriander leaf (Potter, 1996)

RRT*	Compound	% TIC**
0.317	nonane	0.36 – **1.28**
0.541	octanal	0.03 – 0.47
0.595	limonene	0.03 – 0.2
0.812	nonanal	0.07 – 0.27
1.086	*(E)*-9-decenal	0.03 – 0.46
1.118	decanal	**9.25 – 9.45**
1.23	decenal isomer	**0.03 – 0.68**
1.284	*(E)*-2-decenal	0.87 – **12.1**
1.293	2-decen-1-ol	0.03 – **18.8**
1.305	1-decanol	0.89 – 2.09
1.313	undecenal isomer	0.09 – **1.93**
1.405	undecanal	**2.14 – 2.31**
1.565	*(E)*-2-undecenal	**1.18 – 5.32**
1.577	2-decen-1-ol	0.03 – 0.21
1.655	dodecenal isomer	0.31 – 0.34
1.69	dodecanal	**4.96 – 10.3**
1.795	dodecenal isomer	0.47 – 0.51
1.855	*(E)*-2-dodecenal	**15.6 – 21.6**
1.858	2-dodecen-1-ol	0.58 – 0.82
1.955	tridecanal	**1.44**
2.102	*(E)*-2-tridecanal	**1.83 – 2.53**
2.181	tetradecanal isomer	0.26
2.211	tetradecanal	**1.69 – 2.22**
2.25	diallylfumerate	0.03 – 0.25
2.312	tetradecenal isomer	0.36
2.368	*(E)*-2-tetradecenal	**12.7 – 20.2**
2.454	pentadecenal	0.47 – 0.61
2.598	*(E)*-2-pentadecenal	**4.77 – 5.12**
2.748	*(E)*-2-hexadecenal	0.94 –1.58
2.922	phytol	**2.79 – 3.46**
3.005	1-eicosanol	0.4 – **1.48**
3.092	1-docosanol	**1.38 – 2.41**
3.202	1-tetracosanol	0.35 – 0.42

* - Retention time relative to that of the internal standard, naphthalene-*d*8.

** - Percent total ion current; only compounds reached 0.2% of TIC are shown.

5.3.2 Biosynthesis of components

The idea that aliphatic aldehydes and alcohols are synthesised via β-oxidation of fatty acids comes from fruits (pear, banana, apple, melon), where these and shorter compounds are produced during ripening, giving fruits their specific aromas. But even for fruits, this pathway is only suggested and only indirectly proven (Sanz *et al.*, 1997). The pathway of β-oxidation of fatty acids can be found at http://www.gwu.edu/%7Empb/polyunsaturated.htm

5.3.3 Flavour components

An investigation into the aroma of coriander leaf essential oil has recently been published (Eyres *et al.*, 2005). The authors showed that of 81 compounds, 33-38 possibly contribute to the leaf odour. There was, however, a striking disagreement between concentration of compounds in essential oil and their contribution to flavour, the major compound, *(E)*-2-

decenol (26% TIC) contributed only 0.39% to the flavour as assessed by the Charm[5] value. Compounds with very low or even trace concentrations, such as (*Z*)-2-decenal (0.16% TIC), β-ionone (0.02% TIC), eugenol (less than 0.01% TIC), contributed massively (16%, 11%, 4% of the Charm value, respectively) to flavour. It should also be noted, that although aliphatic aldehydes and alcohols were major (73%) contributors to the aroma of the coriander leaf, terpenoids (β-ionone, linalool) and phenyl propanoids (eugenol, camphor) made a significant contribution (26%) even though they are rarely even listed as components as they are only present in trace amounts in the essential oil.

This work demonstrates, again, that the concentration of a substance in essential oil does not necessary correlate with its perception by humans. It is important therefore that any work on the selection of appropriate varieties of coriander should be accompanied by flavour or at least odour evaluation.

5.3.4 Varieties

Potter (1996) showed that between plants of different origin some major compounds vary - for example, *(E)*-2-decenal varied from 1 to12%, 2-decen-1-ol from 0 to 8%, *(E)*-2-undecenal from 1 to 5%, dodecenal from 10 to 5%, *(E)*-2-dodecenal from 22 to 16%, *(E)*-2-tetrodecenal from 20 to 13%, and minor components also vary, as shown for varieties purchased at the local market.

Any work on the selection of appropriate varieties of coriander should be accompanied by flavour or at least odour evaluation, as composition of essential oils, and possibly flavour changes from variety to variety (Gil et al., 2002).

5.3.5 Ontogenesis

The composition of coriander essential oils is different at different stages of development (Ayanoglu *et al.*, 2002).

At bolting/beginning of blooming, Mookherjee *et al*. (1989) showed that the composition of the whole plant essential oil changed compared to the rosette stage, for example, *(E)*-2-decenal increased to 36%, compared with 1-12% present at the rosette stage, nonane increased from 1% to 15%, but *(E)*-2-dodecenal dropped from 21% to 10%, (*E*)-2-tetradecanal decreased from 20% to 4%. Informal tasting also suggests that during bolting the normal slightly bitter taste of coriander leaves becomes extreme and is accompanied by a soapy flavour. It might thus be expected that this increase in unpalatable flavour qualities may be associated with the increase of some aliphatic aldehydes/alcohols in transition to flowering. Potter (1996) suggested, that it could be due to *(E)*-2-decenal. It is an irritant found in the defensive secretions of some insects (Jacobs *et al.*, 1989; Sax and Lewis, 1989) and would be predicted to be aversive for humans (Potter, 1996).

At full blooming, the major substances of whole plant essential oil are: (*E*)-2-decenal (46.6%), also (*E*)-2-dodecanal, decanal, (*E*)-2-undecanal, (*E*)-2-tetradecanal, 1-decanol, and 2-decen-1-ol (Potter and Fagerson, 1990). At later stages, metabolism turns from biosynthesis of aliphatic aldehydes and alcohols towards biosynthesis of terpenoids (linalool, α-pinene, γ-terpinene, geranylacetate, camphor and geraniol), which are the major components of coriander seed essential oil (Diederichsen, 1996). These changes can be seen in Table 4. Comparison of Tables 3 and 4 shows that already at the beginning of flowering, coriander essential oil changes dramatically. Most of major components of leaf oil decrease in concentration (for example, *(E)*-2-dodecenal from 20 to 2%, and *(E)*-2-dodecenal from 21 to 7%) and disappear in later stages, but linalool, α-pinene and others appear and increase in concentration during fruiting.

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[5] panellists inhaled serial dilutions of oils until no odours were detected. Then results were analysed using CharmAnalysis™ using Charmware™ software, which integrated results of individual samplings

Table 4. Changes of the whole plant essential oil composition during fruit ripening in coriander (1-beginning of fruit ripening, 2, 3, 4- stages of ripening, 5-ripening completed) http://viness.narod.ru/coriander.htm

Components	1	2	3	4	5
α-pinene	trace	0.86	**1.76**	**6.61**	**8.23**
camphene	tr	0.13	0.32	**1.17**	**1.35**
sabinene	tr	tr	0.11	0.17	0.14
β-pinene	tr	tr	0.18	0.22	0.22
β -myrcene+octanal	0.87	0.40	0.47	**1.20**	**1.40**
p-cymene	0.39	0.29	1.34	0.79	0.80
1,8-cineol	0.49	0.32	**1.32**	**2.71**	**3.09**
γ-terpinene	tr	0.91	**2.32**	**5.48**	**5.64**
(E)-linalool oxide	**2.68**	0.21	0.15	tr	tr
(Z)- linalool oxide	**2.46**	0.18	0.25	tr	tr
Terpinolene	0.42	0.10	tr	0.60	0.59
Linalool	0.79	**44.89**	**58.24**	**66.01**	**66.15**
Camphor	**1.32**	**3.72**	**4.07**	**4.84**	**4.58**
Dodecanol-2	0.77	0.89	0.35	0.27	0.26
Decanal	**7.98**	**6.89**	**2.60**	0.24	0.10
(E)-2-decenal	0.93	0.58	0.15	tr	tr
(Z)-2-decenal +geraniol	**47.57**	**23.41**	**8.72**	**2.30**	**1.85**
(E)-2-decen-1-ol	**5.33**	**1.57**	0.81	tr	tr
(Z)-2-decen-1-ol	**3.20**	**1.23**	0.56	tr	tr
dodecenal	0.53	0.25	0.17	tr	tr.
(E)-undecenal	**2.60**	**1.42**	0.91	0.13	0.14
Geranyl-acetate	0.16	0.59	**2.46**	**3.76**	**4.13**
(E)-2-dodecenal	**7.26**	**6.30**	**6.44**	**1.17**	0.26
(E)-2-tetradecenal	**2.16**	**1.78**	**3.49**	0.20	0.20

5.3.7 Temperature and photoperiod

No scientific data.

5.3.8 Light of different quality and quantity

No scientific data.

5.3.9 Density

No scientific data.

5.3.10 Nutrition

No scientific data.

5.3.11 Irrigation

No scientific data.

5.3.12 Growth regulators

No scientific data.

5.3.13 Elicitors and stress signals

No scientific data.

5.4 Can growth conditions and diversity improve shelf-life of pot and field grown coriander?

Shelf-life of pot-grown coriander should improve with the use of vegetable varieties (see 5.2.1, above). However, treatment with a variety of hormones or their inhibitors has proved to be effective in slowing down leaf senescence and extending shelf-life (Jiang *et al.*, 2002b). Senescence of cut and bunched coriander leaves was significantly retarded by pre-harvest treatment of plants with gibberellin - GA_3 (sprayed as 50 mM solution). This effect was due to retarded total protein destruction, which was observed during senescence when it was 30-40% slower, than in un-treated plants (Jiang *et al.*, 2002b).

5.5 Recommendations for research to be carried out on coriander

As the review shows, coriander as a herb is not well studied, most research on coriander was performed with the aim of improving seed yield. These works are not shown in the review, but their total number is probably 5-6 times higher, than that on leaf production. The issue is also complicated by fact, that coriander leaf (cilantro) is very popular in cooking only in several places including the UK, in the former USSR, in some countries of Latin America and in the Indian subcontinent. The UK and the former USSR are more difficult places to grow coriander, in terms of climate, than Latin America and South Asia, where coriander grows well in every garden. As a result, no effort or investment have been allocated to investigating cilantro production in Latin America or the Indian subcontinent. The most vigorous research was conducted in the past in the former Soviet Union but this is not the case presently. Since there is no current research programme overseas it is likely that British growers, who are interested in increasing the quality and sales of coriander, should look towards performing their own research.

Key issues to address are: (not in order of preference)

- Search for "best varieties" for pot and field growers in the UK.
- Evaluation of flavour components in coriander and effect of growth conditions on composition of oil and flavour constitutes. There is no knowledge available here at all.
- Research on optimal growth conditions, especially those increasing its shelf-life, as it will improve quality and decrease waste in supermarkets.

6 Dill – *Anethum graveolens* L.

6.1 Diversity of dill

There are many varieties of dill sold in the world and even more are stored in gene-banks. Varieties of dill differ by aroma, by number of branches and leaves, by form of rosette, by leaf colour, area, length and width. But the most important parameter for classification of dill varieties is the time of bolting as this parameter determines date of herb harvest, yield of herb and date of seed ripening. There are also especially selected 'leafy dills' – bush varieties.

6.1.1 Flowering time

Early varieties bolt on 22 – 32nd day and have a harvesting window of about 10 days. Examples of early varieties are: 'à jour', 'Aromatic', 'Esta', 'Grenadier', 'Gribovskii' and 'Umbrella'. *Medium varieties* bolt on 30 – 35th day and have a harvesting window of about 15 days. Examples of medium varieties are: 'Anna', 'Lesnogorodskii', 'Richelieu', 'Super Dukat' and 'Uzory'. *Late varieties* bolt on 35 – 40th days and have herb-harvesting window about of 25 days. Examples of late varieties are: 'Tetra Borée', 'Kibrai'.

6.1.2 Bush varieties

These varieties are very special, because on the base of stem 5-6 internodes are connivent[6] (in other varieties, this applies to just one or two internodes), so branches are produced at a very short distance, giving the plant a 'bushy' appearance. Bush varieties are also characterised by a very big rosette, up to 40 – 50 cm in diameter. Plants look very strong and are 1.5 – 3 m tall, with a leaf length that can reach 40 – 45 cm. Bush varieties produce more biomass than others; they bolt only on 40 – 45th day and have a harvesting window of about 25 days. Because bolting is so late, the aroma of the leaves persists and their texture is tender over a very long period. Examples of bush varieties are: 'Alligator', 'Amazon', 'Bouquet', 'Russian Size', 'Salute' and 'Sultan'.

6.2 Changes in yield and appearance due to diversity and growth conditions

6.2.1 Varieties

Systematic research on dill varieties stored in VIR genebank showed that late varieties have much bigger leaves and biomass, compared to early varieties (Shashilova, 1988).

We recommend a study to determine if bush (see description above) and if late varieties are suitable for dill herb production in the UK.

6.2.2 Ontogenesis

As explained above, the important factor in the development of dill is bolting - the later the bolting the higher is herb yield.

Again, it should be studied if late and bush varieties should be used in the UK to postpone bolting and increase harvesting window.

6.2.3 Mulches

No scientific data. In popular books it is said, that "there is no point of growing dill, if soil is not free from weed" (Hornok, 1980).

We recommend an investigation of the effect of mulches on dill production.

6.2.4 Temperature and photoperiod

Dill is a plant that can tolerate frost down to -6°C and seed germination can start at +3°C. Plant growth is retarded by high temperatures but can occur in very cool conditions (+8 to + 10°C), although the optimal temperature for growth is +16 to +17°C (Alborishvilli, 1984). Indeed, it was shown for three varieties of dill ('Dura', 'Dukat' and 'Mammut') that the average herb yield decreased dramatically the further north dill was grown, being 176, 51 and 10 kg/100 m^2 in southern, central and northern Finland respectively. In southern Finland the growth period was 49 days compared to 74 days in the north. In the north, dill also did not reach the height expected for a first class herb (15-30 cm) before inflorescence formation (Halva, 1987b; Halva *et al.*, 1988).

Temperature and photoperiod have additive effect on production of herb biomass. When several varieties were trialled in Georgia (former USSR), it was shown that plants sown in March (cool short days for Georgia) reached a saleable state twice as slowly as those sown in June (hot long days) to September (hot short days) (Alborishvilli, 1984).

The effect of photoperiod has been extensively studied in Russia on 150 varieties of dill. It was shown that dill was a typical long-day plant and to flower it requires 14 h of photoperiod. In short days (8-10 h photoperiod) only leaves were produced and no bolting was observed (Shashilova, 1988). Thus, productivity of dill as a herb should be greater with a short-day photoperiod. This was confirmed in Finland on field-grown dill where plants sown in short days (March) gave about 60% higher foliage yield than those sown in long

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[6] Converging, arching over so as to meet: Henderson's Dictionary of Biological Terms, 11th Edition. Longman Scientific and Technical, 1995

days (June). At the same time, the rate of development was quicker in long days, so plants sown in midsummer reached maturity and bolted 7-10 days ahead of those sown in spring; however, as noted earlier, these plants produced less foliage (Halva, 1987b). A similar situation was observed in Canada, Nova Scotia where three varieties of dill ('Mesten', 'Dukat' and 'Hercules') sown in May gave three times increase in fresh herb biomass than plants sown in June (Bowes *et al.*, 2004).

The effect of temperature and photoperiod is well studied for dill, and we can conclude that, although dill is a chill-tolerant plant, its development is optimal at mild temperatures; growth is quicker in long days, and biomass production is higher in short days.

6.2.5 Light of different quality and quantity

Although popular books claim that dill is a heliophyte, we did not find proper research demonstrating that herb production increases with light intensity. Halva *et al*. (1992a) once mentioned that growth and essential oil accumulation increased with an increase in light level and was greatest under full sunlight.

We recommend an investigation of the reaction of dill to light intensity.

Dill productivity has been reported to be rather insensitive to light quality, as studies in Finland showed where supplemental red, far-red and blue light revealed no significant differences in biomass yield. The control and blue-light-treated plants had shorter internodes. Red light and far red light induced internode growth, decreased leaf area and increased plant growth rate (Halva *et al.*, 1992a; Halva *et al.*, 1992b).

We conclude that herb yield in dill cannot be improved by morphogenetic light, but appearance (plant height) could be manipulated.

6.2.6 Density

It has been shown, that increasing plant density often decreases green biomass of individual plants, but increases total yield per area (Halva, 1987a). Thick stems in dill cause problems at harvest and are not needed, as the herb is grown mainly for leaves. Thus thinner stems, but higher leaf yield per ha would be an advantage (Halva, 1987a).

In Northeast USA, density proved to be a positive regulator of dill foliage production where maximum production (fresh weight per area) occurred with narrow row widths and close plant spacing within the row (15 cm by 10 cm, respectively). Plant height was not affected by increased density (Garrabrants and Craker, 1987). As expected, fresh weight of individual plants decreased with increase in density. The authors concluded that high density is beneficial for production of dill herb (Garrabrants and Craker, 1987). Conflicting results were obtained on field-grown dill (cv. 'Dura') in Finland. Again height was unaffected by density, and the proportion of leaves to stems was indeed greater at high density (12 cm row spacing versus 25 cm). But, high density, although improving individual plants, brought about a decrease in total herb yield (from 13 to 10 kg/100m^2) and separated leaf yield (from 8 to 6 kg/100m^2) (Halva, 1987a). The authors concluded that row spacing should be further investigated for dill to find optimal practice. It is also worth mentioning, that at high densities, the rate of growth slowed down and dill reached a harvestable stage 2-3 days later than at a low sowing density (Singh and Randhawa, 1991). It should also be noted that bush varieties are affected by high density, as popular books claim, and optimal spacing between individual plants should be 25–30 cm.

The effect of sowing density should be studied for dill, as research performed is controversial. It is also possible that different varieties react differently with increased plant density.

6.2.7 Nutrition

Dill has been shown to be sensitive to fertiliser level. For example, it was shown in Finland for field-grown dill (variety 'Dura'), that without fertiliser, the plants were yellowish and developed slowly. Increase in fertilisation up to 40-10-68 kg NPK/ha increased fresh biomass by three times. This application was declared optimal and further increase in N decreased the yield (Halva *et al.*, 1987). It was also shown in Hungary that with NPK fertilisation (20-20-20 kg NPK/ha) the biomass of fresh herb was twice that without fertilisation. By increasing the level of N supply to N_3PK the herb fresh biomass increased by a further 30%. Increase in P and K (NP_2K and NPK_2) also increased herb biomass production in dill, but not so much as with N. Further increase in P and K had no effect. Thus optimal NPK was declared as 60-40-40 (the difference with previous work could be due to different soils in Finland and Hungary). It was also shown that NPK fertiliser could be substituted by leaf-mould containing an organic source of N (2% Karbamid or 0.1% Wuxal), as it increased green biomass production to a similar extent as N_2PK and N_3PK, respectively (Hornok, 1980). In Northeast USA, the addition of nitrogen was found to be promotional for dill in even greater doses than in Finland. When N was applied at increasing dose (0, 60, 120 kg/ha, the form of N is not given) fresh biomass of dill increased from 49 tons/ha, to 68 tones/ha, to 97 tones/ha, respectively. Increase in P levels also increased growth, but K had no effect (Garrabrants and Craker, 1985; Garrabrants and Craker, 1987).

It should be noted, that N and P, although promoting production of leaves, slows down the growth rate, and plants reached their harvestable stage 7 days later for highest dose of N (120 kg/ha) or 2 days later for P_2O_5 (40kg/ha) (Singh and Randhawa, 1991).

One study from Japan reported on the performance of dill in hydroponics (Udagawa, 1995). Fresh herb biomass was shown to increase by four times, when a nutrient solution was applied in increasing dose (1.2 dS/m, 2.4 mS/cm, 3.6 dS/m; concentrations of macro and micro nutrients are provided). The concentration of N, P, K and Ca in leaves also increased with increase in nutrient level - only Mg had its peak at 2.4 dS/m, and decreased to initial value at 3.6 dS/mm. Thus, the optimal concentration for productivity and nutrient uptake was 2.4 dS/m (Udagawa, 1995). The need for P in hydroponics in gravel culture was investigated in another study. Dill growth was greatly inhibited by the absence of P, but did not show phosphorus deficient symptoms (Shinohara *et al.*, 1992); the optimum phosphorus concentration for those herb plants was estimated to be 4 to 8 meq/l.

In summary, in the field the fertiliser application for dill should be adjusted to local conditions. N, P and K are all essential, but again the ratio of their application for optimal production depends on local soil conditions, so the response of dill to fertiliser should be trialled in UK soils. It is also possible that different varieties, especially bush and late varieties, will react differently to fertiliser, from the medium and early varieties, used in the studies above. From published papers it appears that dill has no preferences for the source of nitrogen. In popular literature organic sources of N are recommended; there is also anecdotal evidence, that growth of leaves is promoted and flowering is postponed when organic sources of N are used. Thus, it sources of nitrogen should be scrutinised for dill. The effects of changes in such important macronutrienst as Ca and Mg, micronutrients and soil pH has not been studied. Nutrition of dill in hydroponics should also be scrutinised, as very little is known.

6.2.8 Mycorrhizas

No scientific data.

Should be studied, as mycorrhizas very often promote growth of herbs.

6.2.9 Irrigation

No scientific data.

Should be studied, because in popular books (Hornok, 1992) dill is described as sensitive to irrigation.

6.2.10 Growth regulators

Plant growth regulators have been shown to increase yield of dill and for the var. 'Balady' in Egypt to improve appearance (El-Khateeb, 1994). The ethylene precursor, ethephone, applied at 50 and 100 ppm, increased the number of primary branches by 30-50%, but did not change plant height. Fresh weight also increased by 20-50% at 50 ppm, and 5-25% at 100 ppm.

Folic acid applied at 25 ppm increased plant height by 15%, number of primary branches by 30-70%, and fresh weight by 30-50% (El-Khateeb, 1994).

Auxin (indole acetic acid applied) at 50 and 100 ppm increased plant height by 10%, number of primary branches by 45%, but did not change the fresh weight. Of the various treatments, highest number of branches was at 100 ppm ethephone, highest plant height at 25 ppm folic acid, highest plant biomass at 50 ppm ethephone (El-Khateeb, 1994).
Thus we recommend investigating application of growth regulators, within the constraints of current regulations, to dill in the UK, as they can improve growth and appearance.

6.2.11 Elicitors and stress signals

No scientific data.
Should be studied, as they are often found to increase production and change the composition of essential oil.

6.3 Changes in essential oil and of aroma due to diversity and growth conditions

6.3.1 Composition of essential oil

Although little investigated, there is a suggestion that the oil content and composition is relatively unaffected by cultivation conditions (Hay and Waterman, 1993). The transition from the vegetative to the flowering stage may alter the composition (see below), but due to the dearth of research it is practically impossible to predict the variation due to changes in the environmental conditions from one year to another. Table 5 indicates the relatively narrow range of the individual compounds within the total oil chemical composition (Bauer *et al.*, 1997).

Table 5. Composition of *Anethum graveolens* (dill)

Major components	Composition (% of total)	Comments
Carvone	30-40	*
p-Cymene	10-20	*
cis/trans-Dihydrocarvone	1-2.5	
Dimethylhexahydrobenzofuran	0.4-12.0	*
Elemicin	1.0-3.0	
3,9-epoxy-*p*-ment-1-ene	10-15	** (Dill ether)
Fenchone	1.0	
Limonene	30-40	*
Methyl-2-methylbutanoate		*
Myrcene	0.2-1.5	
Myristicine	0.5-18.0	*
(+)-(4S)--phellandrene	10-20	
α-Pinene	0.5-5.0	
Pinocarvone	3.0	
γ-Terpinene	3.6-61.5	
α-Terpineol	0.1-2.0	

* main components responsible for flavour and fragrance

6.3.2 Aroma components

The contribution of various chemical components of dill to flavour and aroma has been analysed (Blank *et al.*, 1992). It was shown that in an extract obtained from the fresh material only five compounds, out of more than 20, have any significant impact on odour. Two compounds were major contributors: α-phellandrene was evaluated as the compound dominating the characteristic flavour of dill which was rounded off by an additive effect of dill ether (3,9-epoxy-1-p-menthene = 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran). Some other components, although of relatively high content, did not contribute to flavour significantly (β-phellandrene, α-pinene, p-cymene, apiol), while others - myristicin, methyl 2-methylbutanoate and (R)-limonene - contributed to odour.

This work also shows that concentration of substance in an essential oil does not necessarily correlate with its perception by humans, and substances produced in about the same concentration are not necessarily all perceived. Any work therefore on selection of appropriate varieties of dill should be accompanied by flavour or at least odour evaluation.

6.3.3 Varieties

Dill varieties differ in oil production and composition. In the Canadian varieties 'Mesten', 'Dukat' and 'Hercules' it was found that 'Mesten', depending on the year, produced 1.5-3 times more essential oil, than 'Dukat' and 'Hercules'. The composition of the oil was similar in all three cultivars (Bowes *et al.*, 2004). Oil content and composition can, however, vary between cultivars. In Finland, the total oil content in 'Dura' was 20-40% higher than in 'Mammut' and 'Dukat'. The relative proportion of the components was also different. In 'Dura' the ratio of dill ether - α-phellandrene was 5:3, in 'Mammut' it was 3:5, in 'Dukat', 1:1. The other main components (β-phellandrene, limonene, p-cymene) had similar content in these three varieties (Halva, 1987b). From the study of Blank *et al.* (1992) these three varieties should have different aromas, as their main aromatic components differ in proportion.

We recommend the selection of dill varieties in parallel with an investigation of flavour.

6.3.4 Ontogenesis

The most abundant compounds of the oil from the shoots of dill are α-phellandrene, dill ether, limonene, β-phellandrene and p-cymene; together they comprise 65-80% of all the analyzed compounds and 80-90% of all the identified compounds (Huopalahti and Linko, 1983), see also Table 5.

The composition of the essential oil from dill changes significantly during growth. Huopalahti and Linko (1983) measured, at various growth stages, the composition of essential oil from dill grown in Finland, these were initial herb stage, before bud formation and flowering stage. They showed that α-phellandrene was the major component of the aroma isolated at the initial herb stage (34%) and before the bud formation (47%), but decreased at flowering stage. Also the content of β-phellandrene, α-terpineol, myristicin, and apiol, high at the initial herb stage, decreased in transition to the flower stage. In contrast, the content of limonene, dill ether, terpinen-4-ol, and carvone increased during the growth period. For example, at the initial herb stage dill ether was 11%, while at the flowering stage it was already 32% - the most abundant compound. An increase of carvone content was also remarkable as at the initial herb stage carvone was not even measurable, while at the flowering stage its relative proportion was already 2.5% and was evidently further increasing. In the seed oil carvone and limonene was shown to predominate (Miyazawa and Kameoka, 1974; Scheffer *et al.*, 1977).

As composition of essential oil changes so much with ontogenesis we recommend using sensory evaluation to investigate the stage at which a particular variety is optimal (composition of oil is different in different varieties, see above, and likely to exhibit different changes during growth). It would be also beneficial to develop a

'growth index', similar to the 'flowering index' developed for basil (see above), which will show the best stage of vegetative growth to obtain good flavoured dill leaves.

6.3.5 Temperature and photoperiod

The content, composition and proportion of components of essential oil did not change with temperature, when three varieties of dill ('Dura', 'Mammut' and 'Dukat') were grown in three different locations in Finland (north, central, south) (Halva, 1987b) However, the same authors in another article reported, that degree-days is the most important climatic factor affecting the essential oil content in 'Dura'. The total oil content increased significantly with increasing degree-days, but not dramatically (Halva *et al.*, 1988). Degree-days also changed the composition of the oil, as they increased the concentration of dill ether, a-phellandrene and limonene, but not other components (Halva *et al.*, 1988).
Thus the effect of temperature and day-degree on essential oil production in dill should be additionally studied and correlation between day-degree and temperature should be understood.

6.3.6 Light of different quality and quantity

The production of essential oil in dill was found to be sensitive both to intensity and quality of light. The accumulation of essential oil increased in dill with increases in the light level and was greatest under full sunlight (Halva *et al.*, 1992a; Halva *et al.*, 1992b). In terms of composition, a positive correlation was found for radiation (sunshine hours) and content of p-cymene and thymol, but not other components of dill oil (Halva *et al.*, 1988).
Thus we recommend the further understanding of flavour as high light intensity seems to change the composition of essential oil.

Light of different quality was shown to change oil quality and quantity. In Finland, where the effect of supplemental red, far-red and blue light was studied. Essential oil concentration was greatest in plants grown under far-red treatment. Red light treatment for 4 h also induced more oil production than control and blue light treatments, although decreased biomass (see 6.2.6). Compositionally, the plants exposed to 4 h of red and far-red light produced oil containing more α-phellandrene and less myristicin (Halva *et al.*, 1992a; Halva *et al.*, 1992b). ***We recommend studying the effect of morphogenetic light on essential oil in dill and accompanying this research with sensory evaluation, as light of different quality changes oil composition.***

6.3.7 Density

No scientific data.
Should be studied, as density affects photosynthesis and light quality and may change terpenoid components in dill essential oil.

6.3.8 Nutrition

In the field, production of essential oil in dill is sensitive to fertiliser application. For example, it was shown for var. 'Budakalaszi' in Hungary that without NPK, production of herb essential oil is 15% lower than when fertilised (Hornok, 1980). The optimal dose of fertiliser was found to be different between different countries. In Finland, variety 'Dura' gave 9-10 kg oil /ha on unfertilised plots. Adding fertiliser (40-10-68 kg NPK per ha) increased the yield up to 16 kg/ha when there was no further increase with more N. Oil composition (α-phellandrene, limonene, β-phellandrene, dill ether) did not change consistently with changes in N in fertiliser (Halva *et al.*, 1987). The same amount of N was found optimal in Hungary by increasing the level of N supply (initial 20-20-20 kg NPK/ha) where the essential oil production increased by 25% in combination N_2PK (Hornok, 1980). Further N increase did not give any gain in essential oil production. Altering the ratio of P and K did not change concentration of essential oil when applied in very high doses (NP_3K or NPK_3); it even decreased (slightly) essential oil concentration (Hornok, 1980). Thus in this study, optimal fertilisation was 40-20-20 kg/ha in Hungary, but 40-10-68 in Finland (Halva *et al.*, 1987). In the field in India (in winter) even higher doses of N and P were found to increase oil yield. Increase of N from 0 to 120 kg/ha and P_2O_5 from 0 to 40 kg/ha promoted a 100% and 25% increase in oil yield, respectively (Singh *et al.*, 1987).

Substitution of inorganic N by organic proved to be promotional for dill oil production when NPK was substituted by leaf-mould containing an N-effective agent (2% Karbamid and 0.1% Wuxal), it promoted an increase in essential oil production (2 and 3 times, respectively, Hornok, 1980).

Only one study from Japan has reported the effect of nutrition in hydroponically produced dill. When nutrient solution was applied in increasing dose (1.2 dS/m, 2.4 dS/m, 3.6 dS/m; concentrations of macro- and micro-nutrients are provided), the concentration of essential oil did not change. The main component - α-phellandrene – suffered a decrease at 3.6 dS/m (Udagawa, 1995).

Production of essential oils in dill depends very much on nutrition and organic forms of N may be preferable over inorganic N. We recommend adjusting particular applications of nutrient to particular soils and possibly variety. The effects of Ca, Mg, micronutrients and pH on flavour should be investigated as should the effects of nutrients in hydroponics.

6.3.9 Irrigation

No scientific data.

Should be studied, because in popular books (Hornok, 1992) dill is described as sensitive to irrigation.

6.3.10 Growth regulators

There is no data on how growth regulators affect dill herb oil, but they change dill seed oil significantly. Increasing amounts of folic acid (25, 50 ppm), ethephon (50,100 ppm) and IAA (50, 100 ppm) increased the content of essential seed oil in dill by 30-50% (El-Khateeb, 1994). Application of these growth regulators decreased the carvone content in dill essential oil, but increased the percentage of dihydrocarvone, indicating the affected target at the terminal enzymic transformations in the carvone pathway from limonene. The greatest contents of terpenes - limonene, pinene and dipentene - in the oil were obtained from plants treated with ethephon or IAA, whereas folic acid treatment increased the percentage of phenylpropanoid – apiole (El-Khateeb, 1994).

Thus, we recommend an investigation of the effect of growth regulators on herb oil, as they could increase its quantity and change its quality, such studies should be accompanied by flavour research.

6.3.11 Elicitors and stress signals

No scientific data.

Should be studied, as they are often found to increase production and change composition of essential oil.

6.4. Can growth conditions and diversity improve shelf-life of pot and field grown dill?

No scientific data.

This topic should be investigated, because different growth conditions and use of appropriate varieties should improve dill's shelf life, as was , for example, shown for basil.

6.5. Recommendations for research to perform on dill

Research is performed on early and medium varieties, and rarely on late or bush varieties, which are most suitable for dill herb production. No research has been conducted on irrigation regimes or mycohrryzas with research on fertilisation and light requirements coming from a range of different countries and is rather controversial. There has been relatively little research directly applicable to the UK, being either performed to the north (Finland) or to the south (Hungary, India). The climate of Britain is very suitable for dill herb production, much more so than in Scandinavia or India. With improved quality though research and increase of Slavic immigration, observed now in the UK, dill consumption will rise, as it is the favourite herb in Eastern Europe. Our recommendations are:

- Evaluation of cultivars suitable for different regions of the UK, with further specific research as suggested above.
- Investigation of dill flavour constituents, as they are almost unstudied.

7 Rosemary – *Rosmarinus officinalis* L.

7.1 Diversity of rosemary

Numerous cultivars of rosemary have been selected for ornamental use and are sold in nurseries, but they are not necessarily the best for herb and essential oil production, as they are not characterised for biomass, quality and chemical composition of the essential oil (Mulas and Mulas, 2005). In the last decade it has been recognised that a selection for the food market should be performed. Natural populations of rosemary are found in the Mediterranean basin and show a high genetic variability. In Sardinia and Italy in 1996 a programme was started to select new cultivars for horticulture from wild populations (Mulas *et al*., 2002). Rosemary is now also recognised as a potential crop for Africa (http://www.asnapp.org/resources/plantlist.html) and efforts are being made to establish a collection of the best available horticultural varieties.

7.2 Changes in yield and appearance due to diversity and growth conditions

7.2.1 Varieties

Natural populations of rosemary in the Mediterranean region are characterised by high diversity in terms of appearance and biomass productivity. In Italy, cultivar selection from these natural populations started in 1996 and still continues with the aim of producing new varieties, suitable for the food industry. Thirty one plants from Sardinia were chosen and after vigorous selection the six most promising lines for the herb industry registered, differing in yield and appearance. The cultivar ‘Gerrei’ showed a very upright habit, while ‘Costa Paradiso’ was characterised by compact habit and medium vigour. ‘Sette Fratelli’ was prostrate with low vigour, while the highest vigour (highest shoot length and leaf weight) was observed in plants of ‘Sant Antioco’ (Mulas *et al.*, 2002; Mulas and Mulas, 2005).
Since the Mediterranean and the UK are very different climatically, we recommend assessing cultivars of rosemary for optimal biomass productivity and best appearance under UK conditions.

7.2.2 Ontogenesis

No scientific data.

7.2.3 Mulches

Black polyethylene mulch, black polypropylene ‘transpiring mulch’ and black polypropylene ‘draining mulch’ were trialled in Italy to check if they protect field-grown rosemary from weeds and improve yield. The polyethylene and transpiring mulch controlled 100% of weed. The draining mulch was, from the beginning, perforated by some weed species, which covered 50% of mulch at the end of season. In terms of biomass production transpiring mulch was the best, increasing plant height by 10% and plant canopy diameter by 15%. Polyethylene mulch did not increase these parameters significantly. Thus, authors recommended using transpiring mulch to protect rosemary from weeds and additionally increase yield (Hoeberechts *et al.*, 2004).

We recommend trialling in the UK the use of different mulches on rosemary, as these practices can reduce the need for weed control, increase soil temperature and keep soil from splashing onto leaves.

7.2.4 Temperature and photoperiod

No scientific studies. Anecdotal recommendations are that rosemary suffers at low temperatures and needs to be protected when cultivated in cold-winter areas (Maffei *et al.*, 1993). Rosemary produces maximum leaf growth with high oil content in warm sunny areas – cloudy conditions or low temperatures will adversely affect both the leaf growth and the oil content (ASNAPP).

The effect of temperature, day-degree and photoperiod should be investigated for rosemary.

7.2.5 Light of different quality and quantity

No scientific studies. Anecdotal recommendations are that rosemary is indigenous to the Mediterranean and thrives on arid sunny mountain slopes (ASNAPP[7]). Rosemary prefers sunny (south slopes) locations and is well adapted to full sunlight morphologically and physiologically (Castrillo *et al.*, 2005).

The effect of light intensity and quality on yield and appearance of rosemary should be investigated.

7.2.6 Density

No scientific data.

7.2.7 Nutrition

Very little is known of the response of rosemary to fertilisers. In Spain, in a typical calcareous Mediterranean soil, adult individuals of rosemary increased their vegetative growth and number of flowers in response to a pulsed increase in nutrient supply (N and P). Sometimes (not every year) the increased fertilization advanced the period of flowering by two weeks. The positive effect of the fertilization was mostly due to addition of P, rather than N. Indeed, limiting the role of phosphorus on plant growth and flowering on calcareous soils has been observed in many studies in widespread areas of the different Mediterranean zones. It is due to low availability of soluble phosphates and presence of less soluble diphosphate and triphosphate of calcium in such soils (Sardans *et al.*, 2005).

In the USA, rosemary was shown to be suitable for production in hydroponics (peat/perlite mixtures) and for pot production in a mixture of peat/perlite/soil. Plants grown in the soil-based mix were shorter, shoot fresh and dry weight tended to be lower and essential oil yield was higher compared to plants grown in the soil-less mix (Boyle *et al.*, 1991). Satisfactory growth was obtained in both media when rosemary plants were fertilized with lowest nutrient mixtures: at 9.0 g NPK /pot of controlled-release fertilizer (NPK = 12 : 5.2 : 12.5) or at 150 mg NPK/ l/week of liquid fertilization (NPK = 20 : 4.3 : 16.7). Higher doses of NPK reduced biomass production and plant height (Boyle *et al.*, 1991).

We recommend investigating the nutrient needs of rosemary.

7.2.8 Mycorrhizas

Mycorrhizas promote growth of rosemary and also help it to withstand water-stress in the Mediterranean. The inoculation of *Glomus deserticola* significantly stimulated the growth in colonized plants in both well-watered and stress conditions compared to control (non-mycorrhizal well-watered) plants. Although the decreased soil water potential generated a decrease in the leaf water potential and the relative water content after 14 days, mycorrhizal

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[7] Agribusiness in sustainable natural plant products. Rosemary.

plants exhibited midday values of water potential that were more than 1MPa higher than in non-mycorrhizal plants under drought conditions. This confirms that leaf water potential is often higher in mycorrhizal plants under water stress because of the higher water uptake (Subramanian *et al.*, 1995) which leads to the stimulation of plant growth as reflected mainly in aerial biomass (Sánchez-Blanco *et al.*, 2004).

We recommend considering if mycorrhizas could promote the growth of rosemary in the UK production.

7.2.9 Irrigation

Rosemary is morphologically well adapted to drought (Gratani and Varone, 2004). It was shown that during very dry summers in the Mediterranean region, the water status of rosemary leaves was affected and although the intensity of photosynthesis decreased, no damage occurred to the photosynthetic systems. After the autumn rainfall photosynthetic capacity recovered fully. Thus rosemary is well adapted to drought physiologically and an increase in water deficit is unlikely to have a significant impact on the photosynthetic capacity of leaves (Munne -Bosch *et al.*, 1999; Nogués and Alegre, 2002). Although rosemary is able to withstand drought, its growth is affected, as was shown in Sardinia. Already under mild water stress, stem and leaf dry weight were lower by 80%, leaf area by 45% and plant height by 50%, compared to well-watered plants. Severe water stress reduced all parameters further, but not as dramatically as it might be expected, thus supporting the idea that the plants' natural drought tolerance mechanisms, such as stomatal closure and reduction of biomass and leaf area, helped to minimise water loss (Alarcon *et al.*, 2006).

Irrigation with sweet water proved to increase vegetative growth of rosemary plantations in Spain. The irrigation effect was significant even though the three years of the experiment were relatively wet for the studied site (Sardans *et al*., 2005).

Irrigation with salty water proved to be ineffective as salt in the irrigation water reduced stem and leaf dry weight, leaf area and plant height by 30%-80 %, compared to control plants, depending on treatment and parameter. Thus, salty water brings about the same reduction in rosemary's yield as drought, so that irrigation with salty water is not a good idea even during drought periods (Alarcon *et al*., 2006).

Drought hardening is recommended in the Mediterranean region when small plants are transplanted to their final position, as it was shown to improve survival rate of transplants and their increased ability to adapt to dry climate. Two ways of hardening and their combination were investigated these were deficit irrigation and low air humidity. At the end of the nursery period it was seen that deficit irrigation had altered the morphology of the plants by reducing plant height, stem diameter, leaf area, total dry weight and root length, while air humidity only influenced the parameters related to plant water relations. The survival rate during a dry Mediterranean summer was higher (100%) when plants had a combined hardening, compared to plants exposed to only deficit irrigation (63%) or only low air humidity (31%) or well watered plants at normal air humidity (25%) (Sanchez-Blanco *et al.*, 2004).

All studies on irrigation of rosemary were performed in a dry hot Mediterranean climate, and any conclusions could not be extended to the UK-grown plant. Moreover, we suspect that irrigation is the last thing needed as the UK climate may be too wet for field-grown rosemary where problems of leaf browning and leaf drop are observed. For the UK, a trial of irrigation methods for rosemary grown in a protected cropping environment may be pertinent.

7.2.10 Growth regulators

No scientific data.

We recommend studying the area because in other herbs growth regulators have been shown to promote growth and improve appearance.

7.2.11 Elicitors and stress signal

No scientific data.

***The effects of elicitors and stress signals might be studied, as they have been found to increase biomass production in other herbs*.**

7.3 Changes in essential oil and of aroma due to diversity and growth conditions

7.3.1 Composition of flavour components

The range of individual components is wide (Table 6), and depends on the genotype of the propagule and environmental conditions.

Table 6. Composition of oil of *Rosemarinus officinalis*

Major components	Composition (% of total)	Comments
Borneol	0.4-17.3	*
Bornyl acetate	0.4-17.3	
Camphene	Trace-19.0	
Camphor	0-56.0	*
Caryophyllenes	0-17.0	
1,8-Cineole	3.0-60.0	*
p-cymene	Trace-11.0	
Limonene	Trace-11.0	
Linalol	0-19.0	
Linalyl acetate	Trace-1.4	
Myrcene	Trace-52.0	
α-pinene	1-57.0	*
β-pinene	Trace-9.0	
Terpenene-4-ol	0.2-6.8	
Thujone	0.2-4.0	
Verbenone	Trace-3.0	*

* major components responsible for fragrance

7.3.2 Flavour components.

Not yet evaluated for rosemary. Rosemary oil has a characteristic, refreshing pleasant fragrance, mainly due to cineole, pinene and camphor: Verbenone adds a specific fragrance to the oil.

We recommend investigating flavour components in rosemary as this study supports finding of aromatic varieties for the food industry.

7.3.3 Varieties

In Italy cultivar selection from natural rosemary populations resulted in cultivars, which differ in composition of essential oil. The cultivar ‘Vignola’ was characterized by the typical essential oil composition of most rosemary selections, with 43.8% of α-pinene as major component. The ‘Sant Antioco’ plants were particularly rich in camphor (21.4%) and 1,8-cineole (17.6%), while ‘Sette Fratelli’ and ‘Gerrei’ showed high concentration in verbenone (above 12.0%). ‘Cala Gonone’ stands along because of the abundance of 1,8 cineole (15.0%), borneol (17.5%) and bornyl acetate (12.4%). A high percentage of borneol (26.0%) and bornyl acetate (16.0%) was also observed in ‘Costa Paradiso’ plants (Mulas and Mulas, 2005).

We recommend assessing oil productivity and composition along with a flavour (at least aroma) evaluation in order to select the best rosemary available to the UK growers.

7.3.4 Ontogenesis

The concentration of essential oil depends on plant age and organ and was shown to be highest in young leaves, young soft stems, buds and flowers (ASNAPP). Between organs, the highest percentage of essential oil was obtained from the leaves of the apical part of plants with twice that in flowers and 50 times higher than in stems (Flamini *et al.*, 2002). But, in very young plants (6-7 developed leaves) the concentration of all components of essential oil was 2 to 20 times lower than at the stage of initial flowering (Delfine *et al.*, 2005).

Oil composition in rosemary also depends on ontogenetic stage, as was shown in Spain for four different populations taken into cultivation (Salido *et al.*, 2003). In populations Ro-1 and Ro-2, monoterpenes decreased from full flowering in spring to fruiting in summer, but again increased and reached maximum in winter. In populations Ro-3 and Ro-4, monoterpenes increased from full flowering to fruiting where they reached their maximum. Their concentration decreased during winter, but was still higher than at flowering. Oxygenated monoterpenes in all these populations show the opposite trend to monoterpenes, while sesquiterpenes were more or less stable during the year, slowly decreasing towards winter (Salido *et al.*, 2003).

We recommend selecting rosemary varieties in which the maximum concentration of the most important flavour component coincides with reaching a saleable stage.

7.3.5 Temperature and photoperiod

It was also shown for plants grown in the Mediterranean climate, that production of essential oil decreased when air temperature increased, i.e. maximum concentrations of α-pinene, camphene, β-pinene, β-phellandrene, and the sesquiterpene caryophyllene were found in the coldest periods in spring-autumn and minimum concentrations in the summer (Llusià *et al.*, 2006). However, the authors did not discuss the possible effects of photoperiod – only temperature. Total terpene concentrations followed a similar seasonal and treatment response trend as individual ones (Llusià *et al.*, 2006). Interestingly, sharp increases in air temperature in the field (also a usual stress in Mediterranean) did not affect total production of terpenes, but decreased or retarded production of individual components such as α-pinene, camphene and β-phellandrene, but did not affect β-pinene ((Llusià *et al.*, 2006).

Effect of temperature, day-degree and photoperiod on production and composition of essential oil should be investigated for rosemary in the UK.

7.3.6 Light of different quality and quantity

Emission of essential oils in rosemary in to the atmosphere (as in many Mediterranean plants) showed light dependency, which was distinguishable from temperature dependency. In rosemary, α-pinene, camphene, sabinene, β-pinene, p-cymene, ocimene, γ-terpinene, terpinolene showed a significant increase in emission, i.e. aroma, in light compare to darkness (Owen *et al.*, 2002). Effect of light quality on content and composition of essential oil in rosemary has not been studied.

We recommend investigating how light intensity and quality could improve rosemary aroma in the UK.

7.3.7 Density

No scientific data.

7.3.8 Nutrition

No scientific data.

The Mediterranean region largely features calcareous and siliceous soils. These soils differ in their pH, permeability and nutrient status with moderately calcareous sites having a lesser permeability than acid siliceous soils and a higher nutrient availability, except for Mg^{2+}. Emission of essential oil (aroma of rosemary) depends on soil type, as shown in France. It was shown that quality of essential oils changed significantly when rosemary was grown on different soils (Ormeño *et al.*, 2007). Emissions of monoterpenes, which represent 85% of total emission in rosemary, was 3-fold higher when plants grew in calcareous sites, than in siliceous sites. By contrast, emission of sesquiterpenes (for example α-humulene and α-muurolene) was similar on calcareous and siliceous sites.

The effect of nutrients on content and composition of essential oil in rosemary should be studied for rosemary grown in the UK.

7.3.9 Irrigation

The content of monoterpenes in greenhouse-grown rosemary was shown to increase during drought. At the time of initial flowering all major components, including α-pinene, camphene, sabinene, β-pinene, β-myrcene, α-phellandrene, α-terpinene, γ-terpinene, α-terpinolene, linalool and camphor were more than 50% higher in moderately stressed leaves, and more than 100% higher in severely stressed leaves than in well-watered leaves (Delfine *et al.*, 2005). In contrast, in the field experiments in Mediterranean drought retarded production of individual terpenes (α-pinene, camphene, β-pinene, β-phellandrene, and the sesquiterpene caryophyllene) and also terpenes in total (Llusià *et al.*, 2006).

The effect of irrigation regimes should be studied in the UK, as all studies were performed in a dry hot Mediterranean climate and cannot be extended on the UK-grown plants.

7.3.10 Growth regulators

No scientific data.

7.3.11 Elicitors and stress signals

No scientific data.

7.4 Can growth conditions and diversity improve shelf-life of pot and field grown rosemary?

No scientific data.

7.5 Recommendations for research to be performed on rosemary

Rosemary is little studied, and the few studies that have been published have been performed in a Mediterranean climate and are hardly relevant to the UK growing conditions. However, we consider, that it is probably unnecessary to undertake research relevant to the UK climate, since rosemary grows so well in the Mediterranean region. There is also money allocated to make it a profitable African crop, so British growers will soon likely to find it difficult to compete with cheap products from these regions.

8 Spearmint – *Mentha spicata* L.

Due to its natural plasticity, natural and artificial hybridization and breeding there are many types of 'mint'. Taxonomically, 'spearmint' is *Mentha spicata* L. However a number of other synonyms exist in the literature:

- *Mentha aquatica* L. var. *crispa* (L.) Benth. Note: but not *Mentha aquatica* L. or *Mentha aquatica* L. var. *aquatica*
- *Mentha cordifolia* Auct.

- *Mentha cordifolia* Opiz ex Fresen.
- *Mentha crispa* L.
- *Mentha longifolia auct.*, non (L.) Huds. Note: but not *Mentha longifolia* (L.) Huds. or *Mentha longifolia* (L.) Huds. subsp. *longifolia* or *Mentha longifolia* (L.) Huds. var. *asiatica* (Boriss.) Rech.
- *Mentha viridis* (L.) L.

A bibliography for spearmint is provided in Appendix B.

9 Storage and packaging of herbs

Many post-harvest factors determine the quality of the final product. These include both biological factors and storage factors. Biological factors include respiration, ethylene production, compositional changes, growth and development, transpiration, physiological breakdown, physical damage, and pests and disease. Storage factors include the effect of environmental conditions such as temperature, relative humidity and gas atmospheres (Joyce and Reid, 1986).

Herbs and spices are, ideally, harvested when their aromatic or taste qualities are optimal. However, as living biological entities, they will deteriorate post-harvest, the rate of which will depend on storage and packaging conditions. This deterioration in quality is accentuated by the fact that in today's modern society, production areas are often remote from the urban centres that are the focus of consumer demand. Modern marketing practices may also deliberately introduce a time delay between harvest and sale in order to optimise financial returns during periods of excess, or to increase demand and extend the marketing period into periods of shortage. For herbs that are grown especially under organic conditions, where natural fertilisers are used and where the harvested products are exposed to only minimum washing, contamination with faecal organisms such as *Escherichia coli* or *Salmonella typhimurium* is possible. Packaging to minimise growth of these organisms during storage is important. In addition, when plant material is stored, various volatile products are released, particularly ethylene, and accumulation above critical levels can have a detrimental effect on quality. Methods for ethylene control are therefore important for long-term storage.

9.1 Types of packaging

The role of packaging is to identify, to display, to contain and to protect. An additional role of packaging is extension of shelf life. Simple procedures such as vacuum and/or modified atmosphere packaging serve to extend the shelf life of foods. In recent years various forms of 'active packaging' have been developed, including oxygen scavengers, moisture absorbers and/or colour stabilizers.

Vacuum packaging involves placing a food in a gas-impenetrable package and removing the air with the aim to prevent the growth of spoilage organisms, shrinkage, oxidation and change of colour. However, vacuum packaging alone is not necessarily effective against all spoilage. *E. coli* and *S. typhimurium,* common contaminants in packaged food responsible for many outbreaks of food poisoning, will continue to grow in vacuum packages, and only a combination of vacuum and low temperatures (2°C), will provide effective protection against these organisms. For other organisms, such as *Listeria monocytogenes* or *Yersinia enterolytica*, a combination of vacuum packaging and temperatures as low as –2°C are required to prevent bacterial spoilage. Gas impenetrable storage is also not effective for other reasons. As plant material is stored, respiration will continue, as will the production of ethylene. This means that the atmosphere within the package will change, creating conditions that may enhance rather than delay deterioration, and may induce colour changes in the herbs, as well as changes in aromatic and taste properties. To reduce bacterial spoilage, modified or controlled atmosphere packaging can be effective.

Modified atmosphere packaging (MAP) or controlled atmosphere packaging (CAP) is widely used for red meats (O_2, N_2), baked goods, salad mixes, and cured meats (CO_2). MAP involves packaging, often with plastic films, where the composition of the atmosphere may be altered, but not closely controlled. In contrast, CAP involves more precise atmospheric control where gas composition is changed by removing the air and introducing an inert gaseous atmosphere, such as N_2 or CO_2. The proportion of gases introduced is specific to the product. Under a CO_2 atmosphere, neither *E. coli* nor *S. typhimurium* will grow, even at storage temperatures of 10-12°C. Above that temperature, growth rates are slow, and significantly less that with uncontrolled packaging. A combination of gas packaging and reduced temperature is the most effective for vegetables, herbs and fruits, although the relationship between temperature and gas composition is complex, and varies between species. Temperatures below 2°C induce the development of low-temperature breakdown in fruits, whereas reduction in O_2 levels reduces ethylene production whilst increasing the retention of acid, totals soluble solids, and chlorophyll. Some produce, e.g. partially-ripened tomatoes, can be packaged in 4-6% CO_2 and 4-6% O_2, with a relative humidity of 90% and achieve up to 7 days additional shelf life. Higher levels of CO_2, or lower O_2, will delay ripening, which may reduce product quality. For herbs, a combination of reduced temperature (5-10°C) and gas packaging (CO_2 and/or O_2) may be the most effective. However, the actual concentration of CO_2 and O_2 to use may be species dependent. For example, olives store well at 10% CO_2, 11% O_2, whereas spinach or garlic may do better with 20% CO_2 and 2% O_2. Normal atmospheric air contains 0.035% CO_2, 21% O_2, 79% N_2. However, there are possible dangers involved in using gas packaging, especially for workers exposed to low O_2, high CO_2/N_2 atmospheres. Suffocation is a potential hazard.

Gas permeability of some plastic films used in packaging can also be problematic because the permeability is sensitive to humidity. Gas transmission through polyamides can increase up to three fold when the relative humidity increases from 0 to 100%. Other polymers, e.g. ethyl vinyl alcohol copolymers, can increase permeability by up to 100 times. This means that modified atmospheres may not be stable over extended periods of time, and storage conditions will therefore change, possibly increasing the rate of deterioration.

Active packaging can also involve the inclusion of a desiccant, cholesterol or ethanol absorbers, antimicrobials, or oxygen scavengers, within or as part of the packaging material. They are usually used to control insect damage, mould growth, rancidity or discolouration, and have been used in packaging of herbs. Iron reactions can be used to absorb O_2, whereas ascorbic acid sachets absorb O_2 and also generate CO_2. Potassium permanganate ($KMNO_4$), which has been used to absorb ethylene and in combination with a desiccant, to absorb moisture, can be used to prolong the shelf life of fruits up to 3-4 times that of non-packaged fruit.

Smart/intelligent packaging involves the use of various sensors/indicators to mark or display a change in product shelf life (time/temperature indicators, colour changing dyes, holographic methods, fluorescent controls).

9.2 Optimising packaging conditions for herbs

A number of factors have to be considered in optimizing harvest and postharvest handling procedures for perishable products. There have been only few detailed studies of the postharvest requirements of fresh herbs. The most important factor to be considered is temperature, and typically it involves two phases: pre-cooling (bringing the product to the optimum temperature) and storage (holding it at that temperature). The aim is to maintain turgor, texture, colour, composition and concentration of volatile oils and other aromatic compounds.

The postharvest quality in fresh material depends, to a large extent, on production and harvesting procedures used for the crop. Plants that were grown under an optimum regime of mineral nutrition, moisture and temperature usually do not deteriorate as quickly as plants grown under stress. The Maturity index – the stage of plant maturity at which to harvest for the best quality, is directly related to the concentration of chemical constituents in the plant. Harvesting in the early part of the day is also relevant; this usually minimizes losses of volatile constituents and decreases the cooling required for further storage.

The rate of biochemical reactions (e.g. respiration, that leads to further deterioration) is slowed by approximately 50% for each 10°C decrease in temperature. In addition, the growth of microorganisms is also slowed. Respiration rate can be much higher in freshly harvested flowering herbs (3-30x, depending on the species). The components of the essential oil can change during storage, for example in mint, menthone is decreased by 4% and menthol is increased by 10% during the first 24 hours. Menthofuran and menthyl acetate are also increased by 9% and 23% respectively. Further changes were not followed, however, these data indicate that further research on biochemical pathways and their control is required for more detailed information on individual species.

In contrast to fruits, and with the exception of basil, herb quality was best maintained with a temperature close to 0°C. If the herbs are harvested cool in the early morning, the need for pre-cooling is minimized. A simple forced air pre-cooler can be easily constructed and used for small operations and requires an adequate cool room and fan.

For fresh herbs, storage temperature should be slightly above 0°C because of their high water content and their susceptibility to freezing damage. In addition, herbs are prone to water loss due to their high surface/volume ratio. Continued transpiration through the stomata in freshly cut herbs results in early wilting and loss of quality. Packaging is also an important factor in water loss. Constant temperature is necessary to reduce condensation inside the bag and the consequent risk of fungal and bacterial growth (Corey, 1989).

Freshly harvested plant material has an internal humidity that approaches 100% and consequently water moves outwards to the less saturated atmosphere. A high relative humidity prevents shrivelling, desiccation and discolouration of the tissues and it should be maintained at a high level (90-95%) in the working area, cold rooms and transport vehicles. Humidities over this should be avoided because they may lead to condensation on plant tissues and containers, encouraging the growth and spread of spoilage microorganisms. Bacterial pathogens persist on the green tissues for very long periods. Fresh herbs inoculated with *E. coli* and *S. typhimurium* contained significant numbers of pathogens after 24 days in storage. These results reinforce the concept that, once contaminated, bacterial pathogens will persist on fresh herbs.

Most fresh herbs are susceptible to damage during postharvest handling and this can result in extensive discoloration (particularly mint, basil, coriander) and may initiate microbial infection at damage sites. Breakdown of the tissue and cells contributes to undesirable changes in texture, taste and aroma. Rigid clear plastic containers or partially inflated and sealed bags may be an alternative option.

Decreased oxygen level lowers the rate of respiration and inhibits synthesis of ethylene. Elevated levels of CO_2 also inhibit ethylene activity and prevent growth of certain microflora. Concentrations of 2-4% of oxygen and 5-10% of carbon dioxide are typically used, however the specific requirements may vary considerably for individual plant species and these parameters have not been investigated. Lower oxygen concentrations can lead to anaerobic respiratory activities (fermentative reactions) and an accumulation of compounds such as ethanol, leading to unpleasant odours and a general decline in quality.

Another important factor in packaging of fresh material is the appropriate ratio of product volume to total package volume at any given temperature. The quantity of free gas volume within the package, combined with film permeability, determines the rate at which oxygen is consumed, carbon dioxide is produced, and further chemical changes occur within the plant tissue (Aharoni *et al.*, 1989). Only individual tests for each plant species over the period of time will identify the optimum MAP film type, thickness, temperature and time (Silva *et al.*, 2005; Venere *et al.*, 2002).

Ethylene also has a distinct effect on herb tissues. Sage and rosemary appear to be insensitive to this gas. However, many species (mint, parsley, marjoram, oregano, savoury, basil, thyme) show epinasty (downward bending of the petioles), chlorosis, softening of the tissues and leaf fall, within a period of one week.

Other studies seek to extend shelf life by adding selenium to the nutrient solution one week before harvesting (MinSoon and KuenWoo, 2001; MoonJung *et al.*, 2000). This treatment increased the accumulation of vitamin C and extended shelf life for another three days.

Light and/or dark periods during storage, a N_2 stream, or other modifications of atmosphere can also regulate storage quality (Paakkonen *et al.*, 1990).

9.3 Examples of storage and packaging conditions for fresh herbs

9.3.1 Basil

For basil, at low temperatures, the leaves darkened and became discoloured, and symptoms of chilling injury became evident – the optimum storage temperature for basil is therefore between 10-12°C (Lange and Cameron, 1994, 1998; Meir *et al.,* 1995; Karwowska, 1997).

Basil harvested later in a day (18.00 or 22.00) increased shelf life by almost 100%, when harvested shoots were held at 10 and 15°C (Lange and Cameron, 1994). In addition, good potential exists for pre- or postharvest chill-hardening of basil grown in the greenhouse. For example, chill-hardening of packaged sweet basil for 1 day at 10°C in darkness, before transfer to 5°C, increased average shelf life by 2-4 days (Lange and Cameron, 1997). Reducing oxygen in the atmosphere contributed to better quality of basil leaves after 20 days of storage (Amodio *et al.*, 2005).

9.3.2. Chives

In studies on the storage of chives, 25-30 g bunches were packed in perforated or non-perforated polythene bags (20x25 cm) and stored at 2, 5, 10, 15 or 20°C. The longest satisfactory storage of chives (14-21 days) was obtained in non-perforated bags at 2 °C. The unpacked control kept well for 1-2 days at 2°C but deteriorated rapidly with rising temperature (Aharoni *et al.*, 1989). Turgidity and water potential of leaves, together with lighting regime, are also important for postharvest quality (Niklas and O'Rourke, 1987; Beck *et al.*, 2003).

9.3.3 Coriander

Respiration rates, visual quality, decay, aroma, off-odour, colour, and chlorophyll content in freshly harvested coriander leaves were investigated, together with ethylene production at various temperatures, in the dark, and under controlled atmospheres (Mohamed *et al.*, 2001; Loaiza and Cantwell, 1997). Visual quality could be maintained for up to 20 days with an atmosphere enriched with 5-9% CO_2 and 0°C. However, the typical aroma decreased noticeably after 14 days, regardless of storage condition (Waskar *et al.*, 1998).

A rapid decline in coriander leaf quality due to post-harvest senescence often causes serious commercial losses. As ethylene has been shown to play an important role in senescence of detached leaves, Jiang *et al.* (2002a) determined if ethylene-binding inhibitor 1-methylcyclopropene improves shelf-life of cut and bunched coriander. Coriander branches were constantly kept in air (control), or treated with 100 nl/l 1-methylcyclopropene for 24 h and subsequently kept in air. Chlorophyll content in the control leaves declined continually during storage. By the 9th day more than 74% of the original chlorophyll content was degraded. The level of chlorophyll in leaves treated with 1-methylcyclopropene was, by the 7th and the 9th day, 46% and 31% higher respectively, than that in control leaves. Soluble protein content of coriander leaves declined and free amino acid contents increased during storage in control leaves. Protein degradation was significantly retarded by treatment of 1-methylcyclopropene, and accumulation of free amino acids decreased. Ion leakage (membrane damage) increased constantly in control coriander leaf tissue during storage. On the 4th, 6th or the 8th day ion leakage in leaves treated with 1-methylcyclopropene was, respectively about 40, 40, or 50% lower than that in control leaves. Thus treatment with ethylene-binding inhibitor 1-methylcyclopropene slowed down senescence of cut and bunched coriander. 1-methylcyclopropene was shown to be effective at any storage temperature - at 5, 10, 15 and 20 °C (Jiang *et al.*, 2002a). Package atmosphere, specifically oxygen level, significantly influenced quality of fresh-cut leaves (Luo *et al.*, 2004).

9.3.4 Dill

In studies on the storage of dill, 25-30 g bunches were packed in perforated or non-perforated polythene bags (20x25cm) and stored at 2, 5, 10, 15 or 20°C. The longest satisfactory storage of dill (9-12 days) was obtained in non-perforated bags at 2°C. The unpacked control kept well for 1-2 days at 2°C but deteriorated rapidly with rising temperature. Drying with heated air (and drying generally) significantly influences the odour and taste. Freeze-drying in vacuum packages better preserves both. Frozen dill maintains good quality for 6 months; however, blanching before freezing is important, with subsequent storage at -20°C Paakkonen *et al.*, 1989).

9.3.5 Mint

In packaging tests, mint kept well at 0°C and 95% relative humidity, for up to 4 weeks in perforated polyethylene bags, as opposed to only 4 days in naked bunches (Hruschka and Wang, 1979). Weight loss, respiration rates, vitamin C content and physical properties were measured during this period. In tests using container and top ice, mint kept well for 2 weeks at 0°C (Bottcher *et al.*, 2002).

9.3.5 General

When coriander, dill and chives were packaged in non-perforated polyethylene-lines cartons (NPPE), a marked retardation of yellowing and decay (senescence) was observed. This was mainly attributed to the accumulation of respiratory CO_2 within the package. In addition, chlorophyll degradation within the range of the herb species, was effectively delayed when 5% CO_2 in air was applied. This confirms the ability of elevated CO_2 concentrations to antagonise the effects of ethylene, which is also produced by senescing stored plant material. Beneficial effects of storage conditions could be further improved by vacuum pre-cooling. However, it is important that the temperature is controlled not only during storage but also during transit.

For herbs in particular, insufficient information currently exists to describe optimum storage conditions for each species, and more research is needed to provide detailed information and to suggest the best packaging and storing techniques.

10 Biochemical pathways for volatile oils

Most volatile oils belong to the group of natural plant products that are classified as secondary metabolites. Secondary metabolites have a high degree of specialisation, and can be found in a single plant species, or as a cluster characteristic of a restricted species, genus or family of plants.

The largest, and structurally diverse, class of secondary plant metabolites includes the terpenoids (also termed as terpenes or isoprenoids), and there are about 30,000 terpenes of plant origin. These are predominant amongst the chemicals responsible for the medicinal, culinary and fragrant uses of plants. About 1000 monoterpenes that are present in essential oils have been identified. In the natural environment, they have been recognized as pollinator attractants, feeding deterrents, they may be involved in allelopathy, antibiotic, antifungal and insecticidal activities and as solvents for bioactive compounds.

Most terpenoids are derived from the condensation of branched five-carbon isoprene units and are classified accordingly as monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30) and tetraterpenes (C40). Pigments such as carotenoids, phytyl side chain of chlorophyll, steroids and plant hormones such as gibberellins and abscisic acid also belong to these group of compounds. Recent advances in analytical techniques, such as mass spectrometry and NMR (nuclear magnetic resonance) have made the chemical profiling of complex plant extracts possible.

The fundamental building block in the biosynthesis of all terpenoids is the five-carbon molecule isopentenyl pyrophosphate (IPP), which is synthesized from the mevalonic acid pathway. Each step in the pathway is catalyzed by a specific enzyme. The parent compounds (geranyl, farnesyl, and geranyl geranyl pyrophosphate, squalene and phytoene) may then undergo further transformation by redox reactions, conjugation, cyclization and isomerisation to generate the enormous variety of cyclic monoterpenes found in nature. Despite the vast number and structural diversity of these compounds, practically all of them are derived from one of the following pathways: the acetate, mevalonate, shikimate and 1-deoxyxylulose 5-phosphate (1-DXP) pathway (Figs 1 and 1) (Lichtenthaler, 1999). THE 1-DXP pathway functions in plastids and is responsible mainly for the biosynthesis of monoterpenes, and some sesquiterpenes. The mevalonate pathway operates in the cytosol and leads to the formation of most sesquiterpenes, triterpenes and sterols. There is evidence of a limited degree of cross talk between these two pathways (Kumar *et al.*, 2000; Gao *et al.*, 2002).

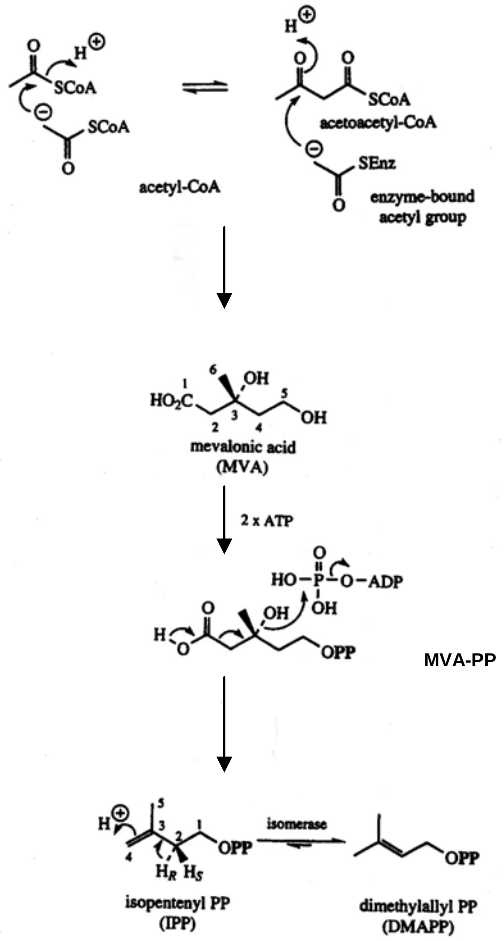

Figure 1. Outline of the mevalonate pathway for the formation of C_5 isoprene units, IPP and DMAPP (adapted from Dewick, 2002)

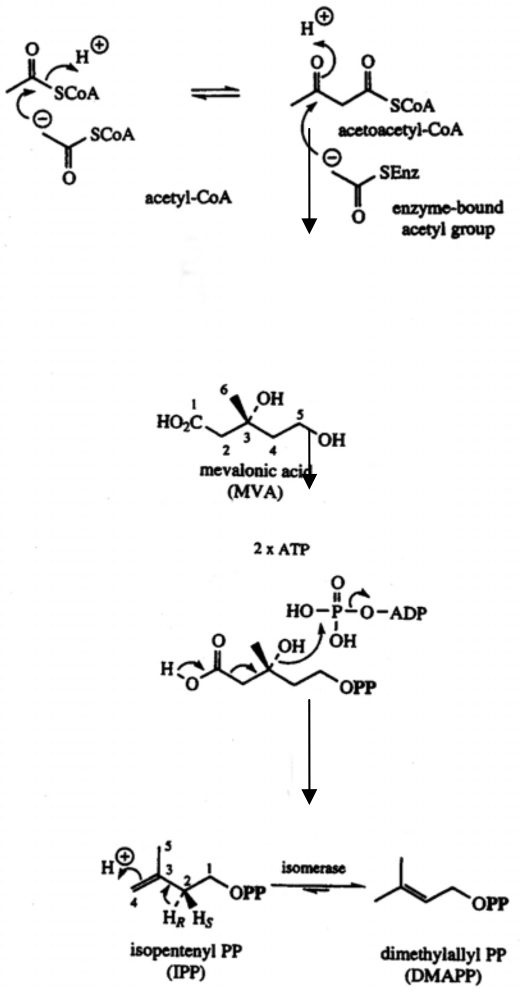

Figure 2 Outline of the deoxyxylulose pathway for the formation of C_5 isoprene units, IPP and DMAPP (pers. comm. Zenk, 2003)

The mechanism of terpene biosynthesis has been studied in great detail, together with the identification of the individual enzymes and their modes of action. This knowledge is fundamental not only for the understanding of plant functions and reactions to environmental influences, but also to the science of biotechnology, that includes tissue culture and genetic engineering (Krens *et al.*, 1997; Vieira *et al.*, 2001). A number of genes encoding these enzymes have been cloned and their DNA sequenced.

11 Potential for genetic manipulation of herbs

In the UK, gene manipulation is defined as the formation of new combinations of heritable material by the insertion of nucleic acid molecules, produced by whatever means outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur. Gene manipulation permits stretches of DNA to be isolated from their host organism and propagated in the same or a different host. This technique is known as cloning. It is possible to clone individual genes for a particular biosynthetic pathway and modify them so that they over-express (e.g. to increase the synthesis of a given chemical) when reincorporated into the plant. Alternatively, it is possible to shut down a particular metabolic pathway and re-direct various intermediates towards a desired end-product (Patterson *et al.*, 1992).

The value of genetic modification or manipulation to the commercial sector has quickly been recognised; e.g. the high commercial value of menthol has led to intensive investigation of transgenic mints with enhanced oil qualities. Certain components of mint, such as menthofuran, can accumulate under poor light conditions and high temperature and can change the mint profile giving it undesirable characteristics. It is possible to suppress the enzyme, menthofuran synthase, at the genetic level and increase the yield of menthol (Croteau *et al.*, 2005). Genetic engineering also targets other enzymes, for example limonene-6-hydroxylase, allowing the biosynthesis of (-)-isopiperitenol instead of (-)-carveol to enhance fragrance characteristics of the oil.

The rapid pace of genetic research in terpene biosynthesis has led to the prospects of genetically engineering plants for improved production of a given terpene.

11.1 Examples of genetic manipulation for herb production

11.1.1 Chives

The distribution of DNA sequences has been studied in the genus *Allium.* FISH (fluorescent *in situ* hybridisation) and PCR (polymerase chain reaction) were used to construct a detailed chromosomal map of all species examined (Friesen and Blattner, 2000). The complete coding sequence of mitochondrial genes of chives was determined and the information can now be used as a specific marker in distinguishing this species from other types (Stevenson *et al.*, 1999). This is important for distinguishing cytoplasmic male sterility in this group, which affects breeding and selection efforts. Similar work has been reported for accessions of various types of basil, where the relationship between different chemotypes was established using RAPD (random amplified polymorphic DNA) and genetic markers (Engelke and Tatkioglu, 2002).

11.1.2 Dill

Dill has been genetically transformed by *Agrobacterium rhizogenes* carrying plasmid pRi 1855. This transformation has not influenced oil yield and composition, although further work with other plasmids is in progress (Santos *et al.,* 2002). A successful protocol has been developed for the rapid and large-scale propagation of dill. Micropropagated plants were uniform and identical to donor plants.

11.1.3 Coriander

Coriander, caraway and fennel are also grown in tissue cultures and transformation efficiency is being tested with different markers. Ethylene has also been shown to play an important role in senescence of coriander promoting leaf senescence. Genetically modified pot-grown coriander (bearing gene of ethylene insensitivity from *Arabidopsis*, ERS1) showed retarded leaf senescence. At 6 weeks after full leaf expansion the leaves of transgenic lines still contained 60% of initial chlorophyll content, although control plants already lost 80% (Wang and Kumar, 2004).

11.1.4 Mint

Menthol is an important monoterpene in various plant species and it is also important as a pure natural product. Biosynthesis and molecular genetics of menthol production in *Mentha* species has been investigated in detail, with individual steps in the pathway being elucidated and key structural genes for relevant enzymes being cloned and characterised. The biosynthesis of menthol requires eight enzymatic steps and involves cyclization of the universal monoterpene precursor (geranyl diphosphate) to limonene. Limonene is consequently changed by four redox transformations and an isomerization step into menthol. Genetic engineering to up-regulate a flux-limiting step and down regulate a side route reaction has led to improvement in the composition and yield of peppermint oil (Inoue *et al.*, 2003; Wildung and Croteau, 2005).

Mentha x *piperita* cultivar Black Mitcham has been transformed by *Agrobacterium* with the limonene synthase gene (Krasnyanski *et al.,* 1999). Resulting plants had a high menthone, low menthol profile. The same mint strain has been transformed by *Agrobacterium tumefaciens* carrying a special promoter gene with the objective to develop peppermint plants resistant to the broad-spectrum herbicide glufosinate (Li *et al.*, 2001). Weed control is a substantial economic input for the production of plants and the development of herbicide resistant varieties would be of significant importance.

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Appendices
Appendix A

FV 290 contracts the following:

Examination of effects of fertilization and irrigation on shelf-life, yield and production of essential oils. Herbs may react very differently to excess or shortage of water (examples: chives is highly tolerant to water stress; for coriander intensive irrigation often increases yield, but decreases shelf-life). Although fertilization promotes yields and quantity of oils, it often affects quality and in particular shelf-life (example: ammonium salts as a source of nitrogen increase yield in coriander, but decrease shelf-life). Thus, whenever possible, a balanced approach should be found and recommended to the industry. In general, the effect of nutrient fertilization on total quality of herbs has not been systemically studied and documented, so “knowledge on the ground” should be gathered and possible directions of research revealed.

Analysis of research performed to find correlations between components of essential oils in herbs and flavour constituents (taste and odour) for humans. Very little has been done in this field. Recommendations need to be provided to the industry for (1) future research on how to find triggers for flavour in herbs; (2) the effect of light, sowing density and photoperiod on morphogenesis of herbs and corresponding biosynthesis of oils, especially terpenes and (3) on either how to escape any negative effects of photoperiod and low light intensity, or to undertake the research.

For many herbs the short shelf-life, poor appearance and flavour is a direct result of storage practices. Collection of information on current storage practices of herbs, along with information on post harvest practices for packed salad vegetables and cut flowers (if found appropriate).

Analysis of influence of different temperature regimes, gas cooling, modified atmosphere packaging on cut and pot herbs shelf-life, appearance and quality of oils. This will also entail examination of possible factors that prolong shelf-life in cut flowers and packed salad vegetables: reduced transpiration, excess of soluble carbohydrates, application of trehalose, gibberellins, volatile ethylene inhibitor, mild salt stress, red light, and recommendations to the industry to undertake research in specific directions.

Appendix B – a bibliography for mint

References marked with an * are for mints other than spearmint, but the data is pertinent to spearmint.

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