EFFECT OF LIGHT AND TEMPERATURE ON VOLATILE COMPOUNDS AND GROWTH PARAMETERS IN SWEET BASIL (*Ocimum basilicum* L.)

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ABSTRACT

The effects of temperature, irradiance, supplementary UV-B and R/FR ratios on volatile oil compounds and plant growth parameters in basil plants have been determined. The base temperature for plant growth, the variation in chemical composition between leaves, the variation in chemical composition during the day and the effect of storage at 4°C for 24 h were also determined.

Basil is a warm climate plant and its base temperature for growth is 10.9°C. The optimum temperature for plant growth is 25°C and this temperature also enhances the volatile oil content in leaves. Plants grown at 25°C for two weeks were taller and possessed more dry matter and larger leaves than plants grown at other temperatures. The total volatile oil content in fresh leaves was three times the level compared with plants at 15°C. Temperature also affected the composition of volatile oils. Warm conditions resulted in the accumulation of eugenol and *cis*-ocimene, whereas cool temperatures resulted in more camphor and trans-*p*-farnesene. There was no effect, however, on the relative contents of 1,8-cineole and linalool. Treatments with alternating temperature that supplied the same accumulated day degrees (ADD), but with a different sequence of temperatures, did not affect most of the plant growth parameters, however, volatile oil content and composition were strongly affected by the temperature regime of the final two weeks. The higher the temperature before harvesting, the higher the volatile oil content and the relative content of eugenol produced.

Basil plants grow well in full sun, however they can tolerate light shade. Heavy shade (75% and 50% shade) resulted in small plants with reduced dry matter, and the volatile oil content in fresh leaves was five times lower than in control plants. Heavy shade significantly increased the content of methyl eugenol, but strongly decreased the contents of eugenol and linalool.

Two weeks treatment with supplementary UV-B (ultraviolet – B) light resulted in short plants with higher dry matter and thicker leaves. It also stimulated the synthesis of volatile oil compounds, i.e. phenyl-propanoids (eugenol) and terpenoids (notably 1,8-cineole and linalool). There was no effect, however, on volatile oil composition.

Use of supplementary light to produce a high R/FR (red / far-red light) ratio resulted in shorter plants with less dry matter and smaller leaves. But the volatile oil content of the leaves was greatly increased. The content of eugenol was decreased whereas the content of β-myrcene was increased. There were no effects on the relative contents of 1,8-cineole, linalool and other compounds.

There were no differences in the volatile oil content and composition of fresh basil leaves harvested during the daytime, i.e. between morning (9.00am) and late afternoon (5.00pm). After storage for 24 h at 4°C in dark conditions, there were no differences in volatile oil content and composition in fresh leaves. There was a great difference, however, in the content and composition of volatile oils between young and mature leaves.

The sensory analysis showed that trained panellists could perceive different intensities of volatile oils and consumers preferred the stronger intensity of volatile oils in fresh basil leaves.

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ABBREVIATIONS

ADD – accumulated day degrees

ANOVA –analysis of variance

CAS – Chemical Abstracts Service

CFCs – chlorinated flurocarbons

cm – centimetre

CO_2 – carbon dioxide

CPT – camptothecin

CST – *p*-coumaroyl-CoA: shikimic acid *p*-coumaroyl transferase

cv – cultivar

CV – coefficient of variation

d - day

d.f. – degrees of freedom

DIF – day temperature minus night temperature

DLI – daily light integral

DNA – deoxyribonucleic acid

DT – day temperature

DW – dry weight

EMOT – eugenol *O*-methyltransferase

FW – fresh weight

g – gram

GC – gas chromatograph

GPP – geranyl pyrophosphate

GST – glutathione – S – transferase

h – hour

HLRG – high pressure mercury vapour lamp

IAA – indoleacetic acid

IUPAC – International Union of Pure and Applied Chemistry

J – joule

l –litre

kg – kilogram

LIS – linalool synthase

LSD – least significant difference

m^2 – square metre

mg – milligram

min – minute

MJ m^{-2} d^{-1} – megajoules per square metre per day

ml – millilitre

mm – millimetre

MS – mass spectrometry

m/z – mass – charge ratio

nm – nanometer (1 nm = 1 x 10^{-9} meters)

NT – night temperature

p – probability

PAR – photosynthetically active radiation

P_{FR} – phytochrome of far-red light absorbing form

PPF – photosynthetic photon flux

P_R – phytochrome of red light absorbing form

psi – pounds per square inch

R/FR – red / far-red light (660nm / 730nm)

R^2 – coefficient of determination

s – second

SED – standard error of difference

SMs – secondary metabolites

SLA – specific leaf area

TD – thermal desorber

TL – lower developmental threshold

TU – upper developmental threshold

UV-A – ultraviolet-A (320-400nm)

UV-B – ultraviolet-B (290-320nm)

UV-C – ultraviolet-C (100-290nm)

W m^{-2} – watts per square meter

µg – microgram

µl – microlitre

µmol m^{-2} s^{-1} – micromole per square metre per second

% – percentage

°C – degree Celsius

Chapter 1 INTRODUCTION

Sweet basil (*Ocimum basilicum* L.), a member of the Labiatae family, is a tender summer and herbaceous annual plant, which originates from tropical and warm areas, such as India, Africa and southern Asia. Plant height varies from 20 to 70 cm. The characteristics of this plant are a square stem and opposite, decussate leaves with many oil glands. The flowers are strongly zygomorphic with two distinct lips, and the stamens lie over the lower (anterior) lip of the corolla rather than ascending under the upper (posterior) lip. The main method of propagation is from seed (Hiltunen & Holm, 1999) with the optimum temperature for germination between 21°C and 30°C (Putievsky, 1983). Basil can tolerate very variable environments as the plants grow in cool moist and tropical rain forest zones with temperatures between 6°C and 24°C and receiving 500-800 mm precipitation (Hiltunen & Holm, 1999). Different cultivars show variation in morphological features, growing characteristics, essential oil production and the composition of oils (Lachowicz, Jones, Briggess, Bienvenu, Palmer, Mishra and Hunter, 1997).

The oil yield and herb yield vary greatly under different environmental conditions. Basil has been shown to produce high levels of phenyl-propanoids, e.g. eugenol and methyleugenol, and terpenoids, e.g. linalool and 1,8-cineole (Gang, Wang, Dudareva, Nam, Simon, Lewinsihn and Putievsky, 2001). The oil content has been shown to be influenced by water stress, temperature (Hiltunnen & Holm, 1999) and UV-B radiation (Johnson, Kirby, Naxakis and Pearson, 1999; Ioannidis, Bonner and Johnson, 2002). Generally the literature shows that increases in stress levels (high temperatures, high levels of UV-B and water stress) leads to higher levels of both phenyl-propanoids and terpenoids. These stress effects may act directly on key enzyme pathways or indirectly by altering the allocation of plant biomass to the oil producing parts of the plant. Studies on the effects of environmental factors on the

volatile oil composition in basil are very limited.

Sweet basil was selected for this study because of its characteristic volatile compounds. It is called the fragrant king of herbs, and its scientific name, *Ocimum basilicum*, means 'fragrant king'. Its volatile oils include both phenyl-propanoid-derived compounds (typically eugenol, methyl eugenol, methyl chavicol, methyl cinnamate) and terpenoids (mainly 1,8-cineole, linalool and limonene) (Grayer, Kite, Goldstone, Bryan, Paton and Putievsky, 1996; Lachowicz *et al.*, 1997; Johnson *et al.*, 1999). In addition, sweet basil is an annual plant that grows rapidly and is easy to cultivate and, therefore, is an ideal model system with which to study volatile oil production.

Research literature shows that variability of volatile compounds and growth parameters may be affected by environmental stress. It was hypothesized that environmental stress (temperature, light and UV-B) could alter the content and composition of secondary metabolites (SMs) and plant growth parameters in basil.

This present study sets out to determine the effects of environmental factors, particularly temperature, light, UV-B light and R/FR ratios on the expression of volatile oil compounds and the growth parameters of basil. The objectives of this investigation can be summarized as follows:

- To investigate the effects of specific environmental factors on the growth of basil plants.
- To determine the influence of environment on the expression of volatile compounds in basil, i.e. oil content and composition.
- To determine differences in volatile oil content and composition between different leaves in basil plants.
- To assess whether an increase in volatile oil content is detectable as an increase in the aroma of fresh basil leaves.

Chapter 2 LITERATURE REVIEW

2.1 Introduction

2.1.1 Taxonomy and botany

The genus *Ocimum* belongs to the Lamiaceae (Labiatae) family, and contains more than 160 species (Bhattacharjee, 2000), native to the tropical and subtropical regions of the world, with a wide diversity in growth characteristics, leaf size, flower colour, physical appearance and aroma (Miele, 2001). *Ocimum* species are cultivated commercially in France, Reunion Island, England, America and India. They are shrubs or herbs, perennial or biennial in habit. Plants are much branched with quadrangular stems, purple, brown or green in colour. Leaves are petiolate, simple, ovate or subovate with serrate or entire margins. Stems, leaves and inflorescences possess glandular hairs that emit volatile oil. Flowers are small in whorls of six to ten in terminal, simple or paniculate racemes. They have a decurrent margin on the upper tooth of the calyx, a flat lower lip on the corolla and a characteristic bifid style. Bracts are rarely longer than the flowers.

Sweet basil (*Ocimum basilicum* L.) is a tender herbaceous annual plant, 20 to 70 cm in height (Xie and Hu, 1995) and propagated from seed (Putievsky and Galambosi, 1999; Phippen and Simon, 2000a). It is valued for its rich and spicy, mildly peppery flavour with a trace of mint and clove and has many names, e.g. Sweet basil, St Josephwort, Basilienkraut (German); Basilic (French); Basilico (Italian); Albahaca (Spanish); Basilkört (Swedish); Raihan (Arabic); Basilicum (Dutc); Manjericao (Portuguese); Bazilik (Russian); Meboki (Japanese); Lo-le (Chinese); Bazylia pospolita (Polish).

2.1.2 The history of basil cultivation

The common name basil is derived from the Greek word basileus, the king of herbs, and because of its special properties it was given regal status. The generic name may

also come from the Greek word okimon, i.e. fragrant-lipped. The ancient Egyptians burned a mixture of basil and myrrh to appease their gods and embalmed their dead with it. In Persia and Malaysia, basil is planted on graves, and in Egypt women scatter the flowers on the resting places of those belonging to them. To the ancient Greeks and Romans, the herb was a symbol of hostility and insanity. They painted poverty as a ragged woman with basil at her side. They believed that to grow truly fragrant basil, it was necessary to shout and swear angrily while sowing its seeds. In French "sowing basil" (semer le basilic) means "ranting". It was introduced to France by Catherine de Medici in 1533 by her Italian chefs and her taste for food well seasoned with basil when she married King Henry II. Other folk traditions have associated the herb with love. During more recent centuries, when an Italian woman placed a potted basil plant on her balcony, it signalled that she was ready to receive her lover. In northern Europe, lovers exchanged basil sprigs as signs of faithfulness. Haitians believe in basil's protective powers, so shopkeepers sprinkle basil water around their stores to ward off evil spirits and bring prosperity.

Basil is a major volatile oil crop; the annual world production of basil oil was estimated at 14 tonnes in 1984, 43 tonnes in 1991 (Lachowicz *et al.*, 1997) and 100 tonnes in 1995 (Runham, 1996). About 15 tonnes come from India, 7 tonnes from Bulgaria, 5 tonnes from Egypt, 4.5 tonnes both from Pakistan and the Comores, 2 tonnes from Israel, 1 ton from Yugoslavia, 1 ton from USA, 1 ton from Madagascar, and smaller amounts from the other countries (Grayer *et al.*, 1996).

The volatile oil of sweet basil possesses insect-repelling (Keita, Vincent, Schmit, Arnason and Belanger, 2001; Kelm and Muraleedharan, 1998; Umerie, Anaso and Anyasoro, 1998), toxic activities and is anti- microbial against a variety of Gram-positive bacteria, Gram-negative bacteria, yeasts and moulds (Wan, Wilcock and Coventry, 1998; Lewinsohn, Ziv-Raz, Dudai, Tadmor, Lastochkin, Larkov, Chaimovitsh, Ravid, Putievsky, Pichersky and Shoham, 2000). In many countries, sweet basil is a popular culinary herb and has been used widely as a food ingredient for flavouring confectionary, baked foods and meat products (Wan *et al.*, 1998). In Brazil, *Ocimum* species are used both in traditional medicine against bronchitis, coughs and sore throats, and in foods and flavourings (Vierira and Simon, 2000). Some studies have shown that basil leaves could increase glutathione-S-transferase

(GST) activity by more than 78% in the stomach, liver and oesophagus, high enough to be considered as a protective agent against carcinogenesis, and glutathione levels were also significantly elevated in the three tissues. The juice of leaves is poured into the ear to cure otitis. The seeds are considered to have refreshing and sedative properties (Aruua and Sivaramakrishnan, 1990).

The essential oil of basil is widely used today because of the antibacterial activity, antifungal activity (Decrose, Seeni and Pushpangadan, 1999), antioxidant activity (Kelm, Nair, Strasburg and DeWitt, 2000), anti-HIV-1 activity (Yamasaki, Nalano and Kawahata, 1998), anti-inflammatory activity, immunomodulating and adaptogenic effects, anticarcinogenic effects, radioprotection effects, central nervous system effects, antiulcerogenic effects, smooth muscle effects and hepatoprotective activity (Holm, 1999; Cimanga, kambu, Tona, Apers, DeBruyne, Hermans, Totte, Pieters and Vlietinck, 2001; Mediratta, Sharma and Singh, 2002; Singh, 1998). Purple basils are an abundant source of acylated and glycosylated anthocyanins and provide a unique source of stable red pigments for the food industry (Phippen and Simon, 1998; 2000b).

From the information at 'www.herbs2000.com', in 2003, over 150 cultivars were grown around the world for their distinctive flavour and volatile oil. Nicky's Nursery, UK (2000-2003) lists 22 cultivars as widely used in the UK (Appendix 1).

2.1.3 Morphology and growth habit of *Ocimum basilicum* L.

Ocimum basilicum L. is an herbaceous species native to warm regions of Asia, Africa and Iran, which emits a warm, sweet and characteristic aroma. This species is commercially cultivated for the extraction of essential oils in southern France, Italy, Spain, Germany, North America, Bulgaria, Egypt, Sicily, Haiti, Comomoros, Madagascar and the Seychelles. It is also cultivated in India. It is an erect glabrous herb up to 70 cm tall with leaves that are petiolate, ovate or oblong, narrowed at both ends, almost entire and possess numerous oil glands, which contain aromatic volatile oil (Xie and Hu, 1995). It bears clusters of small white two-lipped flowers on long terminal racemose inflorescences. The corolla is small inconspicuous and green. The calyx is partly fused with the petiolate bracts and, as it enlarges, remains with the bracts and dries on the plant. Seeds are ellipsoid, black in colour and become

mucilaginous when wet. All above ground parts contain volatile oils. Flowering shoots emit a clove-like scent with a somewhat saline taste.

Basil is suited to warm conditions and develops best under long days in sunny conditions. Even though the optimum temperature for germination is between 21°C to 30°C (Putievsky, 1983), it can tolerate cool moist and tropical rain forest zones with annual temperature between 6°C and 24°C and receiving 500-800 mm precipitation (Putievsky and Galambosi, 1999). Different cultivars show different morphological features, growing characteristics, essential oil production and the composition of volatile oils (Lachowicz *et al.*, 1997).

2.2 Volatile compounds

2.2.1 Volatile compounds in plants

Volatile compounds considered in this study are secondary metabolites (SMs), and there is an enormous variety of such low molecular weight compounds in plants. Although only 20-30% of higher plants have been investigated so far, several tens of thousands of SMs have already been isolated and their structures determined (Wink, 1999) (Table 2.1).

Some of these compounds are volatile and perceived by their comparatively strong-smelling, characteristic, usually pleasant odours. They are produced in small quantities and are typically 'small in volume - high in value' fine chemicals. Plants may contain 0.01 to 20% of extractable compounds based on fresh weight of tissue (Runham, 1996). They are used in perfumes and perfumed products, as well as for flavouring of foods and beverages (Bauer, Garbe and Surburg, 2001).

According to Runham (1996), the world trade in volatile oils increased by 40% from 1984 (£475M) to 1990 (£667M), and at over 6% per annum from 1991 to 1995. The market for herbal products in 1993 was worth £5,600M worldwide and £62M in the UK. There are no official statistics for the area of herb crops in the UK. It was estimated at 1,262 ha in 1992, 1,500ha in 1995 and over 3000ha in 1996.

Table 2.1 Number of known secondary metabolites from higher plants (Wink, 1999)

Type of secondary metabolite	Approximate number of known structures
Nitrogen-containing	
Alkaloids	12000
Non-protein amino acids (NPAAS)	600
Amines	100
Cyanogenic glycosides	100
Glucosinolates	100
Without nitrogen	
Sesquiterpenes*	3000
Monoterpenes*	1000
Diterpenes*	1000
Triterpenes, steroids, saponins*	4000
Tetraterpenes*	350
Flavonoids	2000
Polyacetylenes	1000
Polyketides	750
Phenylpropanoids	500

*** Total number exceeds 22000 at present.**

2.2.2 Volatile oil compounds in basil

The chemical composition of volatile oils in basil has been investigated since the 1930's, and by 1999 approximately 190 chemical compounds had been identified (Hiltunen and Holm, 1999) (Table 2.2).

Due to their high relative content in leaves and extensive uses in plant protection, food stocks and medicine during the past decade, eugenol, 1,8-cineole and linalool have been studied extensively.

Table 2.2 Chemical compounds of volatile oils in basil

Monoterpene hydrocarbons	Sesquiterpene hydrocarbons	Aromatic compounds	Others
3-carene	α-amorphene	*cis*-anethole	*p*-methoxy acetophenone
p-cymene	*cis*-α-bergamotene	*trans*-anethole	*iso*-amyl alcohol
limonene	*trans*-α-bergamotene	anisaldehyde	benzene
myrcene	bicyclogermacrene	benzaldehyde	butanal
α-phellandrene	bicyloelemene	benzyl alcohol	β-damascenone
β-phellandrene	α-bisabolene	benzyl acetate	β-damascone
cis-β-ocimene *trans*-β-ocimene	β-bisabolene	benzyl formiate	2,4-decadienal
cis-allo-ocimene	β-bourbonene	benzyl benzoate	decanol
cis-trans-allo-ocimene	bulgarene	*p*-methoxy-benzaldehyde	dodecanol
α-pinene	α-cadinene	chavicol	δ-dodecalide
β-pinene	δ-cadinene	methyl chavicol	cumin aldehyde
sabinene	γ-cadinene	cinnamyl acetate	ethyl acetate
α-terpinene	calamenene	(E)-methyl cinnamate	2-ethyl furan
γ-terpinene	α-caryophyllene	(Z)-methyl cinnamate	ethyl-2-methyl butyrate
terpinolene	β-caryophyllene	methoxycinnamyl-alcohol	methyl-2-methyl butyrate
α-thujene	β-cedrene	coumarin	furfural
	2-*epi*-α-cedrene	*p*-methoxy-cinnamaldehyde	heptanal
Oxygenated monoterpenes	α-copaene	eugenol	hexanol
borneol	β-copaene	acetyl eugenol	*cis*-hex-3-en-1-ol
bornyl acetate	α-cubebene	ioseugenol	5-methyl-2-heptanone
camphor	β-cubebene, cyclosativene	methyl eugenol	6-methyl-3-heptanone
1,8-cineole	β-elemene	phenyl ethyl alcohol	*cis*-3-hexenol
citronellal	δ-elemene	phenyl ethyl styrene	3-hexenyl acetate
citronellol	γ-elemene	α-*p*-dimethyl styrene	*cis*-3-hexenyl benzoate
citronellyl acetate	α-farnesene	thymol	methyl jasmonate
p-cymen-8-ol	E-β-farnesene	methyl thymol	*cis*-jasmone
fenchone	germacrene-B	vanillin	trans-jasmone
fenchyl acetate	germacrene-D		dibutyl octanediotate
α-fenchyl acetate	α-guaiene		3-octanal
fenchyl alcohol	δ-guaiene		2,4-octadienal
geranial	β-gurjunene		3-octanone
geraniol	γ-gurjunene		octanol
geranyl acetate	β-himachalene		3-octanol
isobornyl acetate	α-humulene		trans-2-octen-1-al
linalool	isocaryophyllene		1-octen-3-ol
linalool oxide	ledene		octyl acetate
cis-linalool oxide	α-muurolene		1-octen-3-yl acetate
trans-linalool oxide	α-santalene		pentanal
linalyl acetate	α-selinene		2-methyl-3-methoxy pyrazine
menthol	β-selinene		tetra-methyl pyrazine
menthone	β-sesquiphellandrene		quinoline
myrtenal	viridifloral		undecylaldehyde
neral			
nerol	**Oxygenated sesquiterpenes**		
neryl actate	α-bisabolol		
trans-ocimene oxide	β-bisabolol		
perilla aldehyde	bulnesol		
cis-sabinene hydrate	T-cadinol		
trans-sabinene hydrate	10-epi-α-cadinol		
terpinen-4-ol	*trans*-cadinol		
α-terpineol	β-caryophyllene oxide		
α-terpinyl acetate	cedrol		
α-thujone	cubenol		
β-thujone	elemol		
	β-eudesmol		
	γ-eudesmol		
	farnesol		
	ledolnerolidol		
	spathulenol		

Linalool is one of the most common compounds in basil oil, its percentage proportion of volatiles ranging from traces up to almost 90%. It may co-exist with methyl chavicol, eugenol, methyl cinnamate, 1,8-cineole and some other monoterpenes (Hiltunen and Holm, 1999). Linalool is a colourless liquid with a flowery-fresh odour, reminiscent of lily of the valley, which is used frequently in perfumery and for many flowery fragrance compositions. It can be used in soaps and detergents because linalool is stable in alkali. Most of the manufactured linalool is used in the production of vitamin E since it is an important intermediate in the synthesis of this vitamin (Bauer *et al.*, 2001). It also plays a role in plant defence against herbivores (Kessler and Baldwin, 2001) and UV protection during growth (Ioannidis *et al.*, 2002).

1,8-cineole is one of the key compounds in the volatile oils of *Ocimum* species, with the amount varying from a trace to over 60% (Hiltunen and Holm, 1999). It is a colourless liquid with a characteristic odour, slightly reminiscent of camphor. 1,8-cineole has a fresh odour and is used in a large quantity in fragrances as well as in flavours (Bauer *et al*, 2001). It is an anti-inflammatory and analgesic agent (Santos and Rao, 2000) and may play a role in UV protection during plant growth (Ioannidis *et al.*, 2002).

Eugenol has been determined to be a predominant constituent in certain volatile oils of *Ocimum basilicum*, varying from a trace to 90% (Hiltunen and Holm, 1999). It is a colourless to slightly yellow liquid with a spicy, clove odour. Eugenol is used in perfumery in clove and carnation compositions (Bauer *et al.*, 2001). It is an antibacterial compound that inhibits the growth of many significant food-borne pathogenic bacteria, and an effective antifungal agent, acting as a fungistatic or fungicidal compound. Nematodes are susceptible to eugenol and are killed at low dosages. It also has a marked insect anti-herbivory effect (Fakae, Campbell, Barrett, Scott, Teesdale-Spittle, Liebau and Brophy, 2000; Gang, Wang, Dudareva, Nam, Simon, Lewinsihn and Putievsky, 2001). Eugenol is also used as a component in dental cement for temporary fillings (Kaufman, Cseke, Warber, Duke, Briemann, 1999). During plant growth, it can act as a sunscreen, providing protection against UV radiation (Ioannidis *et al.*, 2002).

2.2.3 Chemotypes of sweet basil

Different cultivars have different plant morphology and chemical composition of volatile oils (Hiltunen and Holm, 1999). Researchers have attempted to establish relationships between plant morphology and volatile oils, and although it is not always possible to observe a correlation between morphological characteristics and chemotype, some evidence for correlations has been presented (Marotti, Piccaglia and Giovanelli, 1996). Since 1930, the following classifications of sweet basil have been suggested:

In 1930, Guillaumin classified sweet basil oil into four categories (Guillaumin, 1930 cited by Hiltunen and Holm, 1999).

(1) Common basil oil contains linalool and methyl chavicol as the main compounds, with 1,8-cineole and eugenol present but no camphor and cinnamate.

(2) Camphor-type basil oil is rich in camphor but it also contains smaller amounts of α-pinene, cineole, linalool and methyl chavicol.

(3) Methyl cinnamate-type basil consists of 15-75% methyl cinnamate.

(4) Eugenol-type basil oil is rich in eugenol (30-80%).

In 1949, Gunther divided sweet basil into four types (Gunther, 1949 cited by Hiltunen and Holm, 1999).

(1) European type: Principal constituents are methyl chavicol and linalool, but no camphor.

(2) Reunion type: Principal constituents are methyl chavicol and camphor, but no linalool.

(3) Methyl cinnamate: Principal constituents are methyl chavicol, linalool and methyl cinnamate.

(4) Eugenol type: Principal constituent is eugenol.

In 1982, Sobti and Pushpangadan divided sweet basil oil into five chemotypes.

(1) Type 1: geraniol (40-50%) and eugenol (20-30%).

(2) Type 2: eugenol (20-40%).

(3) Type 3: camphor (10-15%).

(4) Type 4: methyl cinnamate (60-65%).

(5) Type 5: geraniol (20-30%), linalool (30-35%) and eugenol (20-30%).

In 1987, Boniface *et al.* classified sweet basil into three categories (Boniface *et al.*, 1987 cited by Hiltunen and Holm, 1999).

(1) Group 1. Asian (oriental) oils. Methyl chavicol (68.9-89.0%), linalool (0.5-16.7%).

(2) Group 2. European oils. Linalool (23.0-75.4%), methyl chavicol (0.4-43.6%), methyl cinnamate (0.1-15.5%), 1,8-cineole + cis-β-ocimene (2.7-13.6%).

(3) Group 3. African oils. Eugenol (5.9-19.2%), methyl chavicol (2.4-26.6%)

In 1989, Holm *et al.* classified sweet basil into five types (Holm *et al.*, 1989 cited by Hiltunen and Holm, 1999).

(1) Linalool – methyl chavicol type.

(2) Methyl cinnamate type

(3) Linalool type

(4) Linalool – eugenol type

(5) 1,8 – cineole type

In 1992, Baritanx *et al.* divided the sweet basil oil into four types.

(1) Type A: principal constituent linalool (59.6%).

(2) Type B: principal constituent methyl chavicol (84.1-86.3%).

(3) Type AB: linalool (39.9-41.1) and methyl chavicol (23.4-28.2%).

(4) Type BA: methyl chavicol (56.6-77.5) and linalool (10.0-19.0%).

In 1996, Grayer *et al.* classified sweet basil into five types.

(1) Linalool as the major compound

(2) Methyl chavicol as the major component

(3) A mixture of linalool and methyl chavicol as the two major compounds

(4) A mixture of linalool and eugenol as the two major components

(5) A mixture methyl chavicol and methyl eugenol as the two major compounds

Most of the classification methods were based on the main compounds of volatile oils and their origin. There are still some problems with those approaches to classification. One is the fact that the compositions of volatile oil can be different

before and after drying, and the length of storage may also affect this (Lawrence, 1992; Baritaux, Richard, Touche and Derbesy, 1992). Apart from using fresh or dried plant material, other factors affecting the composition of volatile oils include the method for extraction and analysis (Ayala and Luque de Castro, 2001; Chester, Pinkston and Raynie, 1998; Ehler, Nguyen, Quirin and Gerard, 2001; Gamiz-Gracia and DeCastro, 2000), the organ of the plant analysed and plant growth stage (Bettelheim Dudai, Putievsky, Ravid, Saadi, Katzir, Michaelovich and Cuabi, 1993; Bonnardeaux, 1992), environmental factors (Bettelheim *et al.*, 1993), use of fertilisers (Alder, Simon and Wilcox, 1989) and water supply (Hiltunen and Holm, 1999). For these reasons, chemotype classification should not be based only on one major compound because frequently there are two or more major compounds that may be present in almost equal amounts. It would be better if classification were based on all of the major compounds (Grayer *et al.*, 1996).

2.3 Effects of environmental factors on plant growth and volatile compounds

The principal interest in the cultivation of the crop for its volatile oil is the maximisation of economic yield returns. A crop may be grown solely for its volatile oil or for the oil and other products. One example is blackcurrant, which can be used for berries and its buds for valuable volatile oil (very expensive and currently used in luxury perfumes). Basil can be used for fresh leaves and also for its production of volatile oil. Therefore, in the cultivation, plants should be grown to maximise the yield of both volatile oil and fresh leaves.

2.3.1 Temperature

Temperature plays an important role in controlling the growth and development of all plants (Yeh, 1996) and responses are very dependent on the species and growth conditions (Lawlor, 1993).

Putievsky (1983) stated that sweet basil was not sensitive to temperature for germination and germinated well at temperature regimes between 18/13 and 30/25°C

(day/night) with +5°C DIF (day temperature minus night temperature), however, with increasing daytime temperature between 21 and 30°C, plant height increased, and the highest yield (dry matter) was obtained at 30°C. Pogany (Pogany, 1968 cited by Putievsky and Galambosi, 1999) also reported that the optimum temperature for growth was 27°C, with reduced yield at lower or higher temperatures.

Some reports compared plant yield in different places and suggested that higher yields were produced in warmer conditions. Plant weight of sweet basil grown in the Italian plains (380m altitude) was two to three times higher than that grown in the cooler mountain conditions (1050m altitude). Also the volatile oil content of the plants grown in the mountains was lower than in the plain (Putievsky and Galambosi, 1999). Hay, Svoboda and Barr (1988) found that, in Scotland, the fresh yield of sweet basil was 4.6-6.8 kg/m^2 and the dry yield was 0.5-1.0 kg/m^2. These yields were comparable with the yields possessed in southern countries only in glasshouses at a temperature of 18°C. In Denmark, Sorense and Henriksen (1992) covered the soil in the field with 0.05 mm thick transparent plastic sheet to increase soil temperature, and found increases in the number of surviving plants by 100% and in the fresh yield by 35-70%. At 60° and 61° northern latitude in South Finland, Agryl P17 fibre cloth mulches increased the fresh weight of basil over three times. However, further north at 69° latitude, basil cannot grow (Putievsky and Galambosi, 1999). Also in Finland, warm greenhouse conditions increased the fresh yield and doubled the volatile oil content (Nykänen, 1989).

Previous research directly or indirectly proved the warm temperature requirements of basil, however, there are still some important questions that need answering. Firstly, all temperature experiments reported were based on different day and night temperatures for imitating natural conditions. This temperature, however, differential would lead to difficulties in interpretation, since DIF, defined as day temperature (DT) minus night temperature (NT), in a wide range of species has a strong effect on plant morphology (Myster and Moe, 1995) and may influence phytochrome controlled chlorophyll synthesis and the ratio of chlorophyll a/chlorophyll b (Vågen Moe and Ronglan, 2003). Secondly, most of the previous research was focused on fresh yield or dry matter, but there have been very few studies on the effects of temperature on volatile oil compounds, especially in basil plants.

2.3.2 Irradiance

Irradiance that impinges on plants has multiple effects on plant growth, development and physiology, depending on the intensity, duration, direction and spectral quality of the light (Nilsen and Orcutt, 1996). A principal reason for this is that light enables photosynthesis (Salisbury and Ross, 1992), supplies the energy needed to fix carbon, catalyses the synthesis of anthocyanin pigment and plays an important role in the biosynthesis of medicinally important metabolites (Kaufman *et al.*, 1999).

Lee, Seo and Chung (1994) used three different shading nets to study the effects of irradiance on plant size, weight and oil content in basil. Plants were either shaded with white cheesecloth (21% shading), black cheesecloth (50%) or Gariso (a black material also giving 50% shading) or were not shaded (control). Growth was generally better in plots shaded with white cheesecloth and in control plots than in the more shaded ones. Plant height was greatest under black cheesecloth. Leaf area was greatest (5600 cm^2/plant) under white cheesecloth and only 2384 cm^2 under black cheesecloth and 892 cm^2 under Gariso. No significant difference in total fresh weight (FW) was observed between plants under white cheesecloth and the controls; the total dry weight (DW) and DW/FW ratio were highest (44.78 g and 9.8%, respectively) for the control plots. The essential oil content of the leaves was higher and the total oil content/plant was greater in plots under white cheesecloth than in control plots. From these results, it was suggested that light shading was beneficial to growth and oil production. The results were different, however, when the same shade percentage was achieved with different shading materials. This was probably due to the shading materials influencing the light quality, especially the R/FR ratio which could lead to changes in plant morphology (Morelli and Ruberti, 2002). Thus, it is difficult to conclude whether the effects observed were due to light quality or light intensity.

2.3.3 Supplementary UV-B light

Due to the decline in stratospheric ozone concentration, the increase in ultraviolet-B (UV-B) radiation reaching the surface of the earth has led to much research on the effects of enhanced UV-B radiation on some changes in plants (Karousou, Grammatikopoulos, Lanaras, Manetas and Kokkini, 1998). Thirty years ago virtually

nothing was known about the effects of UV radiation on plants. Even today, knowledge is principally limited to the effects on a few agricultural crops. In general, the response of plants to UV radiation include physiological, biochemical, morphological and anatomical changes, with deleterious effects on plant growth and leaf size, thus limiting the area available for energy capture (Maffei and Scannerini, 2000). UV-B (280-320nm) radiation damages DNA and it is toxic and mutagenic to cells (WMO report No.37, 1995; Landry, Stapleton, Lim, Hoffman, John, Hays, Walbot and Last, 1997). A number of plant molecules, such as lipids and proteins, strongly absorb UV-B and induce specific changes in tissue and whole-plant structure and function (Caldwell, Teramura and Tevini, 1989). UV-B can reduce plant growth and yield through reductions in biomass, seed yield and yield quality (Barnes, Jordan, Gold, Flint and Caldwell, 1988). It can alter plant morphology through reductions in plant height and leaf area, increased tillering, and changes in plant geometry (Barnes *et al.*, 1988). Plant physiological processes are also affected by UV-B, e.g. photosynthesis is often reduced and the production of plant secondary metabolites is increased (Caldwell *et al.*, 1989; Renger, Volker, Eckert, Fromme, Holm-Viet and Graber, 1989; Teramura and Sullivan, 1994). UV light has effects on secondary compounds of the phenyl-propanoid pathway via action on key regulatory enzymes (Kuhn, Chappell, Boudet and Hahlbrook, 1984; Bharti and Khurana, 1997). It stimulates production of flavonols and flavonoids (Cuadra and Harborne, 1996) and these compounds have been implicated both in plant defence and as protection against UV light (Chappell & Harborne, 1984).

There have been only a few studies on the effects of UV-B on aromatic plants. In a study of the effects of UV-B radiation on oil content and composition in *Mentha spicata* it was determined that essential oil production could be increased by UV-B radiation (Karousou *et al.*, 1998). The essential oil content of peppermint was slightly increased by UV-B radiation, and the menthol content was significantly decreased because of increased synthesis of menthone, menthofuran and menthyl acetate. Plant morphology was altered due to a significant inhibition of stem elongation and an increase in total leaf area (Maffei and Scannerini, 2000).

To date, there have been a few conflicting reports on the responses of plant growth and volatile oil compounds in basil to UV-B light. UV-B light enhanced the levels of

most of the major volatiles in sweet basil, i.e. phenyl-propanoids and terpenoids, and there were only small effects on plant height, a reduction in leaf area and little effect on leaf number. After treatment for two weeks, the leaves were also changed in shaped, becoming slightly longer and more pointed (Johnson *et al.*, 1999). Ioannidis *et al.* (2002) studied effects of UV-B on the composition of essential oils, and noted that there were no significant differences in the qualitative or quantitative composition.

2.3.4 Supplementary red light

In the early 1960s, Siegelman and other protein chemists purified phytochrome from homogenates of cereal-grain seedlings by using column chromatography and other routine techniques for the purification of proteins. They demonstrated that isolated phytochrome changes colour reversibly upon exposure to either red or far-red light (Salisbury and Ross, 1992). Since then, R/FR ratio has been widely studied, and it has been suggested that phytochromes mediate responses during the entire life of a plant (Genick and Chory, 2000). Many plants, including the model plant *Arabidopsis thaliana* (Gyula, Schäfer and Nagy, 2003; Morelli and Ruberti, 2002; Parks, Folta and Spalding, 2001), cucumber and *Fuchsia* (Moe, Morgan and Grindal, 2002), white clover (Héraut-bron, Robin, Valet-grancher and Guckert, 2001), *Eragrostis curvula* (Wan and Sosebee, 1998), pepper (Schuerger, Brown and Stryjewski, 1997), *Trifolium repens* L. (Robin, Hay, Newton and Greer, 1994), bean (Barreiro, Guiamet, Beltrano and Montaldi, 1992), soybean (Kasperbauer, Hunt and Sojka, 1984) and tobacco (Kasperbauer, 1971; Kasperbauer and Peaslee, 1973) have been used to clarify the effects of R/FR ratio on plant growth.

Moe, Morgan and Grindal (2002) grew *Fuchsia* plants at a PPF (Photosynthetic Photon Flux Density is the number of photons in the waveband between 400 and 700 nm that are incident on a surface in a give time period, the units are μmol m^{-2} s^{-1}) of 200 μmol $m^{-2}s^{-1}$ in growth cabinets and showed that plant height was reduced by about 20% when the R/FR ratio in the light spectrum was changed from 1.1 to about 8.0, while a combination of high R/FR ratio and additional blue light reduced plant height about 30%. However, this light combination resulted in a delay of flowering. A high R/FR ratio resulted in less accumulation of dry matter in the plants and more dry matter distribution to the leaves than to the stem compared to plants grown under

a low R/FR ratio.

Barreiro *et al.* (1992) suggested that high R/FR ratios are responsible for decreases in leaf thickness, however, Schuerger *et al.* (1997) cast doubts on such work by suggesting that the changes may have been due to decreased absolute levels of blue photons and not caused by changes in total PPF (photosynthetic photon flux) or R/FR ratio. This suggestion was supported by their results that pepper plants displayed no significant difference in leaf thickness between those grown at 660 nm (red light) and those grown at 660/735 nm (far-red light). They reported, however, that an increase in R/FR ratio reduced the thickness of mesophyll tissue and that leaf thickness was most modified by a combination of R/FR ratio, blue light and PPF. The disputes over the validity of such studies leave a gap for clearer, more precise experimentation on the effects of spectral composition on leaf thickness.

Although there has been no systematic research to study the effects of light quality (R/FR ratio) on basil plants, two reports (Loughrin and Kasperbauer, 2001, 2003) used different coloured mulches (black, red, green, blue, yellow and white) to observe changes in the volatile compounds in basil leaves. It was noted that the reflection from the different coloured mulches could change the R/FR ratio and resulted in the different concentrations of volatile compounds emitted from basil leaves, and the concentration of volatile compounds from fresh leaves was about 50-fold higher than those found in the air-dried leaves. This method, however, strongly affected soil surface temperature between 32.3°C (white coloured mulch) and 64.1°C (black coloured mulch), so it is difficult to explain which factor, temperature or light, lead to this result.

This chapter provides an overview of the roles of some environmental factors on plant growth and volatile compounds in basil, particularly temperature and light. This was not an exhaustive review of all environmental factors. There are some others, e.g. soil water (Simon, Reiss-Bubenheim, Joly and Charles, 1992), types of soil, nutrient supply and harvesting method (Putievsky and Galambosi, 1999; Gupta, 1996; Randhawa and Gill, 1995), which may be associated with plant growth parameters and volatile compounds in basil.

Chapter 3 GENERAL MATERIALS AND METHODS

The experiments described in this thesis were carried out at the University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, UK, during the period September 2001 to April 2004. In this chapter, general materials and methods are described and any specific modifications to the following procedures adopted in any particular experiment are described in the relevant chapter.

3.1 Controlled environments for experiments

All experiments were carried out in either glasshouses or controlled environment rooms. Temperature in the glasshouses was controlled and recorded using a microcomputer, TomTech HC 45 (TomTech, Spalding, Lincolnshire, UK), connected to a temperature sensor inside an aspirated screen. Mean daily temperature was within a range ±3°C. Between September and April, 16 h of supplementary lighting was provided by 400 Watt high pressure sodium lamps (Poot Lichtenergie, Holland), because basil develops best under long days and highest yields have been obtained with 16 h treatment (Putievsky, 1983).

Experiments including temperature treatments, UV-B light treatments, R/FR ratios and plants for sensory analysis were carried out in controlled environment rooms. Air temperatures were maintained to within ±1°C and measured using screened thermocouples. Illumination was provided by 400 Watt high pressure mercury vapour lamps (HLRG; Philips, Holland). Light intensity was 450±25μmol m^{-2} s^{-1} and a 16 h photoperiod was provided.

3.2 Plant materials

Seeds of four basil genotypes, namely Basil Sweet Genovese, Basil Bush, Basil Ruffles Purple and Basil Ruffles Green, were obtained from Nickerson-Zwaan Ltd for a preliminary comparison of plant growth parameters and volatile oil content. Based on the results (Appendix 2), basil cv Basil Sweet Genovese was selected for use in this study.

3.3 Seed germination, plant raising and management

All plants used in this study were raised from seeds. Seeds were sown on the surface of Levington F2s Compost (Fisons Horticulture Ltd, Ipswich, UK) in plastic trays. Seedlings with one pair of unfolded leaves were transplanted to 12 cm diameter pots containing Levington M2A compost. The mean daily temperature in the glasshouse was set at 21°C for seed germination and plant raising.

3.4 Measurement of plant growth parameters

Plant height, plant weight (fresh and dry), leaf weight (fresh and dry), number of axillary shoots, number of leaf pairs on the main stem and leaf area were measured. Plants were cut off at the compost surface, and placed in plastic bags to avoid loss of moisture during transport to the laboratory for analysis of these growth parameters.

Plant height was measured as the distance between the cotyledon node and the apex of the main stem.

Leaf area was measured using a leaf area meter (Plate 3.1). It provided a rapid, easy-to-use system for precise measurement of all sizes of leaves with resolution available at either 1 mm^2 or 0.1 mm^2. Perforated leaves and those with irregular margins were correctly measured using the LI-3100. Samples were rapidly measured by placing them on a moving transparent belt and allowing them to pass through the

instrument. As the sample travelled under the fluorescent light source, the projected image was reflected by a system of three mirrors to a solid-state scanning camera within the rear housing. An adjustable press roller flattened the curled leaves and fed them properly between the transparent belts. The accumulating area in cm^2 was shown on the LED display.

Plate 3.1 Leaf area meter (LI – 3100, Li-Cor Ltd., Lincoln, USA)

After measurement of plant height, leaf area and fresh weight, shoots and leaves were separated and then oven-dried at 80°C for 48 h and weighed. Specific leaf area (SLA) was calculated by dividing total leaf area by total leaf dry weight.

3.5 Extraction and analysis of volatile oils

Fresh leaves harvested from plants were immediately homogenized in a blender modified with a Universal Tube packed with Tenax / Carbograph ITD / Carbaxen 1000 (Plate 3.2). Nitrogen gas at 1.5 psi was passed through the blender to pump the volatile oils into Universal Tubes and prevent oxidation. These Tubes were designed for an analyte volatility range from n-C2 to n-C30, with sorbent strength weak (Tenax), medium (Carbograph ITD) to very strong (Carbaxen 1000).

Plate 3.2 Modified blender. Two holes on the top, left one for connecting nitrogen gas and right one for connecting Universal tube.

3.5.1 Determination of volatile oil collecting time

A 5 g sample of fresh leaf was blended for 5 s and using three tubes the volatile oils released were collected from 0.0 to 1.5 min, from 1.5 to 3.0 min and from 3.0 to 4.5 min. During the first 1.5 min, 112 peaks were detected, but only 18 and 10 peaks during the second and the third 1.5 min, and all of the peaks could be detected in the first 1.5 min. According to the sum of corrected peak areas, the third 1.5 min of collecting was only 0.57 % of the first 1.5 min. The total collecting time was 4.5 min so, to avoid any loss of oils, the collecting time was standardised at 5 min in this study.

3.5.2 Determination of sample weight

Three fresh leaf weights, 2.5, 5.0 and 10.0 g, were tested as the sample size for extraction of volatile oils. The total peak areas of 2.5, 5 and 10 g fresh leaf samples were 2.2×10^8, 5.4×10^8 and 10.3×10^8 respectively, i.e. total volatile oils obtained from 5.0 g fresh leaves was 245% of the amount of 2.5 g sample and 52% of the amount of 10.0 g sample. This suggests that the 2.5 g and 10.0 g samples were not blended thoroughly. With the 2.5 g sample, some pieces stuck to the top of the blender and were not in contact with the blades, and with the 10.0 g sample, much of

the leaf fragments adhered together. Thus, 5.0 g was selected as the sample weight for blending to release volatile oils.

3.5.3 Identification of chemical compounds

A Markes International Thermal Desorption (TD) unit plus Agilent 5973 Network MSD and Agilent 6890N Network GC (Plate 3.3) were used to identify volatile compounds present.

Plate 3.3 Markes International Thermal Desorption (TD) UNITY system plus Agilent 5973 Network MSD combine Agilent 6890N Network GC system

Thermal desorption conditions were as follows:

- Prepurge time 1 min
- Tube desorb 5 min
- Trap low temperature -10°C
- Trap high temperature 275°C
- Flow path temperature 150°C
- Minimum carrier pressure 10.0 psi

GC/MS conditions were as follows:

- Capillary GC/MS measurements were carried out on a DB-5MS (0.25mm x 25m x 0.25μm) column coupled directly to Agilent 5973 Network MSD.
- Flow rate of helium gas through column, 0.6ml/min.
- Average velocity, 30 cm/s.
- The pressure was set at 0.0 psi., however, the actual flow rate appeared not to get down to 0.0 psi but instead ran between 0.36-0.37 psi.
- Oven programme: Initial temperature 40°C, ramped at 10°C/min to 180°C and held 10 m.
- Carrier gas helium.
- Electron energy was 70 eV
- The mass spectra was set at 1306 emV (electron multiplier voltage)
- Scanning speed was 3.39 scans/s from 35 to 250 m/z.

Split ratio: 390:1 (0.256%), based on the following calculation:

Primary Desorb

Desorb Vent = 129.9

Split Vent = 270.0

The percentage of the total flow that went on to the cold trap

129.9/ (270.0+129.9) = 0.32

Trap Desorb

Split Vent= 235.7

Column flow = 2ml/min

The percentage that went onto the column

2/(2+235.7) = 0.008

Total Split = 1st split x 2nd split

0.32 x 0.008=0.00256

Therefore 0.256% of the original sample went onto the GC column.

Chemical compounds in basil were identified on the basis of relative retention times, using standard samples and comparing peaks with a library (Wiley7n.L) Figures 3.1, 3.2, 3.3 and 3.4 give examples of chemicals, which were identified using GC/MSD.

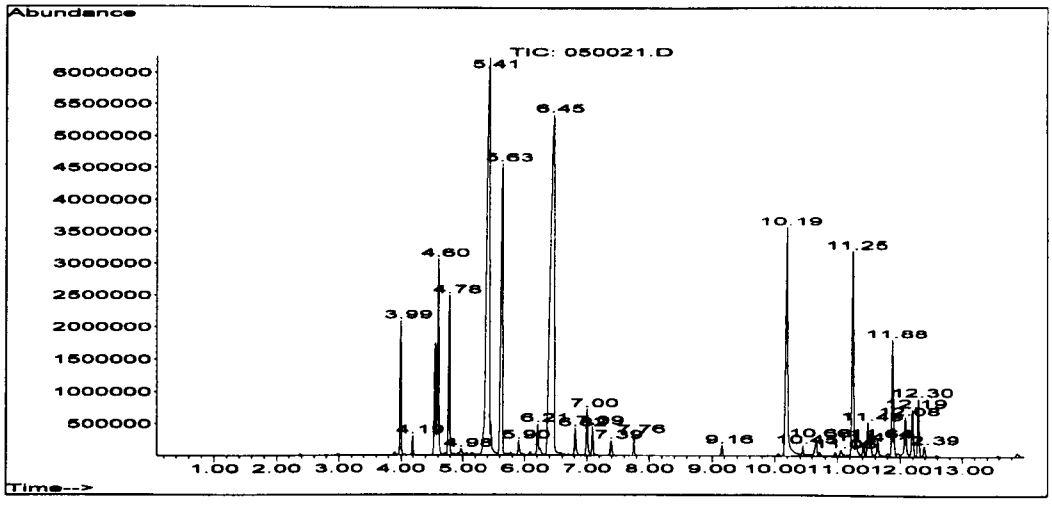

Figure 3.1 GC/MSD Chromatogram of Ocimum basilicum L.

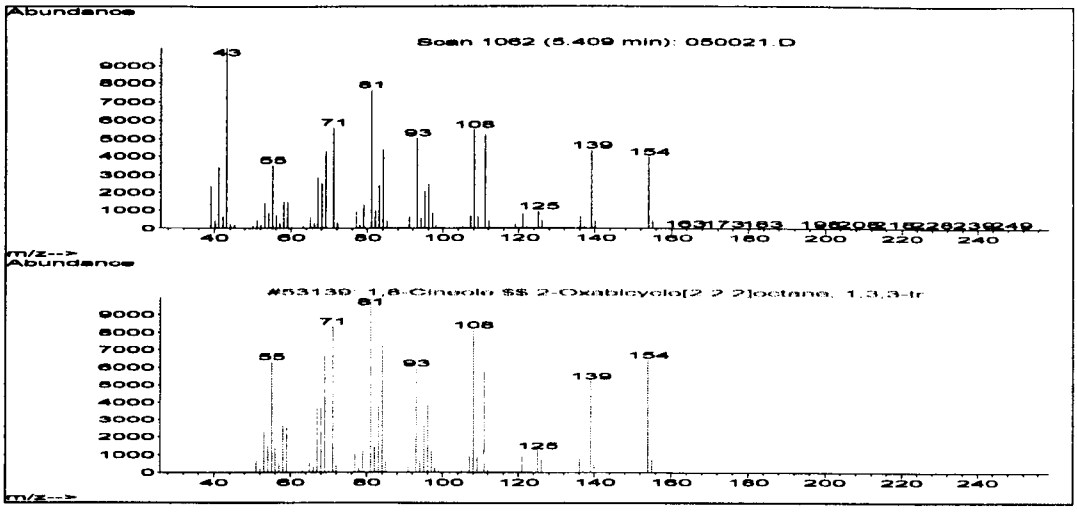

Figure 3.2 Comparison between reference (1,8- Cineole) and unknown spectra (Quality 98)

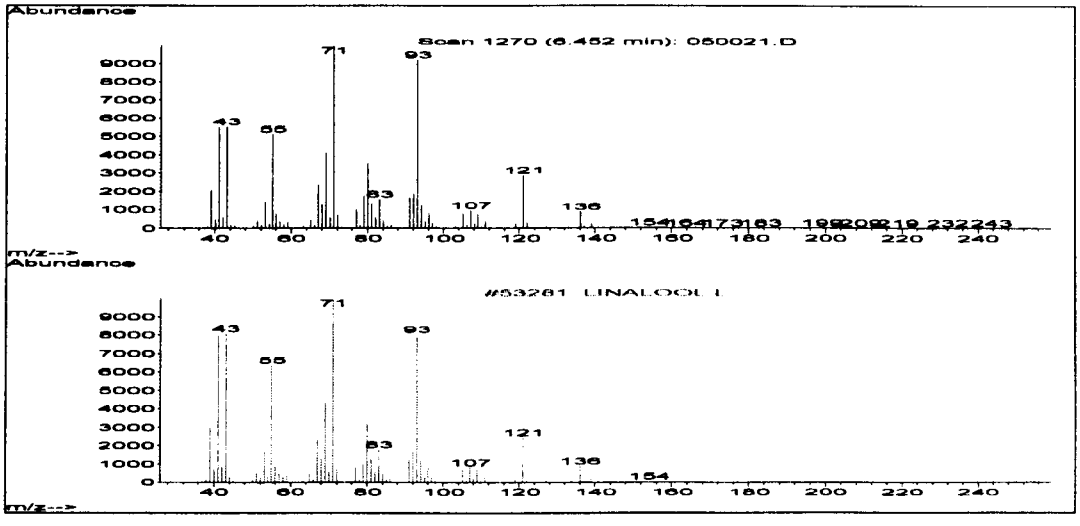

Figure 3.3 Comparison between reference (Linalool) and unknown spectra (Quality 99)

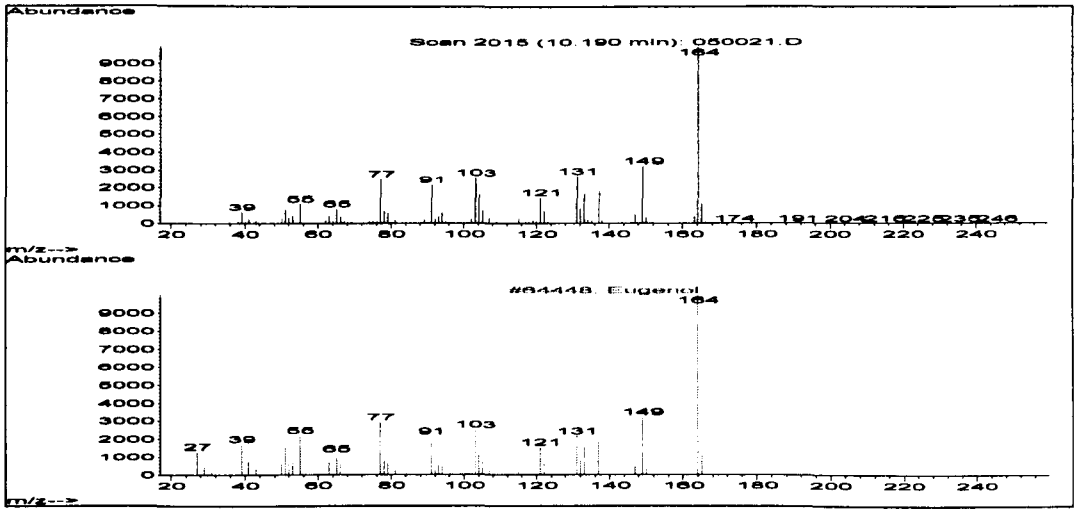

Figure 3.4 Comparison between reference (Eugenol) and unknown spectra (Quality 97)

3.5.4 Calibration using standards

3.5.4.1 Preparation of standards

Due to the nature of the samples analysed by TD/GC, it can be difficult to introduce an internal standard, therefore external standards for calibration are more common (Markes International Limited, 2002). Calibration Method 2 was used, i.e. liquid standard was introduced directly. This was the simpler method for loading calibration standards and involved the use of a syringe to introduce the sample to the rear of the tube through the quartz wool and deposit it as a liquid droplet on the sorbent bed.

Selection of a pure (chromatographic grade) solvent that is at least partially retained by the tube and cold trap sorbent is essential to avoid the components of interest being carried through the short sorbent bed as the unretained solvent migrates through the tube in liquid form. Ethyl acetate is compatible with Tenax sorbent. As ethyl acetate will appear in the chromatogram, it is preferable to use smaller injection volumes, i.e. < 1µl.

The standard must be introduced onto the rear of the sample tube, through the glass wool, to ensure that no volatile components are lost during the initial pressurisation and purge stage of desorption.

3.5.4.2 Preparation of calibration curves

0.025%, 2.5%, 5% and 7.5% standard solution of 1,8-cineole, linalool plus eugenol were prepared by mixing, and ethyl acetate was selected as solvent. There were three replicates for each density and a small volume (1 μl) was injected for each replicate. From the known amounts of the pure chemicals and the peak areas, calibration curves were calculated (Figures 3.5, 3.6 and 3.7).

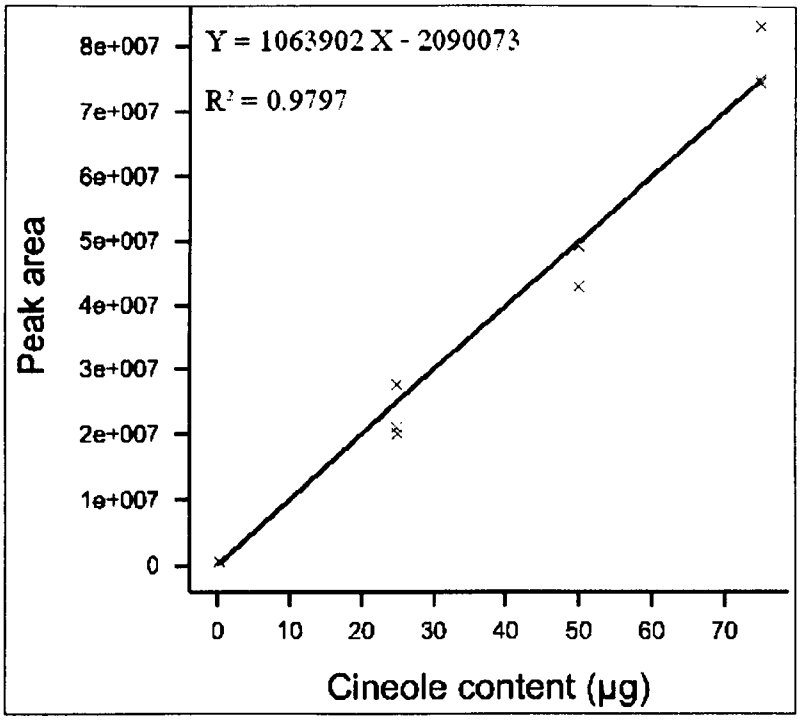

Figure 3.5 Calibration curve of 1,8-cineole

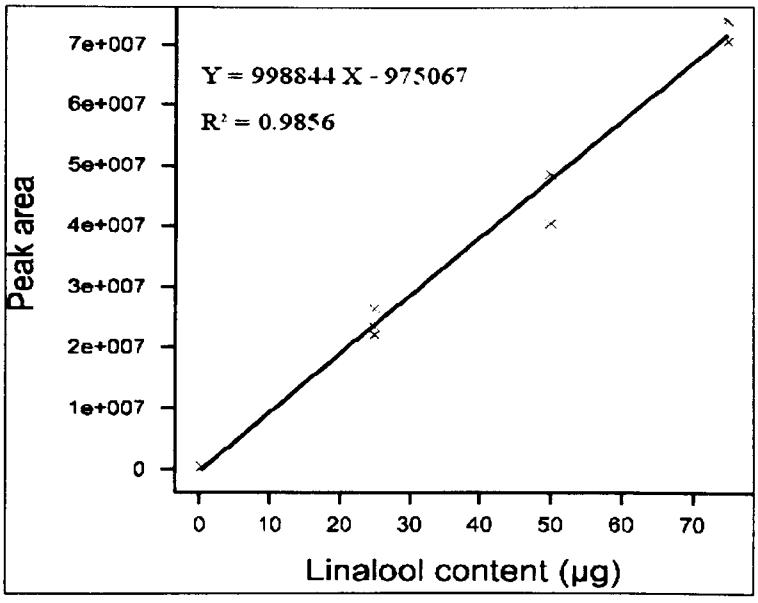

Figure 3.6 Calibration curve of linalool

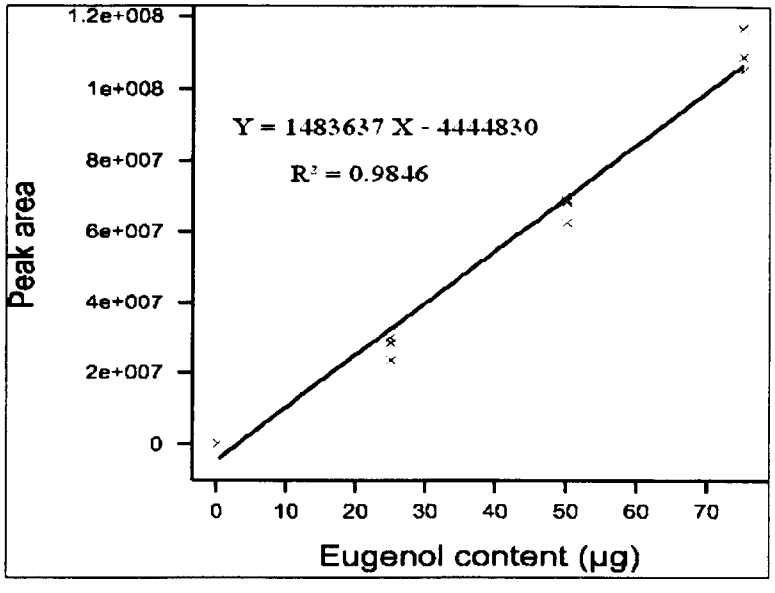

Figure 3.7 Calibration curve of eugenol

3.6 Measurement of light

A Skye light meter (Plate 3.4) with a Skye 200 PAR sensor was used to measure photosynthetically active radiation (PAR) between 400 nm and 700 nm, the light energy used by plants for photosynthesis. With a Skye 660/730 Sensor, this meter was used to measure the amounts of red and far-red light and the red / far-red ratio. This was a two-channel radiometer with a cosine corrected head that was fully sealed and suitable for external use. It had a common light collecting area for both channels.

Plate 3.4 Skye light meter **(Skye Instruments LTD, UK)**

UV-B light was measured using a digital UVX Radiometer (Plate 3.5) with one of three interchangeable sensors for measuring the intensity of 254 nm (UV-C), 302 nm (UV-B) and 365 (UV-A) nm UV wavelengths.

Plate 3.5 UVX Radiometer (UVP Inc, USA)

3.7 Measurement of leaf temperature

Leaf temperature was measured using an Inferatrace Infrared Thermometer (Plate 3.6). This provided a non-contact measurement of surface temperature that was both rapid and accurate.

Plate 3.6 Inferatrace Infrared Thermometer (Kane-May KM823, Comark Ltd, England)

3.8 Measurement of photosynthesis

An Infrared gas analyser (CIRAS –1, UK) was used to measure photosynthesis rate (Plate 3.7).

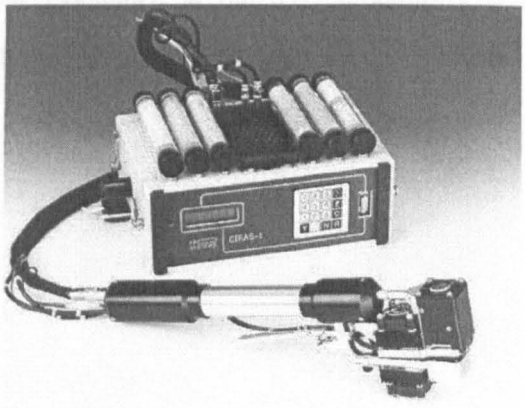

Plate 3.7 Infrared gas analyser (CIRAS – 1, UK)

It measured gaseous exchange in plants and, by use of an integrated microprocessor, calculated the net rate of photosynthesis (A), stomatal conductance (gs) and transpiration (E). It involved passing a continuous stream of air of known carbon dioxide and water concentration through a chamber containing a known area of intact leaf. Photosynthesis and transpiration by the enclosed leaf tissue depleted the carbon dioxide in the air passing through the chamber, and enriched it with water vapour relative to the air entering the chamber. Measurements were expressed as absolute concentration of a reference and the difference between the reference and sample concentrations.

3.9 Experimental design and statistical analysis

In most experiments, plots were arranged in a completely randomised block with 3 replicates for each treatment and each replicate consisting of 18 plants, i.e. 6 plants for growth parameter measurements and 12 plants for volatile oil extraction. Particular care was taken to ensure uniform irradiance and photoperiod between the growth rooms.

An analysis of variance (ANOVA) table was calculated for each growth parameter measurement and volatile oil content and composition in all experiments. Differences between treatments were assessed using the F-test, and the Least Significant Difference (LSD) was calculated at the 0.05 probability level. GenStat 2003-04 version was used with the exception of the data from the sensory analysis.

Chapter 4 PRELIMINARY INVESTIGATIONS

Due to the shortage of published information, some elementary protocols concerning basil plant growth and volatile oil content were not clear. The preliminary investigations in this chapter were to determine:

- Base temperature for plant growth
- Uniformity of growth parameters and chemical composition between plants
- Variation in chemical composition between leaves
- Variation in leaf chemicals during the day
- Effect of storage of plant samples at 4°C for 24 h

4.1 Base temperature investigation

4.1.1 Introduction

Temperature is one of the major environmental factors, which affect plant growth and development. There is a threshold temperature at which plants begin to grow, and it is called the "**base temperature**". Plant growth and development are most rapid around a narrow range of optimal temperatures and slow quickly at higher and lower temperatures. Development stops altogether when the temperature falls below the lower developmental threshold (TL) or rises above the upper developmental threshold (TU) (Figure 4.1).

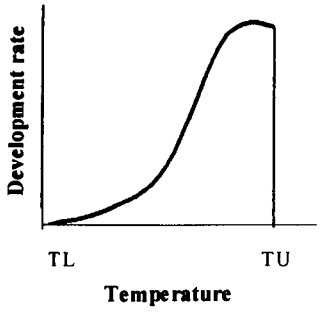

Figure 4.1 Relationship between development and temperature (Herms, 1998)

The purpose of this investigation was to determine the base temperature for basil plants.

4.1.2 Materials and Methods

This investigation was carried out in controlled environment rooms at constant temperatures maintained to within ±1°C to provide 15°C, 20°C, 25°C and 30°C (day/night). Seeds were sown on 1 November 2002. Temperature treatments were applied at the three leaf-pair growth stage on 29 November 2002, and ten plants from each treatment were harvested and analyzed on 9 December 2002. Seed germination, plant raising and management before temperature treatment were as described in

section 3.3. Before, and ten days after temperature treatments, plant dry weight was measured. The data were regressed and the intercept provided the base temperature (Mo, 1992). Plant growth rate (g / d / plant) was calculated as follow:

Growth Rate = (Weight $_{after}$ – Weight $_{before}$)/t

Where,

Weight$_{after}$: the value of plant dry weight after treatment

Weight$_{before}$: the value of plant dry weight before treatment (0.15 g)

t: the duration of the treatment (10 d)

This investigation was repeated 20 d later and a similar result was obtained.

4.1.3 Results and discussion

Before the temperature treatments, mean plant dry weight was 0.15 g. After ten days of temperature treatments, all plants in each treatment were harvested, oven dried at 80°C for 48 h and weighed separately.

Liner regression analysis was used to give a straight line of the type y = mx + c, where m is the gradient and represents the response factor (value of dry weight per gram increased) and c is the y intercept which represents the value of temperature when no dry weight is increased (Figure 4.2). The value of the y intercept of the formula Y = 86.74 X + 10.92 gave the base temperature of this basil cultivar.

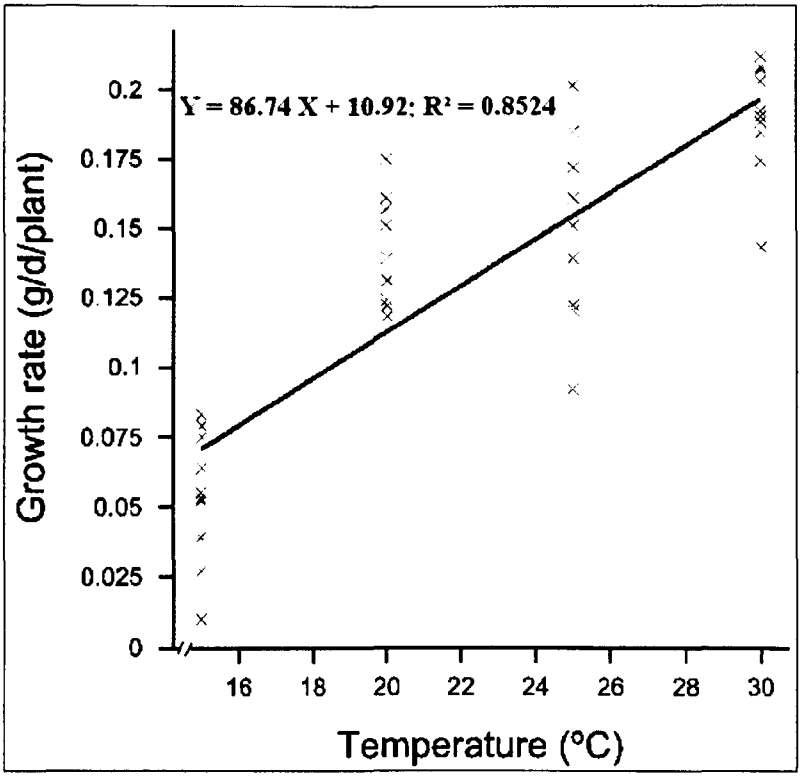

Figure 4.2 The relationship between basil plant growth rate and temperature (x: observed values)

4.2 The uniformity of basil cv Basil Sweet Genovese

4.2.1 Introduction

The purpose of this investigation was to understand if the plant growth parameters, individual chemical composition and total volatile oil content of Basil Sweet Genovese were relatively uniform.

4.2.2 Materials and methods

This investigation was carried out in a glasshouse. Plant materials, seed germination, plant raising and management and the measurement of plant growth parameters were as described in Chapter 3. This investigation started on 23 May 2002, and plant growth parameters and chemicals were measured and calculated at the six leaf-pair growth stage. There were eighteen plants for the determination of plant growth parameters and twelve plants for the chemical compounds.

Trapping of volatile oils on sorbent tubes and TD-GC/MS analysis were used in this study. All fresh leaf samples were cut from the fourth and fifth pairs of leaves, i.e. two leaves from the fifth pair and one leaf from the fourth pair.

Coefficient of variation (CV) was used to judge the relative uniformity of this cultivar.

CV = (Standard deviation / mean) x 100

4.2.3 Results and discussion

All of the CV values for plant growth parameters were quite low (most were less than 5%), especially pair of leaves and number of shoots (table 4.1). Some of the basil leaves were not very flat, especially young leaves, making leaf area measurement difficult, however, leaf area CV was still less than 10%.

Table 4.1. Variation in plant growth parameters at 6 leaf-pair growth stage

Parameter	Mean	SD	CV %
Height (cm)	48.5	1.6	3.4
Leaf area (cm^2)	900.4	63.8	7.1
Leaf dry weight (g)	4.418	0.159	3.5
Leaf fresh weight (g)	26.070	1.286	4.9
Pair of leaves	6	N/A	N/A
Plant dry weight (g)	6.612	0.171	2.6
Plant fresh weight (g)	47.680	0.918	1.9
Number of shoots	8.0	N/A	N/A

For estimating variation of volatiles, total peak area and four major compounds were measured and calculated (table 4.2). This result was obtained from twelve individual plants, and all the procedures were kept under the same conditions. Plants were harvested in the morning at 9.00am, and kept at 4°C in a refrigerator until oil collection. All samples were analysed within ten hours, from 9.00am to 7.00pm.

Table 4.2. Variation in chemical compounds at the six leaf-pair growth stage on 11 July 2002

Chemicals	Retention time (minutes)	Peak area		
		Mean (% of total)	Standard deviation	CV (%)
1,8-cineole	6.578	23.29	1.62	7.0
Linalool	7.622	19.34	1.72	8.9
Camphor	8.385	1.31	0.10	7.8
Eugenol	11.535	14.64	1.14	7.8

Note: The mean of total peak area, standard deviation and CV were 1.085×10^9, 1.022×10^8 and 9.4 respectively.

The CV values were slightly higher than plant growth parameters but they were all less than 10%.

The use of CV lies partly in the fact that the mean and standard deviation tend to change together in many experiments. CV was created to measure population

variability, however, its most common use is to measure validity of field experiments. The CV can be used to measure variability in genetic populations, to determine the best plot size in uniformity trials, to measure stability of phenotypes or to measure variation in other individual or population attributes.

A CV of 10 to 15% for yield in experiments is generally expected by most field crop researchers and the value is often between 5% and 15% (Bowman, 2001).

4.2.4 Conclusions

The results proved that this cultivar, Sweet Basil Genovese, could be used in the further experiments. Plant growth parameters and volatile oil content and composition were relatively stable under the same growth conditions.

4.3 Variation in chemical content and composition between leaves

4.3.1 Introduction

Basil oil is a volatile substance extracted mainly from the leaves of basil. This study was designed to reveal differences in oil content and composition from the basal leaves to the top leaves. The principle purpose of this investigation was to design the sampling procedure to compare chemical content and composition between treatments.

4.3.2 Materials and methods

This investigation was carried out in a glasshouse. Plant materials, seed germination, plant raising and management were as described in Chapter 3. This investigation was commenced on 23 May 2002.

There were three replicates of twelve plants. Each 5 g sample composed three to six leaves, depending on the size of the leaves. Sorbent tube trapping extraction and TD-GC/MS volatile oil analysis were used in this study.

4.3.3 Results and discussion

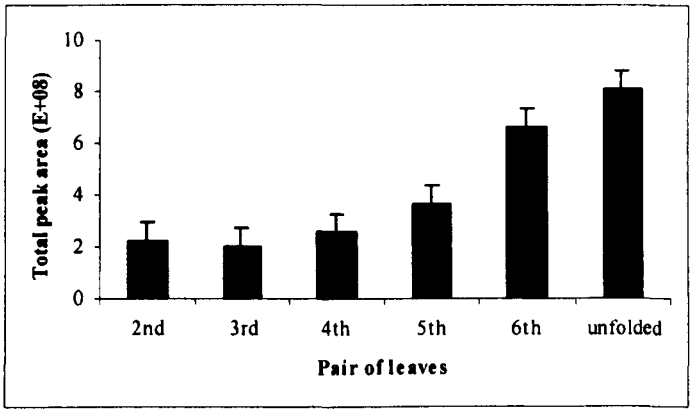

Figure 4.3 Total content of volatile oils in different leaves of basil

Great differences were found in the content (Figure 4.3) and composition (Figure 4.4) of volatile oils between young and mature leaves.

There were no differences in the total content of volatile oils between the second, third and fourth pair of leaves, however differences between the fifth, sixth and unfolded leaves were significant. The unfolded leaves had a fourfold content compared to the second, third and fourth pair of leaves. This supports the findings of Putievesky and Galambosi (1999) that young leaves had a higher content of essential oil per unit area compared to old leaves, and that, as the leaf size increased, the essential oil content decreased.

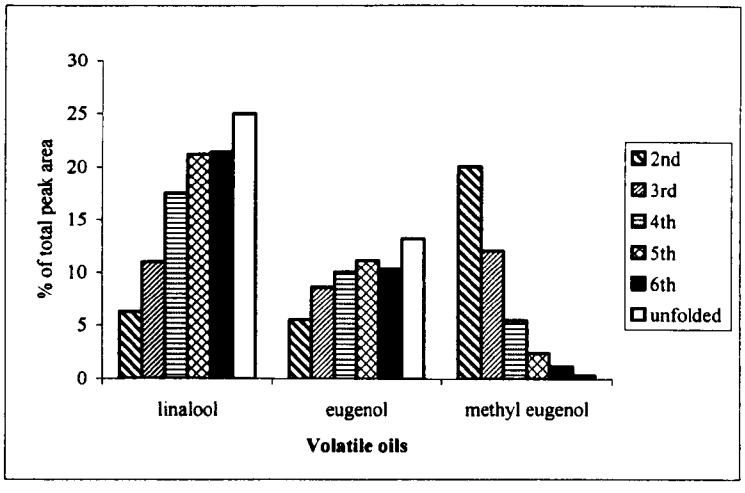

Figure 4.4 Selected volatile oils in different leaf pairs

Analysis of the composition of individual chemical compounds showed that most did not differ significantly and that three main volatile oil compounds, linalool, eugenol and methyl eugenol, showed significant differences ($p = 0.005$, 0.021 and <0.001 respectively) (Figure 4.4). It was clear that the oil of the young leaves was rich in linalool (Werker *et al.*, 1993) and eugenol, while methyl eugenol was higher in the older leaves. Eugenol could be the precursor of methyl eugenol. It was assumed that eugenol was synthesized in the young leaves but, as the plant grew, leaves became

older and some eugenol was methylated to methyl eugenol. According to a previous report on the biosynthesis of phenylpropenes in basil, eugenol O-methyltransferase (EOMT) could be the enzyme that controls this synthesis (Gang *et al.*, 2001).

4.3.4 Conclusions

Great differences were found in the content and composition of volatile oils between young and mature leaves. Thus, it is important to define sampling procedures when comparing the volatile oils between treatments.

4.4 Variation in leaf chemicals during the day

4.4.1 Introduction

There are many different factors which influence the quality and quantity of volatile oils in basil. The content and composition of volatile oils in plant organs may change over time as shown by differences in the oil content and composition due to leaf size and age (Putievesky and Galambosi, 1999).

The purpose of this investigation was to reveal the differences in volatile oil content and composition during the day from 9.00am to 5.00pm. It was hypothesized that volatile oil content as well as composition would be different due to the continuous change in solar light and temperature during the day.

4.4.2 Materials and methods

This investigation was carried out in a glasshouse. Plant materials, seed germination, plant raising and management were as described in Chapter 3. This investigation was commenced on 17 March 2003, and chemical analysis was carried out at the six leaf-pair growth stage on 9 May 2003.

There were three replicates of twelve plants. All leaf samples (5 g fresh leaves per sample) were collected from the fifth pair of leaves. Irradiance and temperature were recorded automatically each hour with a Campbell CR10 data logger. It was a clear day and cloud cover was present only between 10.30am and 1.00pm.

This experiment was repeated twenty days later and similar results were obtained.

4.4.3 Results

The glasshouse temperature was relatively stable during this experiment with a mean temperature of 21 ± 1.5°C. The solar intensity ranged from 37 to 519 watts/m^2 (Table

4.3).

Table 4.3 Temperature and irradiance in glasshouse on 9 May 2003

Time of recording	Temperature (°C)	Irradiance (watts/m^2)
7.00	21.0	37.81
8.00	19.5	110.6
9.00	19.5	247.9
10.00	21.7	227.5
11.00	23.0	195.3
12.00	23.0	154
13.00	22.7	179.2
14.00	22.2	246.4
15.00	22.4	260.4
16.00	21.7	432.6
17.00	22.1	519.4
18.00	23.0	131.6

Differences in total volatile oil content measured throughout the day were not significant ($p = 0.928$) (Figure 4.5).

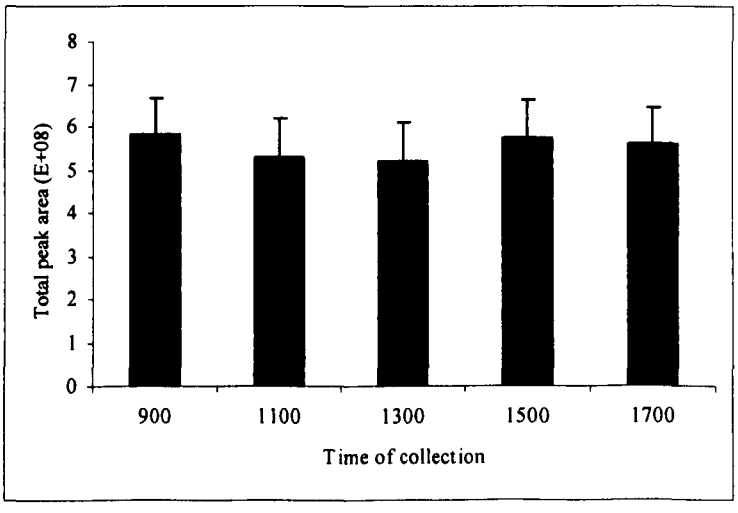

Figure 4.5 Total volatile oil content in basil leaves during the day

Thirty chemical compounds were identified, and they did not show any significant differences, as illustrated by three main compounds, eugenol (Figure 4.6), 1,8-cineole and linalool (Figure 4.7).

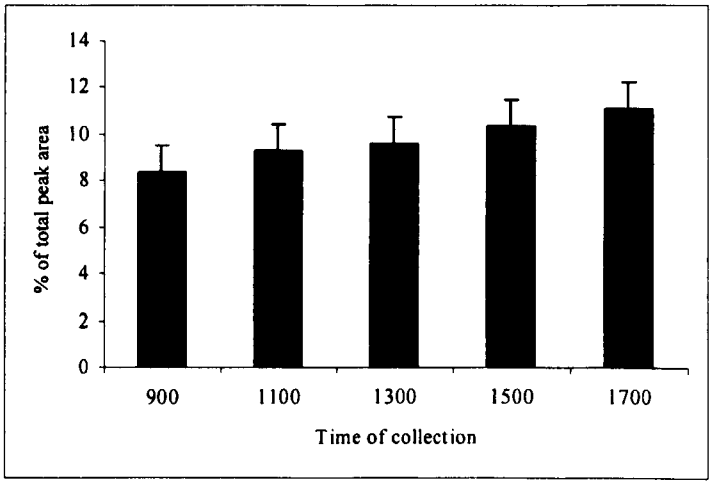

Figure 4.6 Eugenol composition during the day

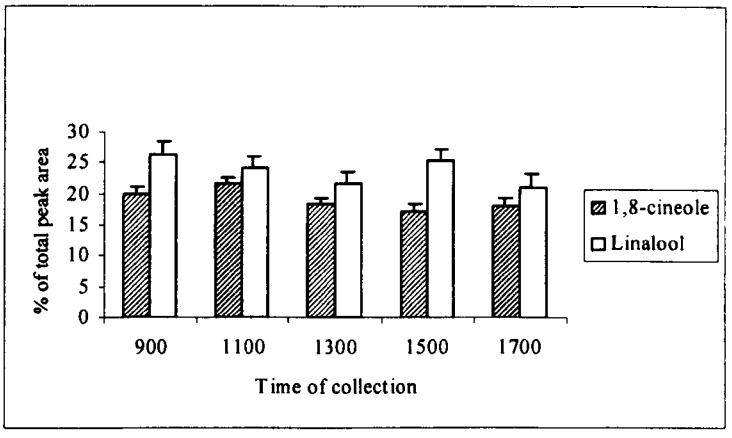

Figure 4.7 Selected volatile oil composition during the day

4.4.4 Discussion

Previously, DeVasconcelos *et al.* (1999) reported variation during the daytime in the chemical constituents of the essential oil of *Ocimum gratissimum* leaves, however this was not supported by the present study. They showed a considerable variation in the eugenol yield, i.e. 98% at 12.00am to 11% at 5.00pm, the opposite of the present

results where eugenol was slightly increased from 9.00am to 5.00pm. Careful study of the previous report raises some questions. Firstly, there was no indication of whether they repeated the experiment, and there was no mention of replicates in their sampling. Secondly, the report stated that they extracted 30 g of fresh leaf samples, but did not mention where the leaves came from. The present investigation has shown significant differences in the volatile oil content and composition between leaves. Thirdly, there was no information about the environmental conditions, i.e. temperature and irradiance. The report just mentioned that samples were collected each hour during the day from plants growing under sunlight on 18 November 1995. Finally, the numbers were erratic, whereas a trend would have been expected. For eugenol from 2.00pm to 6.00pm, the values were 80.3, 27.5, 67.0, 11.4 and 39.5 and there was no trend. For 1,8-cineole, the results were similar (0, 60.7, 32.5, 75.5 and 22.5), and again no real trend.

Regarding the fluctuations in the data, this could have been due to operator error, clouds passing over and lack of uniformity of sampling.

It may be that a light dependent cultivar was used in the previous research because the composition of 1,8-cineole was 52.1% at 8.00am and 0.0% at 12.00pm, and eugenol was 14% at 8.00am and 98% at 12.00pm. This could be very relevant for crop breeding and production, however it was not considered in that report.

4.4.5 Conclusions

During daytime from 9.00am to 5.00pm, there were no differences in the total volatile oil content and composition although the percentage of eugenol increased slightly. It can be considered that the original hypothesis was not correct.

4.5 Effect of storage at 4°C for 24 h

4.5.1 Introduction

The purpose of this investigation was to determine if the volatile oil content and composition of leaves changed during storage at 4°C for 24 h in the dark because, in the present study, leaves usually had to be stored for 10 h prior to the extraction and analysis of volatile oils.

It was hypothesized that there were no changes in the volatile oil content and composition of fresh basil leaves after storage at 4°C in darkness for 24 h.

4.5.2 Materials and methods

Plants for this experiment were grown in a glasshouse. Plant materials, seed germination, plant raising and management were as described in Chapter 3. Seeds were sown on 12 May 2003, and plants transplanted on 23 May 2003. Volatile oil compounds were analyzed at the six leaf-pair growth stage on 16 and 17 July 2003.

There were three replicates of six plants. All leaf samples (5 g fresh leaves per sample) were collected from the fifth pair of leaves. All of the plants were harvested by cutting between the third and fourth pairs of leaves on 16 July 2003 at 9.00am and then stored at 4°C. After storage for 0, 3, 6 and 24 h, the content and composition of volatile oils were analysed by TD-GC/MS after trapping in sorbent tubes.

4.5.3 Results and discussion

Comparing the sum of peak area after storage for 0, 3, 6 and 24 h, there was no change within 24 h. The values for the sum of peak area were between 5.21 and 5.83E+08 ($p = 0.928$) (Figure 4.8) and showed that there was no difference in the total volatile oil content of basil leaves kept at 4°C in darkness.

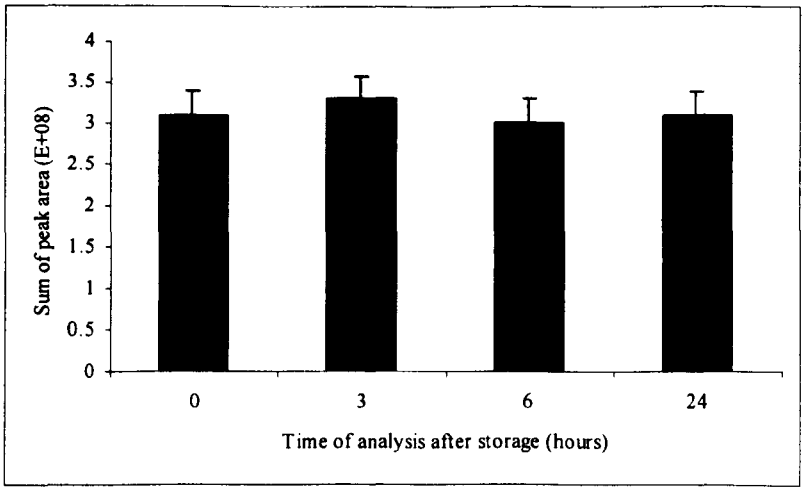

Figure 4.8 Comparison of total volatile oil content during storage

None of the thirty six chemical compounds identified, including 1,8-cineole, linalool and eugenol (Figure 4.9), showed any differences after storage of the leaves for 0, 3, 6 and 24 h at 4°C in darkness.

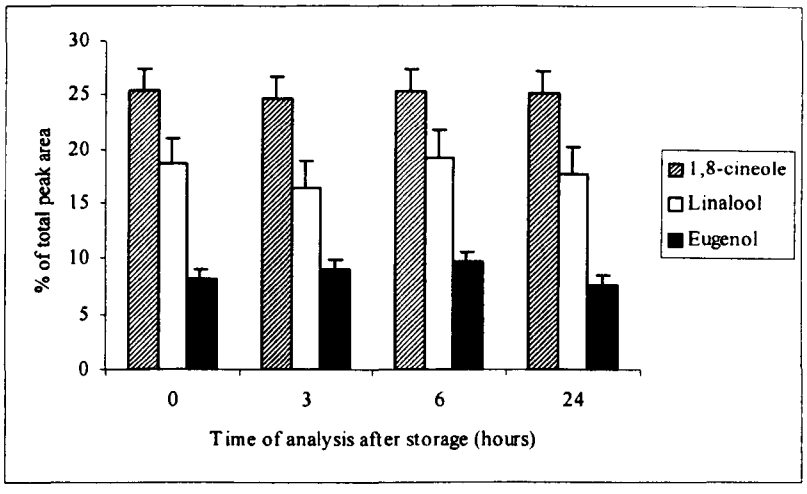

Figure 4.9 Selected volatile oil composition after storage

The absence of any changes in the content and composition of volatile oils in fresh basil leaves after 24 h storage at 4°C in darkness was probably due to the inactivity of most enzymes at low temperatures (Kaufman *et al.*, 1999).

4.5.4 Conclusions

Fresh leaf samples of basil can be stored at 4°C for 24 h without any adverse effects on the content and composition of volatile oils.

Chapter 5 TEMPERATURE EFFECTS

5.1 Introduction

Temperature is an important environmental factor that regulates plant metabolism, growth and development (Lawlor, 1993; Kaufman, 1999). Temperature responses of plants are dependent on the species and growth conditions (Lawlor, 1993). Many tropical or subtropical crops are sensitive to low temperature, which can lead to water loss and wilting and reduced accumulation of dry matters in roots, but such plants are generally less sensitive to moderately high temperatures between 15 and 32°C (Basra, 2001). Basil is cultivated in a range of climatic and environmental conditions, but the most favourable conditions are found in warm climates as shown by numerous publications from temperate climate countries (Hiltunen and Holm, 1999). In Chapter 4 the base temperature of basil was determined to be 10.9°C, showing that basil grows best under warm conditions.

Previous research has focused on the effects of temperature on the morphology of plant and oil yield, with limited information about the volatile oil composition. The experiments reported in this chapter were carried out using constant day and night temperatures to study the effects of temperature on plant growth parameters, volatile oil content and composition in basil. There were two experiments, the first comparing constant temperatures and the second comparing alternating temperature treatments. It was hypothesised that warm temperatures would increase plant height (Putievsky, 1983), leaf number, leaf area and plant weight as well as the yield of volatile oil, because the rate of chemical reactions generally doubles with every 10°C increase in reaction temperature (Copeland, 2002).

5. 2 Effects of constant temperature

5. 2.1 Materials and methods

Basil plants were raised from seeds as described in chapter 3 and then transplanted to controlled environment rooms at constant temperatures maintained to within ±1°C with a 16 h photoperiod to provide three different temperatures, i.e. 15/15°C, 25/25°C, 30/30°C (day/night) for one week and two weeks at different plant growth stages. The one week of treatment was applied to plants at the four and six leaf-pair growth stages and the two weeks treatment to plants at the three and four leaf-pair growth stages. There were three replicates of eighteen plants for each treatment and the experiment was carried out on 12 July 2002 and repeated on 24 November 2002.

Plant growth parameters and the content and composition of volatile oils in fresh leaves were measured after one and two weeks of treatment. Fresh leaf samples (5 g) were cut from the fifth pair of leaves and immediately homogenized in a modified blender with Universal Tubes packed with Tenax / Carbograph ITD/ Carbaxen 1000. Extraction, analysis and identification of volatile oils were as described in Chapter 3.

5.2.2 Results

5.2.2.1 Growth

Some differences in growth parameters were observed in the different temperature treatments, however there were no differences between one week and two weeks of treatments applied at different plant growth stages. Data presented is from the one week's treatments. The plants at 25°C were taller, heavier (fresh and dry weight) and possessed larger leaf area, more leaves and more shoots compared to the 15°C

treatment, but for some differences between 25°C and 30°C treatments were not significant.

After one week, plants in the 30°C treatment were the tallest (all $p < 0.001$) but differences between plants at 25°C and at 30°C were not significant (Figure 5.1).

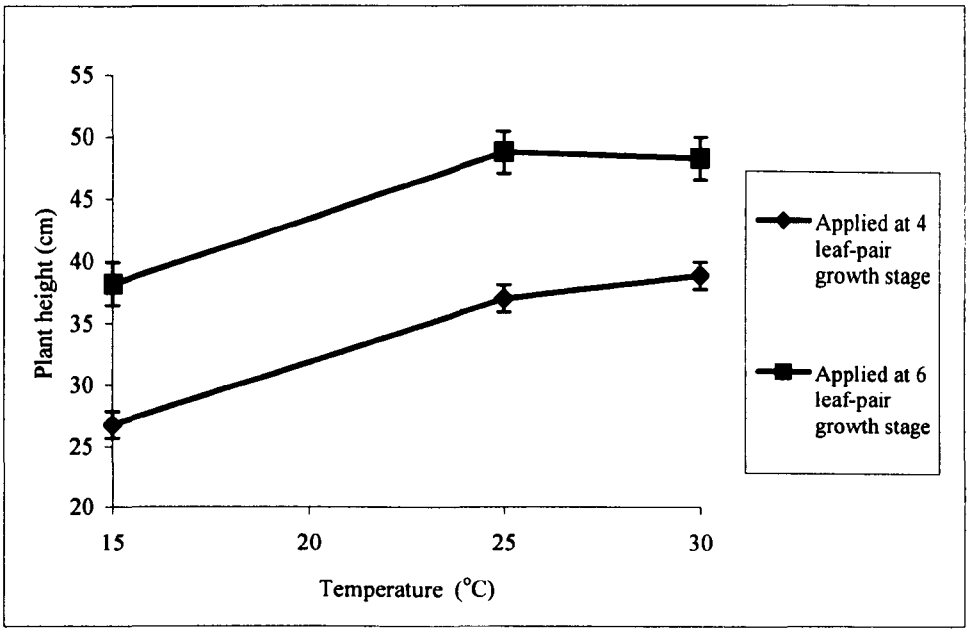

Figure 5.1 Comparison of plant height after one week of temperature treatment applied at certain growth stages

Leaf fresh weights at 25°C were the highest and differences between plants at the four and six leaf-pair growth stages were significant ($p<0.001$). Comparing plants at the two growth stages, plants with six leaf-pairs showed no difference between 25 and 30°C, however, those with four leaf-pairs showed differences, suggesting that young plants were more sensitive to temperature (Figure 5.2).

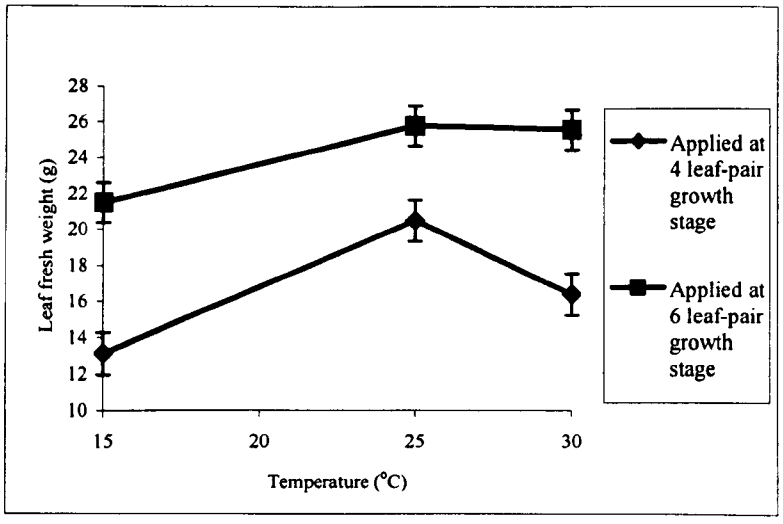

Figure 5.2 Comparison of leaf fresh weight after one week of temperature treatment applied at certain growth stages

Leaf dry weights at 25°C were the highest and differences were significant (for all $p<0.001$) between plants at both growth stages (Figure 5.3). Leaf water content increased with temperature and differences between temperature treatments were highly significant (Figure 5.4). This may explain the significant difference in leaf dry weights between 25°C and 30°C.

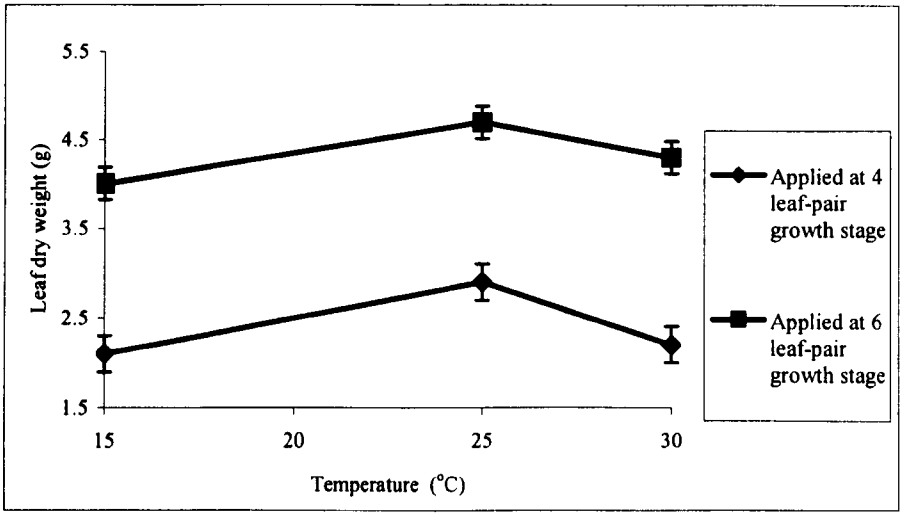

Figure 5.3 Comparison of leaf dry weight after one week of temperature treatment applied at certain growth stages

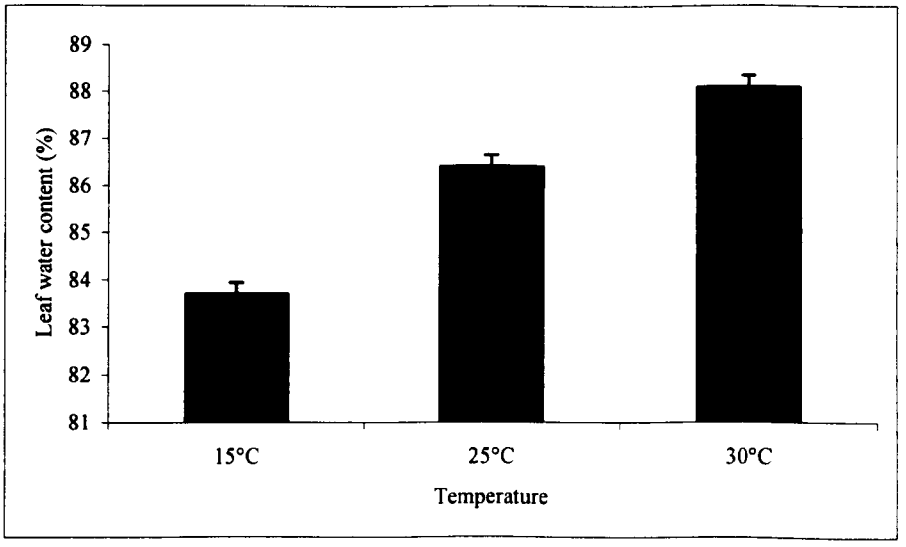

Figure 5.4 Leaf water content after one week of temperature treatment applied at the four leaf-pair growth stage

Leaf area was highest for plants grown at 25°C and differences were significant, however plants at the 4 leaf-pair growth stage did not show any difference between 25°C and 30°C (Figure 5.5).

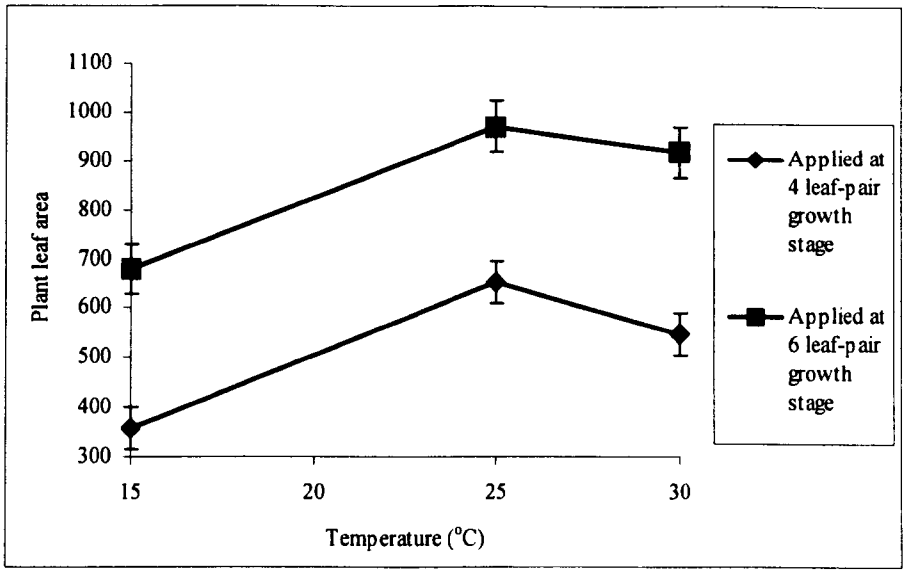

Figure 5.5 Comparison of leaf area after one week of temperature treatment applied at certain growth stages

It was also clear that the area of individual leaves on the main stalk was influenced by treatments applied at different growth stages. For the six leaf-pair growth stage, after one week of treatment the areas of the sixth and seventh pairs of leaves were significantly different (Table 5.1).

Table 5.1 Comparison of leaf area after one week of temperature treatments applied at the 6 leaf-pair growth stage

Leaf area	Temperature (°C)				
(cm^2)	15/15	25/25	30/30	SED	Pr
Total leaf area	679.0	969.0	917.0	51.800	<0.001***
3rd leaf-pair	80.4	71.6	83.8	5.270	0.089
4th leaf-pair	72.4	68.9	72.0	5.310	0.772
5th leaf-pair	50.3	59.9	55.9	4.730	0.158
6th leaf-pair	25.8	45.2	41.4	4.640	0.002**
7th leaf-pair	0.0	31.0	23.9	2.900	0.035*

Specific leaf area (total leaf area divided by total leaf dry weight) was increased by increasing temperature after one week of treatment ($p<0.001$) (Figure 5.6).

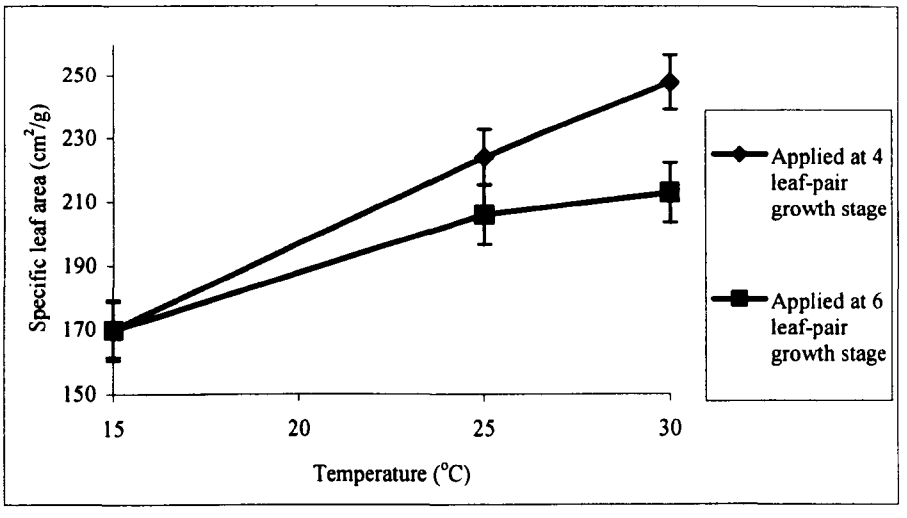

Figure 5.6 Comparison of specific leaf area after one week of temperature treatment applied at certain growth stages

Compared with plants grown at 15°C, more lateral shoots were produced at 25°C and 30°C ($p<0.001$), but differences between 25°C and 30°C were not significant

(Figure 5.7).

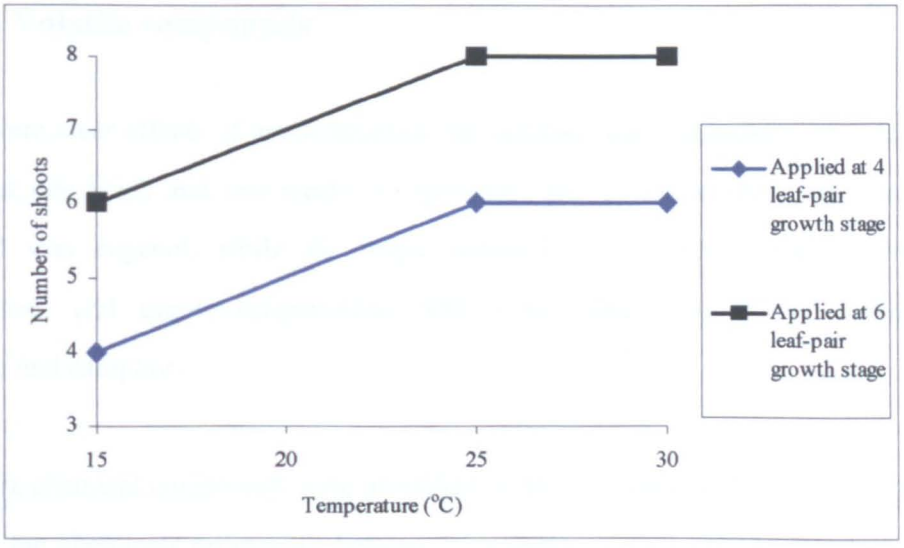

Figure 5.7 Comparison of number of lateral shoots after one week of temperature treatment applied at certain growth stages

Leaf colour was affected by temperature. Plants grown at 15°C exhibited paler leaves (more yellow) after only two or three days compared with plants grown at 25 and 30°C (Plate 5.1).

Plate 5.1 Comparison of leaf colour of basil plants at the 6 leaf-pair growth stage grown for one week at 15°C (left) and 25°C (right)

5.2.2.2 Volatile compounds

There were clear effects of temperature on the quantity and composition of volatile oils after one week and two weeks of treatment. The principal phenyl-propanoid detected was eugenol, while the major terpenoids were 1,8-cineole, linalool, cis-ocimene and trans-α-bergamotene, with some small amounts of pinenes, myrcene and camphor.

Thirty six chemical compounds were identified in this experiment (Appendix 3). For most of the chemicals differences between treatments were highly significant. The relative content of individual compounds was used to compare the composition of volatile oils, and the sum of peak areas was used to compare the total oil content in the basil leaves under different conditions.

Differences in the total oil content between temperatures for both one week and two weeks of treatment were highly significant. Leaves grown at 25°C produced the highest oil content (Figure 5.8).

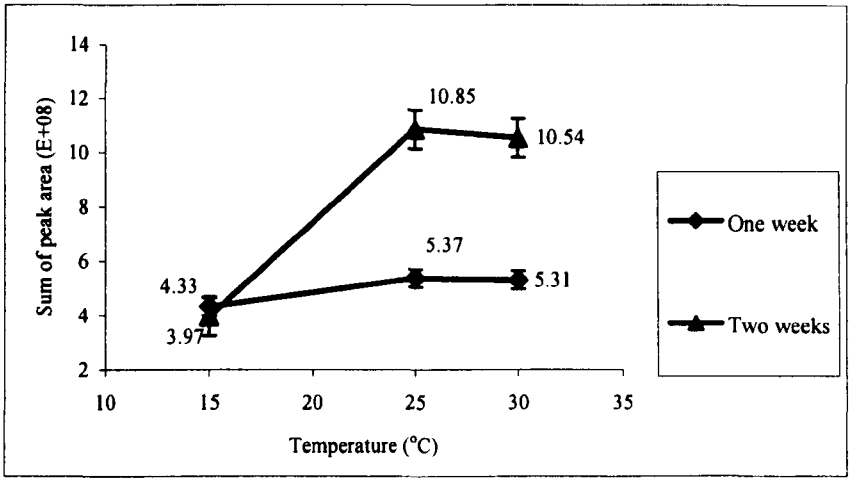

Figure 5.8 Volatile oil content in 5 g fresh leaves after one week and two weeks of treatments applied at the four leaf-pair growth stage

Compared with one week of treatment, much more oil was produced after two weeks of treatment. For two weeks of treatment, the fresh leaves of plants at 25°C and 30°C contained three times as much oil as those at 15°C. For one week of treatment, the increase was only 25%. There was no difference in oil content between plants grown at 25°C and 30°C.

With temperature treatment, plants showed significant differences in the relative content of ten compounds. They were the phenyl-propanoid eugenol, and the terpenoids α-terpinene, cis-ocimene, γ-terpinene, trans-sabinene hydrate, camphor, α-terpinenol, trans-α-bergamotene, trans-β-farnesene and δ-cadinene.

Some of the chemicals were enhanced at cool temperatures whereas some were enhanced at warm temperatures (Figure 5.9). For example, eugenol, α-terpinene, *cis*-ocimene, γ-terpinene, trans-sabinene hydrate, δ-cadinene and α-terpinenol were increased by warm temperature, while camphor, trans-β-farnesene and trans-α-bergamotene were decreased by warm temperatures.

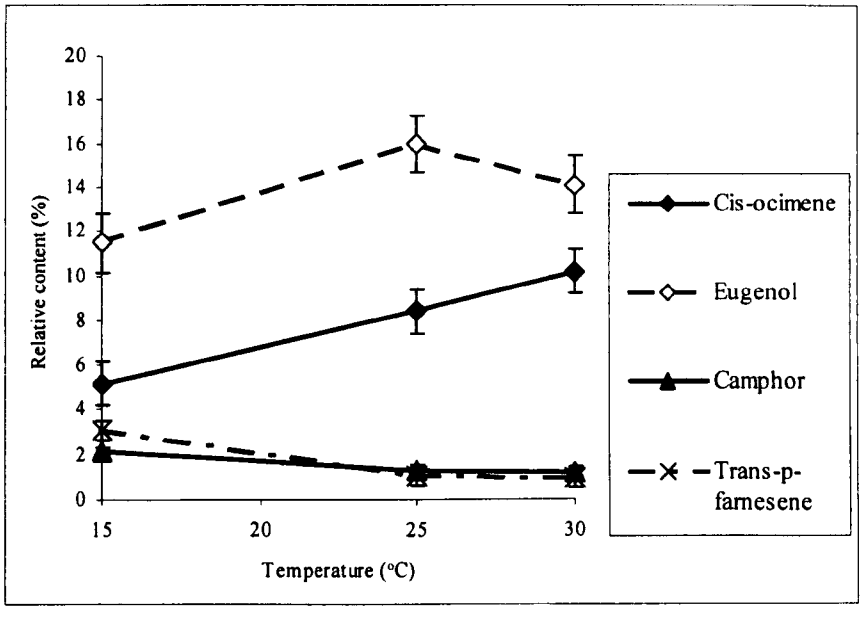

Figure 5.9 Relative content of selected volatile oil compounds after two weeks of temperature treatment applied at the four leaf-pair growth stage

For both one week and two weeks of treatment, there was no difference observed in the relative contents of 1,8-cineole and linalool, the two main compounds in basil leaves. These two compounds represented approximately 41% of the basil oils with 1,8-cineole at 23% and linalool at 18%.

Three main compounds, 1,8-cineole, linalool and eugenol, were selected to calculate their absolute contents using peak areas and calibration curves.

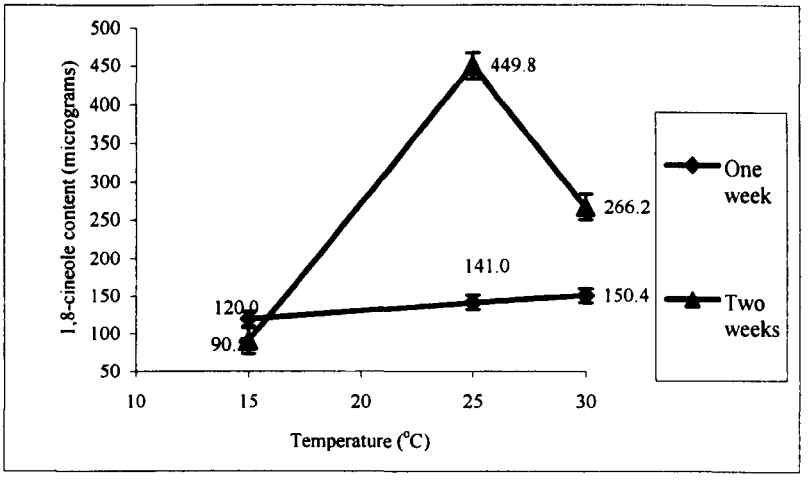

Figure 5.10 1,8-cineole content in 5 g fresh leaves after one and two weeks of temperature treatments applied at the four leaf-pair growth stage

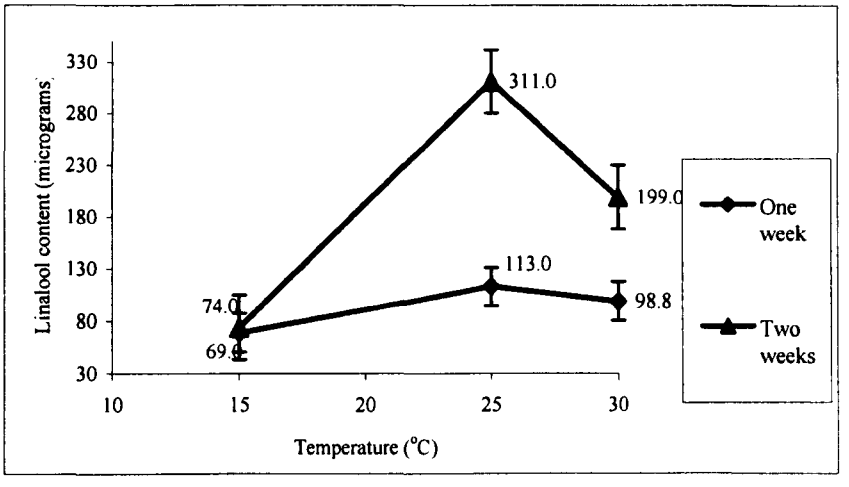

Figure 5.11 Linalool content in 5 g fresh leaves after one and two weeks of treatment applied at the four leaf-pair growth stage

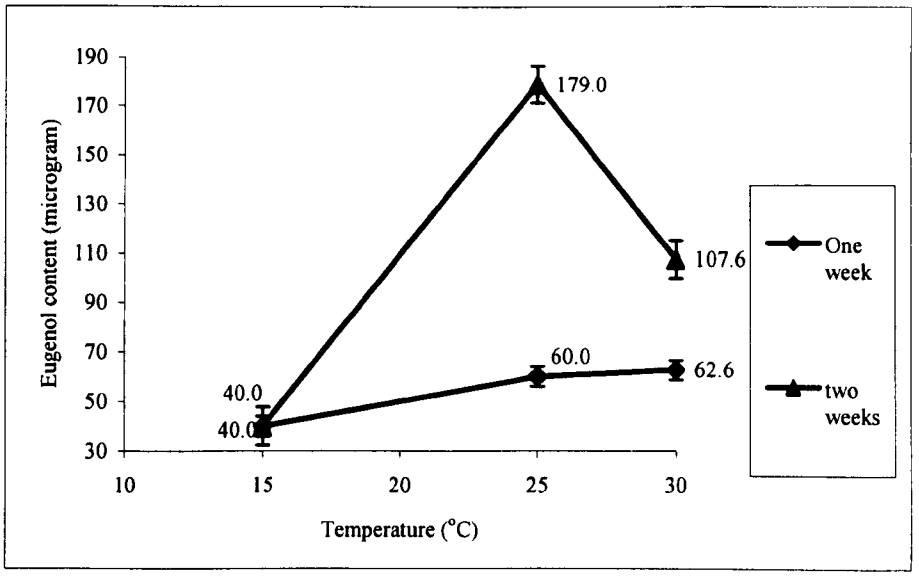

Figure 5.12 Eugenol content in 5 g fresh leaves after one and two weeks of treatments applied at the four leaf-pair growth stage

Plants grown at 25°C produced the highest yield of these three volatile oils and differences between one and two weeks of treatment were highly significant (Figures 5.10 to 5.12). Although the oil content in the leaves of plants grown at 30°C temperature was lower than those at 25°C, it was still significantly higher than the amount at 15°C.

For one week of treatment, there were no differences between 25°C and 30°C for all three compounds. Plants at 15°C produced less volatile oils ($p<0.001$). The effect of temperature, however, was much less compared with that resulting from two weeks of treatment.

Although there were no differences in the relative contents 1,8-cineole and linalool, differences in the absolute content in fresh leaves were highly significant, both for one week and two weeks of treatment. With the longer treatment, the difference was bigger.

5.2.2.3 Photosynthesis, transpiration and stomatal conductance

In this study, irradiance (PAR) was constant between different temperature treatments; they were 349.7, 351.2 and 382.8 μmol m^{-2} s^{-1} at 15, 25 and 30°C respectively (differences not significant).

Plants grown at 25°C possessed the highest photosynthetic rate and stomatal conductance to water vapour. The highest transpiration rate, however, was at 30°C but there was no significant difference between 25°C and 30°C (Table 5.2).

Table 5.2 Photosynthesis, transpiration and stomatal conductance of leaves at different temperatures for two weeks of treatment applied at the 4 leaf-pair growth stage

Parameters	15°C	25°C	30°C	SED	Replicates	P
Photosynthetic rate (μmol m^{-2} s^{-1})	3.92	6.00	5.10	0.554	12	0.003**
Transpiration rate (mmol m^{-2} s^{-1})	0.26	1.38	1.79	0.181	12	<0.001***
Stomatal conductance (mmol m^{-2} s^{-1})	34.90	78.50	75.20	9.840	12	<0.001***

5.2.3 Discussion:

5.2.3.1 Plant growth parameters:

There were clear effects of temperature on the growth of basil plants, notably plant height, weight, leaf area and specific leaf area. Environmental factors influence photosynthesis and thereby inhibit or stimulate growth. Light is the driving force for production, and other factors, such as temperature and moisture, regulate production (Lawlor, 1993). In this experiment, irradiance was constant between the temperature treatments, but there were significant differences in photosynthetic rate, transpiration rate and stomatal conductance.

The primary effect of temperature is via changed rates of enzyme reactions, metabolite transport and diffusion; at low temperatures these processes are slower than at high temperatures. For example, the rate of supply of phosphate ions to chloroplasts at low temperature probably limits photosynthesis, and hence a reduction in organ growth (Lawlor, 1993). In the present study, plant weight, plant height, total leaf area and all non-expanded leaves (before treatment) were significantly decreased in cool conditions compared to warm conditions. Cool conditions lead to decreased stomatal conduction (Table 5.2) and reduced net rate of photosynthesis, thereby inhibiting growth. Lower transpiration rate and stomatal conductance in cool conditions inhibited water uptake from the soil, therefore plant water content at 15°C was significantly lower than that at 25 and 30°C.

The data reveal that there were no significant differences in the weight, height, and total leaf area of plants grown at 25 and 30°C. Photorespiration is a small part of net photosynthesis in cool conditions but, with increasing temperature, the photosynthetic rate rises and photorespiration increases more than gross photosynthesis (particularly above 30°C), resulting in a small increase in net photosynthesis (Table 5.2). This result supported the findings of Pogany *et al.* (1968) in a growth chamber experiment, which measured the fastest growth rate at 27°C.

In this study, the leaves of plants grown at 15°C became yellow from the second or third day of treatment. At low temperatures, the content of carotenoid, plastoquinones and cytochromes is increased (Lawlor, 1993) and, a very short time after a temperature drop, leaves may contain lower concentrations of both chorophyll a and chorophyll b (Vagen *et al.*, 2003).

In the present study, plant leaf area was strongly affected by temperature, i.e. the higher the temperature, the bigger the leaf area. With the plants grown at 15°C, specific leaf area was decreased because the leaves were thicker. Tomato leaves have also been shown to be smaller and thicker at low temperature and Hoek *et al.* (1993)

attributed this to slower growth of leaves due to a low rate of cell division or lower number of leaf cells. A similar situation occurred in basil, however, this requires further study to determine the rates of cell division.

5.2.3.2 Volatile oils

The levels of camphor, trans-α-bergamotene, trans-β-farnesene, α-cis-bergamotene and α- humulene were enhanced in plants grown at 15°C. Some volatile oils were increased by warm conditions, i.e. eugenol, cis-ocimene and cadinene. This result supports previous evidence that the eugenol content of basil plants in the open field is less than in those grown in warm plastic houses (Nykanen, 1989).

Temperature is an important factor that regulates plant metabolism. At temperatures around 0°C most enzymes are inactive but, as the temperature increases, the rate of enzyme activity also increases up to about 40°C, after which most plant enzymes become inactivated and even permanently damaged (Kaufman *et al.*, 1999). At high temperature, the largest class of plant secondary metabolites, terpenoids, play a role in stabilizing membranes (Wink, 1999). This could explain why with increase in temperature the total content of terpenoids was enhanced. Combined with increased eugenol content, there was more than a threefold increase in volatile oil content after two weeks of temperature treatment at 25°C compared with plants at 15°C.

Since plants at 25°C possessed heavier fresh leaves than those at 15°C or 30°C, combined with higher oil content per weight unit, after two weeks at 25°C they had produced seven times as much volatile oil as those at 15°C. Although there was no difference in oil content per weight unit between 25°C and 30°C treatments, the difference in oil content per plant was highly significant because of the difference in leaf weights.

Since the total oil content was significantly increased by increasing temperature,

absolute contents of most of the individual compounds were significantly different even if there were no differences in their relative contents, for example, 1,8-cineole and linalool.

5.2.4 Conclusions

- ✧ Plant height, weight (fresh & dry) and leaf area were significantly increased by increasing temperature after one and two weeks of treatment.
- ✧ Specific leaf area was strongly affected by temperature, i.e. the higher the temperature, the bigger the specific leaf area.
- ✧ Thirty six volatile compounds were detected, most of which were terpenoids. The phenylpropanoid eugenol was also detected.
- ✧ Plants grown at 25°C produced the highest content of volatile oils.
- ✧ Relative amounts of some compounds were significantly affected by warm temperatures, i.e. eugenol increased and camphor and trans–p–farnesene decreased, however 1,8-cineole and linalool were not affected.
- ✧ Although there were no differences in relative amounts of 1,8-cineole and linalool, the absolute contents in fresh leaves were significantly different, and plants at 25°C produced the highest amounts.

5.3. Effects of alternating temperature

The previous experiments (section 5.2) showed that temperature strongly affects plant growth parameters and volatile oils in basil. It is not known, however, if the effects were due to accumulated day degrees or the last temperature experienced. So far, there has been no publication on how alternating temperatures affect morphology and volatile oils in basil. This experiment was designed to study how temperature change affects plant growth and volatile oils in basil. Treatments were arranged so that after three weeks all plants had received the same accumulated temperatures, but in a different temperature order.

It was hypothesised that using alternating temperature treatments, all plants would be the same size, but they would differ in their content and composition of volatile oils.

5.3.1 Materials and methods

Plant materials, measurement of plant growth parameter, sample preparation and compound identification were as described in chapter 3, except that plant height, leaf number and shoot number were also measured after one week and two weeks of treatment.

Alternating temperature experiments were carried out in controlled environment rooms at constant temperatures maintained to within ±1°C with 16 h daytime photoperiod. There were three temperatures, 15°C, 25°C and 30°C (day/night), and six different treatments (Table 5.3).

This experiment was commenced on 6 January 2003. Plant growth parameters and chemicals were measured and calculated after three weeks of treatments. All fresh leaf samples were collected from the fifth pair of leaves.

Table 5.3 Alternating temperature treatments

Treatments	Temperatures (°C)		
	First week (ADD)	Second week (ADD)	Third week (ADD)
1	15(28.7)	30(162.4)	25 (261.1)
2	25(98.7)	15(127.4)	30 (261.1)
3	30(133.7)	25(232.4)	15 (261.1)
4	30(133.7)	15(162.4)	25 (261.1)
5	25(98.7)	30(232.4)	15 (261.1)
6	15(28.7)	25(127.4)	30 (261.1)

ADD = $\Sigma (T_r - T_b)$,

Where,

ADD: accumulated day degrees

T_r: recorded temperature

T_b: base temperature (10.92°C)

5.3.2 Results

5.3.2.1 Plant growth parameters

After one week, plants grown at the warmer temperatures were taller (Figure 5.13) and possessed more leaves (Figure 5.14). After two weeks, there were no differences in leaves and plant height between the treatments with the same accumulated day degrees. With higher accumulated day degrees, however, plants were taller (Figure 5.13), possessed more leaves (Figure 5.14) and more shoots (Figure 5.15).

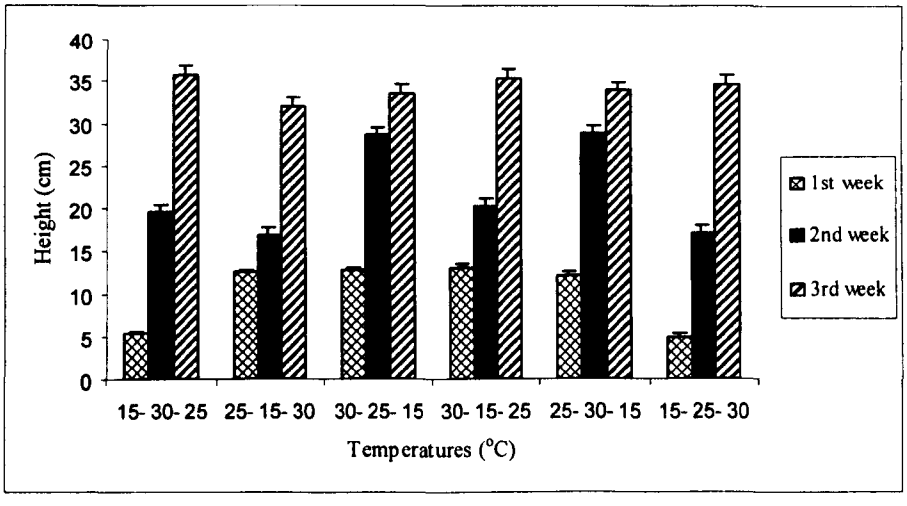

Figure 5.13 Plant height after alternating temperature treatment applied at the one leaf-pair growth stage

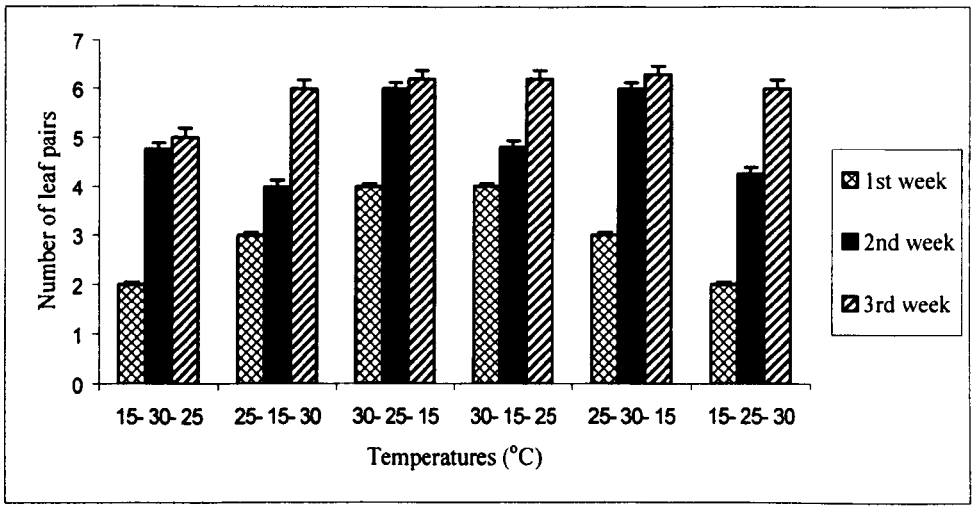

Figure 5.14 Leaf number after alternating temperature treatment applied at the one leaf-pair growth stage

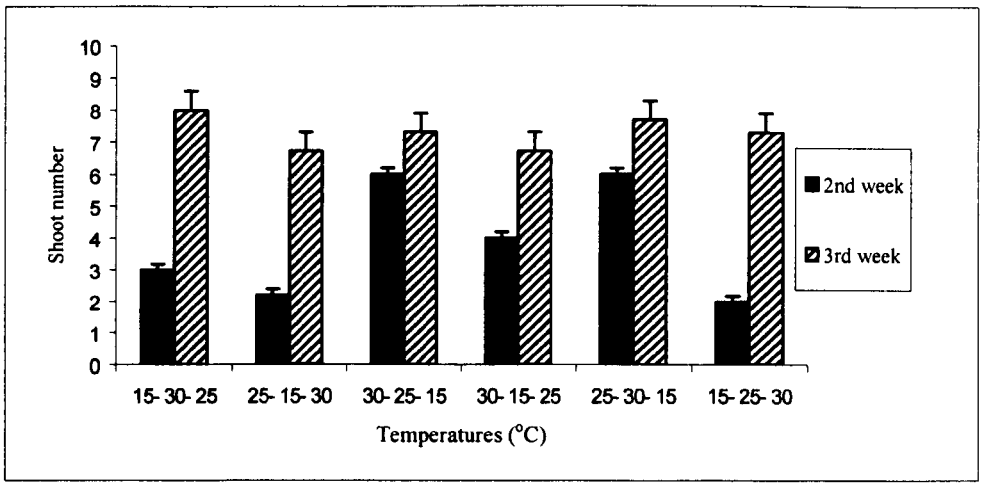

Figure 5.15 Number of shoots after alternating temperature treatments applied at the one leaf-pair growth stage

After three weeks, plants in all of the treatments had received the same accumulated day degrees, i.e. 261.1, but in different order. There were no significant differences in number of shoots (Figure 5.15) and fresh weights (Figure 5.16) between treatments. Due to significant differences, however, in the water content of plants at different temperatures, differences in dry weights between treatments were highly significant (Figure 5.17). With cool conditions in the last week and warm temperatures in the first two weeks, plants possessed more dry matter, while cool

conditions in the first week but warm conditions in the last two weeks resulted in taller plants.

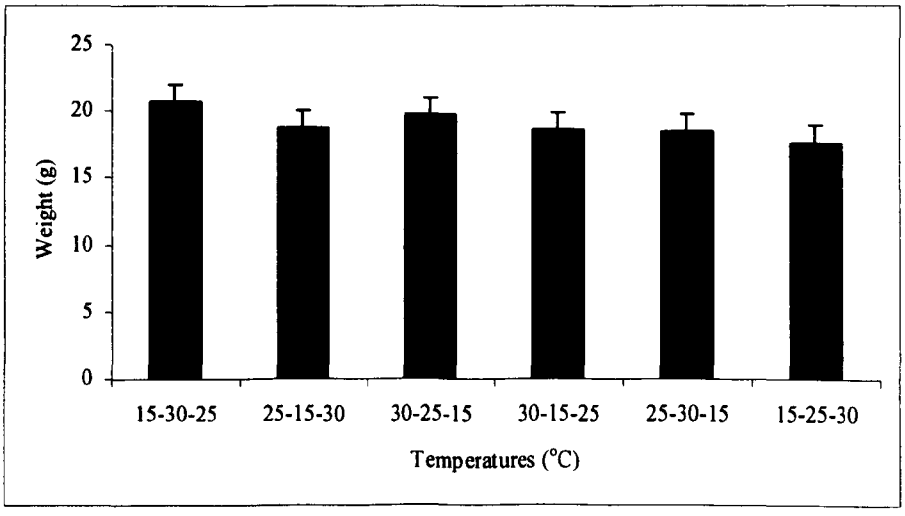

Figure 5.16 Leaf fresh weights after alternating temperature treatments applied at the one leaf-pair growth stage

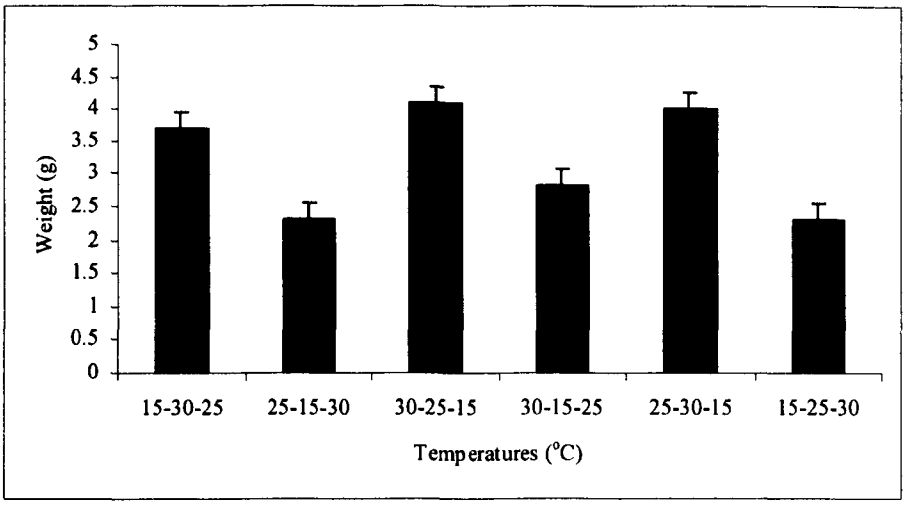

Figure 5.17 Leaf dry weights after alternating temperature treatments applied at the one leaf-pair growth stage

Both whole plant water content and leaf water content were strongly affected by the last temperature experienced. Plants that had experienced 15°C as the last

temperature had the lowest water content and those at 30°C had the highest water content (Figure 5.18).

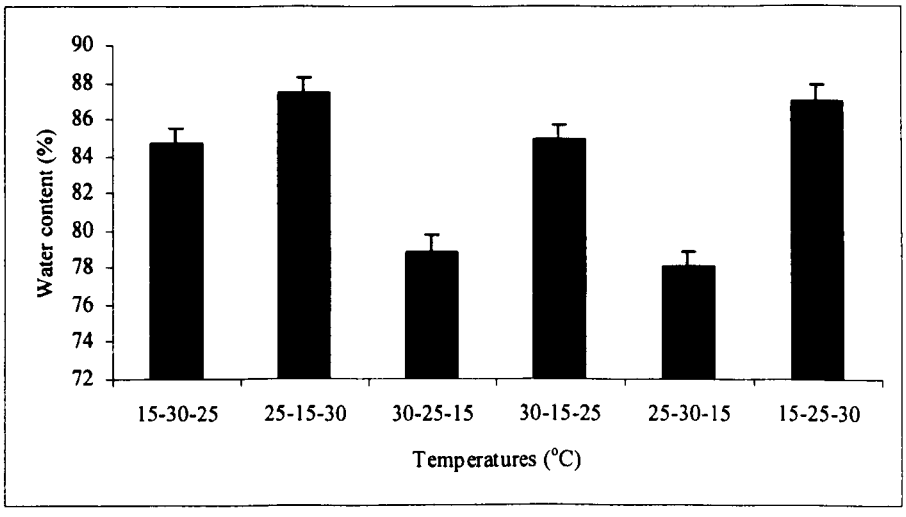

Figure 5.18 Leaf water content after alternating temperature treatment applied at the one leaf-pair growth stage

At the time of harvest, all plants were at the early flower initiation growth stage, and no more new leaves would be produced. Normally there are six to seven pairs of leaves in this cultivar, but when plants were grown in cool conditions (15°C) for the first week, then moved to higher temperatures, there was approximately one leaf less than in the other conditions (Figure 5.14).

Differences in leaf area between treatments were highly significant, but it was not possible to make any conclusions (Figure 5.19). Specific leaf area, however, was strongly affected by the last temperature experienced, with significant differences between treatments (Figure 5.20). With the last temperature experienced, the higher the temperature, the bigger the specific leaf area. This was the same trend as shown for constant temperature treatments.

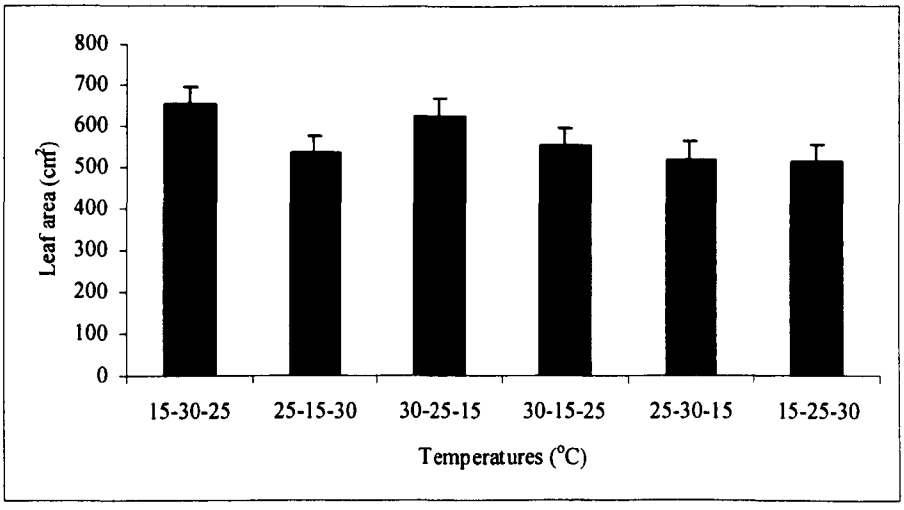

Figure 5.19 Leaf area after alternating temperature treatments applied at the one leaf-pair growth stage

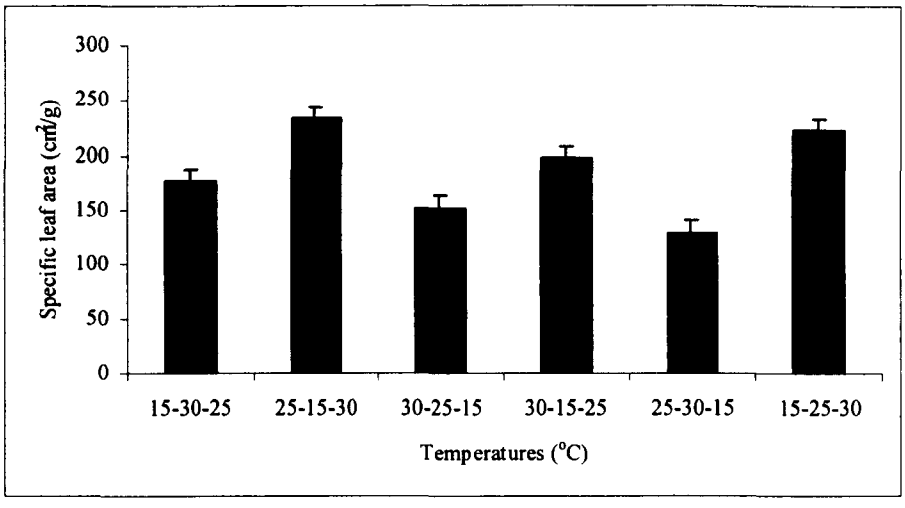

Figure 5.20 Specific leaf area after alternating temperature treatments applied at the one leaf-pair growth stage

5.3.2.2 Volatile compounds

Thirty three chemical compounds were identified, of which sabinene, β-pinene, β-myrcene, phellandrene, α-terpinene, 1,8-cineole, cis-ocimene, α-terpinolene, linalool and eugenol showed significant differences in their relative contents in the different treatments. All of them are monoterpenes except eugenol. In contrast to the constant temperature treatments, three major compounds, i.e. 1,8-cineole, linalool

and eugenol, showed significant differences after three weeks of alternating temperature treatments. As with the constant temperature treatments, the higher the temperature before harvesting, the higher the eugenol content of the plants (Figure 5.21).

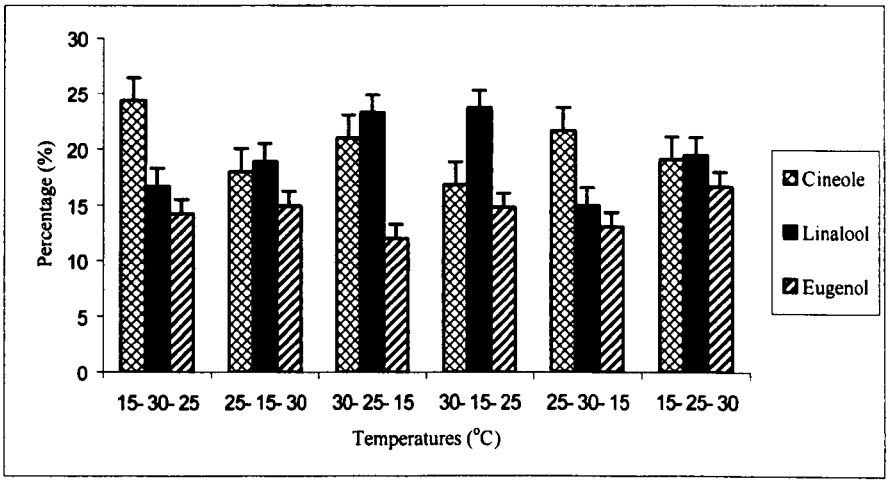

Figure 5.21 Relative contents of selected volatile oil compounds after alternating temperature treatments

For 1,8-cineole, warmer temperatures in the first week followed by 15°C in the second week, resulted in a decreased in the relative content. For linalool, when the plants were grown initially at 30°C, the relative content was significantly higher than in the other treatments (Figure 5.21).

Oil content in fresh leaves and oil yield per plant were strongly affected by the alternating temperature treatments. Plants grown in warm conditions for two weeks prior to harvest possessed higher oil content (Figure 5.22).

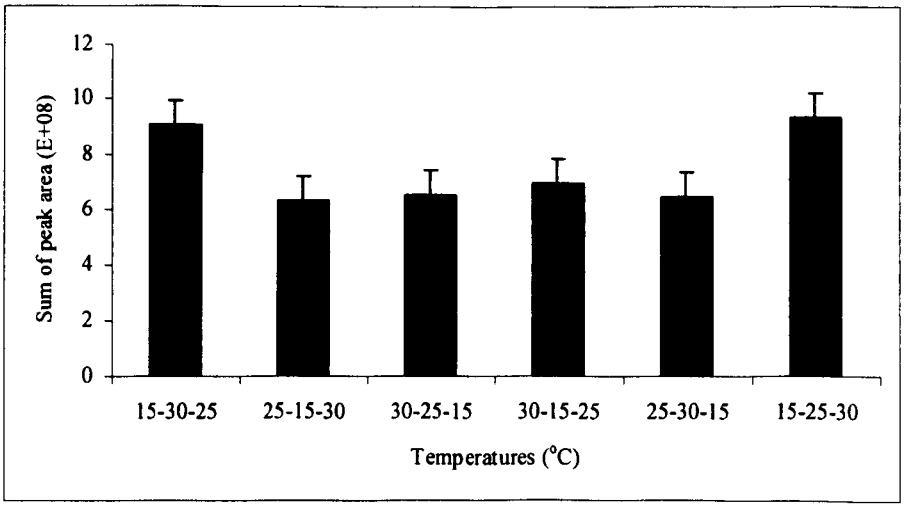

Figure 5.22 Comparison of volatile oil content in 5 g of fresh leaves after alternating temperature treatment

From the sum of peak area in fresh leaves and leaf fresh weight per plant, total peak area per plant was calculated to compare the difference in oil contents per plant between treatments. Since there was a large difference in the oil content in fresh leaves and there was no difference in leaf fresh weight between treatments, there was a highly significant difference in the total volatile oil content between treatments. Warm conditions in the last two weeks significantly increased the total oil yield (Figure 5.23).

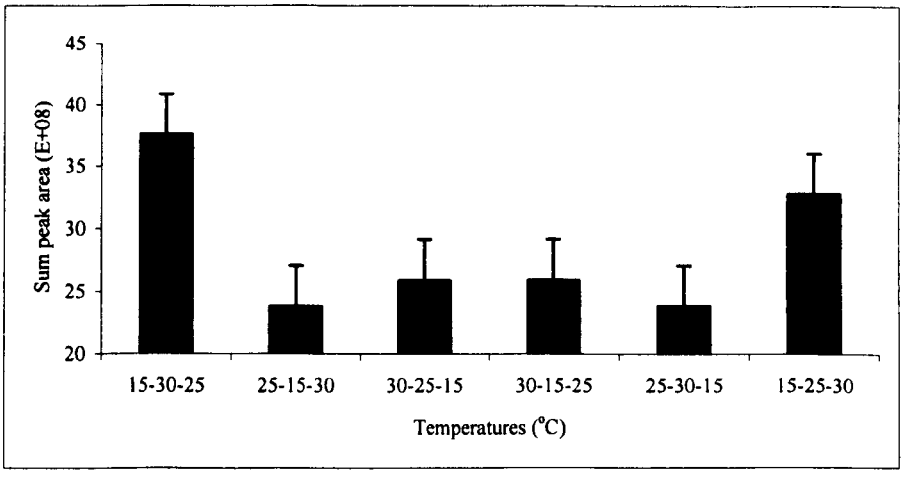

Figure 5.23 Comparison of volatile oil content in individual basil plants after alternating temperature treatments

Plants grown in warm conditions in the last two weeks before harvest produced more eugenol ($p<0.001$) (Figure 5.24).

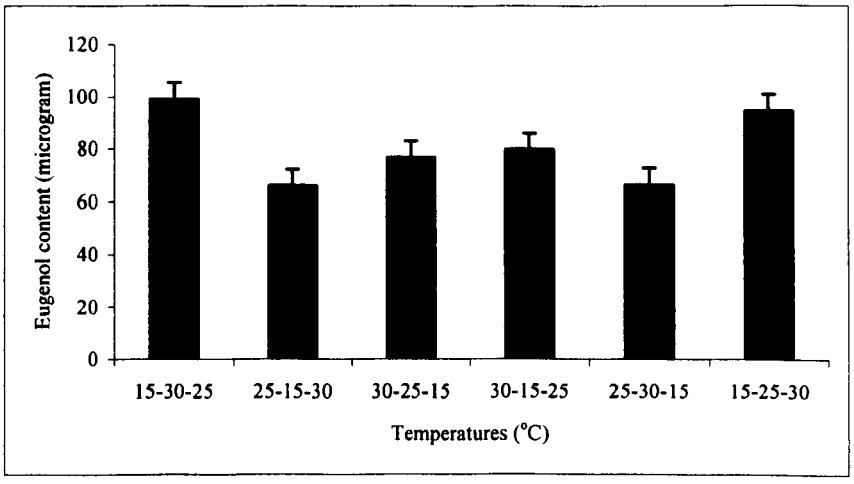

Figure 5.24 Comparison of eugenol content in 5 g of fresh leaves after alternating temperature treatments

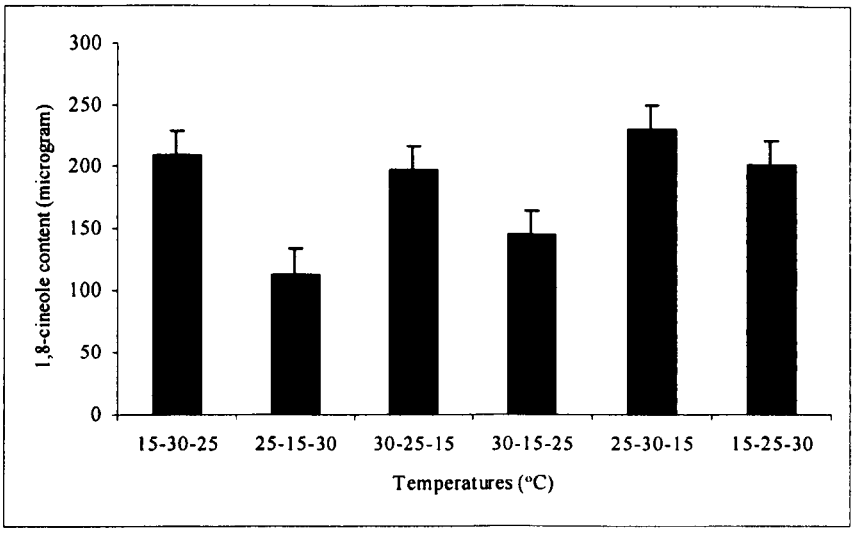

Figure 5.25 Comparison of 1,8-cineole content in 5 g of fresh leaves after alternating temperature treatments

The temperature treatments affected the contents of both 1,8-cineole and linalool (Figures 5.25 and 5.26). When plants were grown at 15°C for the second week of the treatment, the absolute content of 1,8-cineole was significantly decreased, however, there was no difference between the other four treatments (Figure 5.25). For linalool,

as with the result for relative content, plants grown initially at 30°C, produced more linalool than with the other treatments (Figure 5.26).

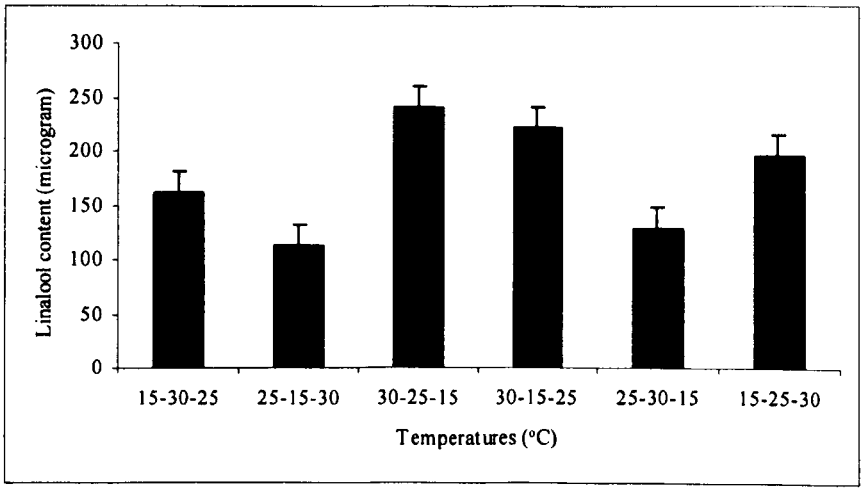

Figure 5.26 Comparison of linalool content in 5 g of fresh leaves after alternating temperature treatments

5.3.3 Discussion

Plant height was strongly affected by accumulated day degrees. When the plants received different accumulated day degrees during the first one or two weeks, very different heights were noted. With higher accumulated day degrees, the plants were taller. After three weeks of treatment, all plants had received the same accumulated day degrees and, although there were still differences in plant height, the differences were much less than in the first two weeks.

There were no differences in plant fresh weight and leaf fresh weight between treatments but, because cool temperature resulted in lower water content (Nilsen and Orcutt, 1996), there were significant differences in plant dry weight and leaf dry weight between treatments. Plants that received more accumulated day degrees in the first two weeks produced more plant dry matter.

Number of shoots was strongly affected by accumulated day degrees. As with plant height, when plants received different accumulated day degrees during the second week of treatments, the number of shoots was significantly different between treatments, and with higher accumulated day degrees, the plants possessed more shoots. After three weeks, differences between treatments were not significant.

Low temperature has been shown to stimulate the initiation of carnation flowers and increased temperature will normally result in more rapid development, with more leaf pairs initiated prior to flower initiation (Beisland and Kristoffersen, 1969). Temperature also affected basil leaf and flower initiation. Before harvesting, all plants were in the early flower growth stage, and no new leaves would appear on the main stem. The plants possessed fewer leaves below the initiated flowers when grown at 15°C in the first week of treatment. Treatment 1 (15-30-25°C) and treatment 6 (15-25-30°C) possessed 5.0 and 5.8 pair of leaves respectively, but the other treatments had more than six leaves. In the first two weeks of treatments, as with plant height and number of shoots, the number of leaves was strongly affected by accumulated day degrees, and with higher accumulated day degrees, more leaves were produced.

Of the three major volatile compounds selected to compare the differences between treatments, the content of eugenol was strongly affected by temperature conditions before harvesting, the highest content being produced in warm conditions one week before harvest. Unlike eugenol, the contents of 1,8-cineole and linalool were significantly different between treatments. Linalool and 1,8-cineole have the same precursor, geranyl pyrophosphate (GPP), and the enzymes linalool synthase (LIS) (Kanfman, 1999) and 1,8-cineole synthase (Gang *et al.*, 2001) that catalyse the conversion of GPP directly to linalool and 1,8-cineole have been identified. How environmental factors stimulate these enzyme activities, however, is still not clear. Based on the studies from Dewick (2002), Gang *et al.* (2001) and Kaufman *et al.*

(1999), a network of metabolic pathways leading from D-glucose to three major volatile oil compounds is proposed (Appendix 4).

Oil contents, both in fresh leaf samples and yield per plant, were strongly enhanced by accumulated day degrees two weeks before harvesting, and strongly reduced by cool temperature (15°C) one week before harvesting. For oil production, it would be better to grow plants under warm conditions, especially during the two weeks prior to harvest.

5.3.4 Conclusions

- ✧ Plant height, dry weight and number of shoots were strongly affected by accumulated day degrees; the higher the accumulated day degrees, the higher the above parameters.
- ✧ The growth of young plants in cool conditions may reduce the number of leaves.
- ✧ Specific leaf area was strongly affected by the last temperature experienced; the higher the temperature, the bigger the specific leaf area produced.
- ✧ Eugenol, i.e. both relative content and absolute content, was strongly affected by the last temperature experienced; the higher the temperature, the more eugenol produced. 1,8-cineole and linalool, however, showed significant differences between treatments but the mechanism and the conditions that increase or reduce the relative content are still not clear.
- ✧ Oil content of fresh leaves and oil yield per plant were strongly affected by temperature two weeks before harvest; higher temperature resulted in higher oil content.

Chapter 6 IRRADIANCE EFFECTS

6.1 Introduction

Light is the main factor controlling plant production and growth and development. Plants are affected in a complex manner by irradiance at all times. The architecture of plants is dependent on the quantity, direction, duration and quality of light. Photosynthesis is dependent on light for energy and for induction of enzymatic processes. High irradiance affects plant processes directly through its effect on factors such as enzyme activity and photosynthesis, but it also affects plant physiological processes indirectly by its impact on thermal attributes of the tissues. It is also suggested that long day treatments frequently promote an increase in dry weight in plants that otherwise grow in short days (Adams and Langton, 2005). Leaf temperature is regulated primarily by the influences of net radiation, latent heat exchange, conduction and convection, and air temperature. Some leaves can have as much as 10 to 15°C difference between air and leaf temperature under certain conditions. Most leaves, however, will have 5°C or less difference between air and leaf temperature (Nilsen and Orcutt, 1996).

Plant responses to light include a variety of adaptations at physiological (photosynthesis, nutrient uptake) and biochemical (pigments, carbohydrates) levels. Such responses are translated into alterations of growth rate, plant architecture and morphological characteristics (Peralta *et al.*, 2002). Exposure of plants to excessive light is a well known cause of photoinhibition (Sorrentino *et al.*, 1997).

So far, no publications show how light affects the morphology and volatile oil

content and composition of basil. One of the aims of this study was to evaluate the effect of irradiance on plant growth and volatile oil content and composition in plants of sweet basil. It was hypothesized that low irradiance would decrease the content of volatile oils and change the oil composition as well. Plants grown under reduced irradiances would also possess less dry matter, fewer leaves and shoots and reduced plant height.

6.2 Materials and methods

Plants of basil were raised from seed and grown as described in Chapter 3. A shading experiment in a glasshouse was commenced on 17 March 2003 (first experiment) and repeated on 13 June 2003 (second experiment).

6.2.1 Shading treatment design and data collection

Three levels of reduced total irradiance were achieved by covering metal support frames (140x70x70cm) with green 'Rokolene' shading net of different percentage light transmissions. Rokolene netting has been previously shown to have no effect on light quality (Wright and Sandrang, 1995). In this way, four levels of irradiance were provided in the glasshouse, i.e. no shade (control), 25, 50 and 75 percent glasshouse irradiance. Each treatment had two replicates with eighteen plants.

The light irradiance and temperature for each treatment were measured at plant height (50cm) using a data logger (Campbell CR10) each hour automatically. Irradiance readings were subsequently converted to Watts m^{-2}. Daily light integral (DLI) (moles m^{-2} d^{-1}), i.e. the total quantity of light delivered over the course of an entire day, was used in this study, and calculated by the formula (Faust, 2002):

DLI (moles m^{-2} d^{-1}) = [Sum of data logger reading each hour x 0.0036] x 2

Where: 0.0036 is derived come from 60 s/min x 60 min/h ÷1000000 micromole/mole

Moles m^{-2} (≡Watts m^{-2} x 2)

6.2.2 Measurement of photosynthesis

An infra-red gas analyser (CIRAS I) was used to measure photosynthesis rate in the first experiment on 16 May 2003. Measurements were made on the fifth pair of leaves of plants that, at the three leaf-pair growth stage, two weeks of shading treatments. The rate was calculated from the difference between the CO_2 concentration entering (C_{in}) and leaving (C_{out}) and the flow rate through the cuvette using the following formula:

$$A = C_{in} \times W - C_{out} \times (W + E)$$

$$= - [W \times (C_{out} - C_{in}) + C_{out} \times E]$$

Where:

A: Rate of CO_2 exchange in the cuvette (μ mol m^{-2} s^{-1})

C_{in}: CO_2 concentration of air into cuvette (μ mol mol^{-1})

C_{out}: CO_2 concentration of air leaving cuvette (μ mol mol^{-1})

W: Mass flow of dry air per unit leaf area (mol m^{-2} s^{-1})

E: Transpiration rate (mol m^{-2} s^{-1})

C_{out} - C_{in}: The CO_2 difference (CO_2 Diff) that was calculated and displayed by the CIRAS

6.2.3 Plant measurements

Plants used in the irradiance treatments were carefully selected for uniformity. The measurements of growth parameters and methods were as described in Chapter 3.

6.2.4 Sample preparation and analysis of volatile oils

Volatile oils in leaf samples were extracted using the sorbent tube trapping system and analysed by TD-GC/MS as described in Chapter 3. Depending on the plant growth stage, the collection of leaf sample for volatile oil analysis was varied. When the irradiance treatment was applied at the one leaf-pair growth stage for two weeks, fresh leaf samples were collected from the third pair of leaves, and when applied at the three leaf-pair growth stage, samples were collected from the fifth pair of leaves. There were three samples analysed for each replicate of the irradiance treatments.

6.2.5 Measurement of leaf temperature

An infrared thermometer (Kane-May KM823, Comark Ltd, England) was used to measure leaf temperature. The data were collected on the third pair of leaves when treatment was applied at the one leaf-pair growth stage and on the fourth pair of leaves when the treatment was applied at the three leaf-pair growth stage. There were ten replicates (ten individual plants) for each treatment.

6.3 Results

The shading treatments produced a range of mean daily light integrals (Table 6.1).

Table 6.1 Mean daily light integrals (moles m^{-2} d^{-1}) during shading treatments

Experiments	Date (2003)	no shading	25% shading	50% shading	75% shading
First	09 /April-06/May	19.8	12.2	8.8	4.3
Second	02/July-23/July	24.9	13.5	11.3	5.3

6. 3. 1 Growth

Differences in plant morphology as well as plant weights (fresh and dry) and leaf weights (fresh and dry) were observed following two weeks of shading treatments. Since both experiments produced similar results, only data from the second experiment are presented.

6.3.1.1 Plant height

Plant height increased ($p<0.001$) with daily light integral from 5.4 moles $m^{-2} d^{-1}$ to 11.3 moles $m^{-2} d^{-1}$ in plants treated at the one leaf-pair growth stage and the three leaf-pair growth stages (Figure 6.1). No increase was observed with daily light integrals greater than 11.3 moles $m^{-2} d^{-1}$ applied at the first leaf-pair growth stage and 13.5 moles $m^{-2} d^{-1}$ applied at the third leaf-pair growth stage, suggesting a light saturated response at these levels.

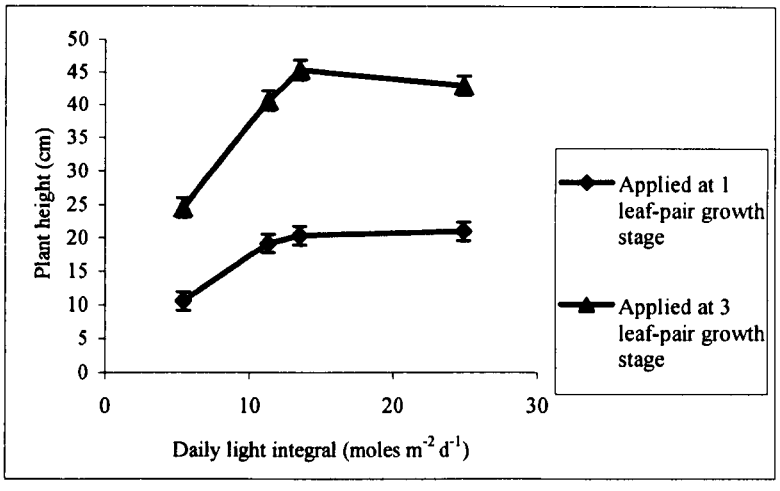

Figure 6.1 Plant heights after two weeks of shading treatments applied at the one and three leaf-pair growth stages

6.3.1.2 Weight

The fresh and dry weights of plants and leaves were significantly increased ($p<0.001$) with increases in daily light integral for two weeks at both plant ages (Figures 6.2 to

6.5). However, the differences were not significant between the control and 25% shading treatments suggesting a saturation of the light level. Compared with treatments applied at the one leaf-pair growth stage, differences in the effects of the 11.3 and 13.5 moles m^{-2} d^{-1} light integrals applied at the three leaf-pair growth stage were significant.

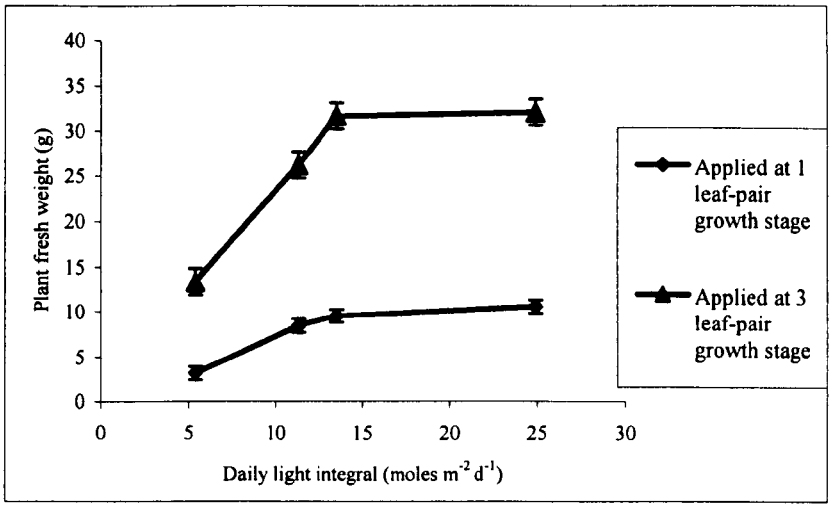

Figure 6.2 Plant fresh weights after two weeks of shading treatments applied at the one and three leaf-pair growth stages

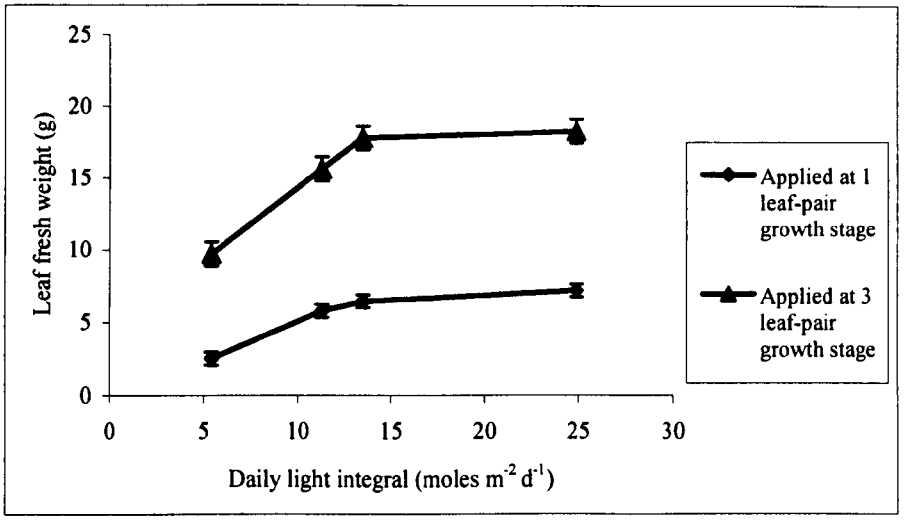

Figure 6.3 Leaf fresh weights after two weeks of shading treatments applied at the one and three leaf-pair growth stages

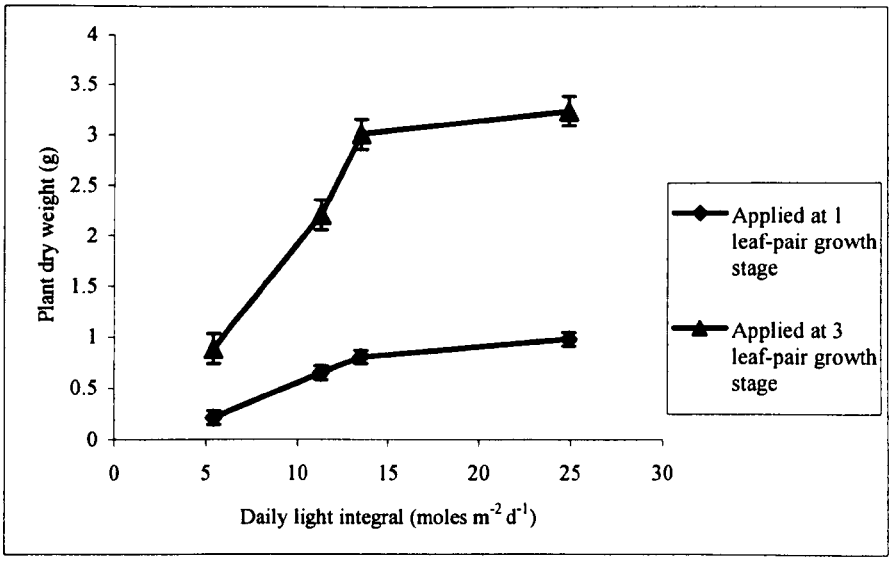

Figure 6.4 Plant dry weights after two weeks of shading treatments applied at the one and three leaf-pair growth stages

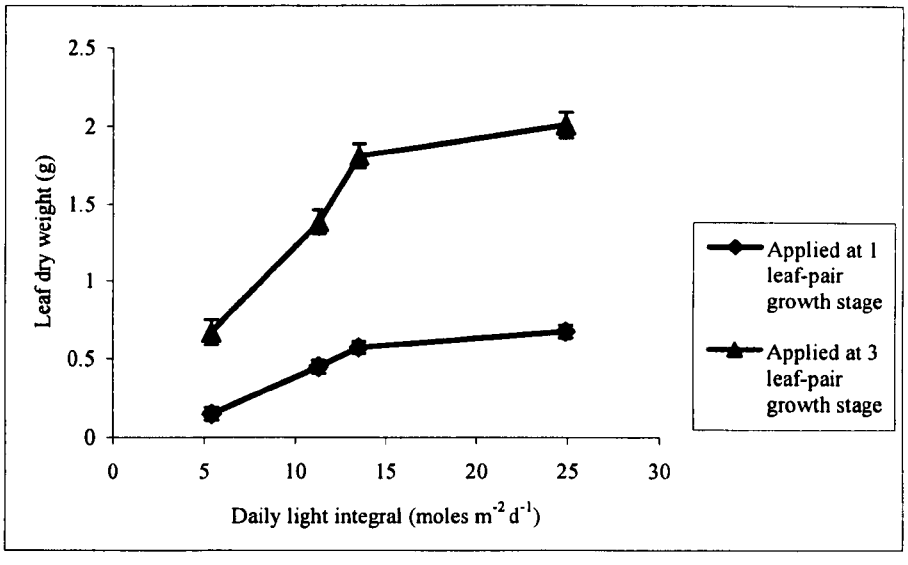

Figure 6.5 Leaf dry weights after two weeks of shading treatments applied at the one and three leaf-pair growth stages

6.3.1.3 Leaf number

The number of leaves on the plant main stem did not vary with light integrals from 11.3 to 24.9 moles m^{-2} d^{-1}, remaining constant at four pairs and six pairs of leaves with the treatments applied at the one and three leaf-pair growth stages respectively (Figure 6.6). Differences in leaf number, however, between 5.4 and 11.3moles m^{-2} d^{-1}

were significant ($p<0.001$). There was one leaf pair less on plant main stems under 5.4 moles m^{-2} d^{-1} compared with the higher daily light integrals.

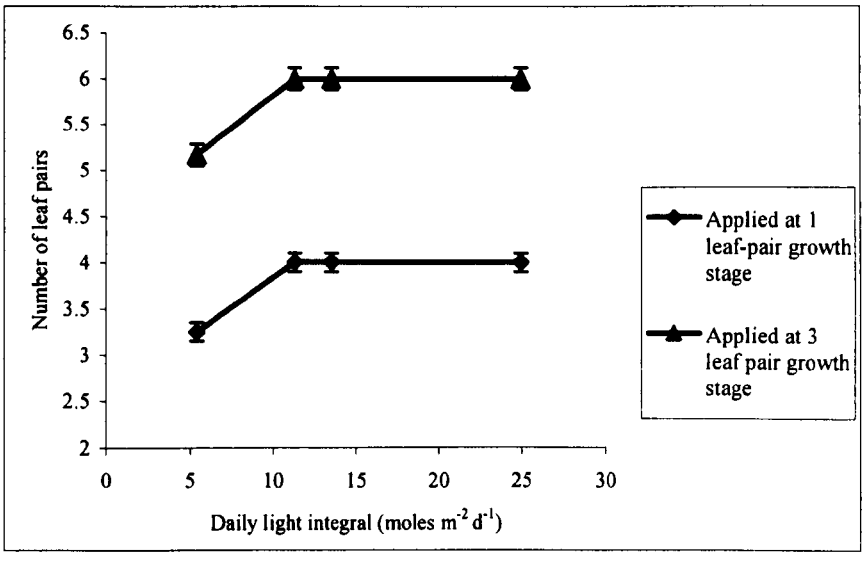

Figure 6.6 Number of leaf pairs on the plant main stem after two weeks of shading treatments applied at the one and three leaf-pair growth stages

6.3.1.4 Leaf area

There were highly significant differences ($p<0.001$) in total mean leaf area between different daily light integral conditions (Figure 6.7). They ranged from 110 cm^2 to 237 cm^2 and 356cm^2 to 714 cm^2 when treatments were applied at the one and three leaf-pair growth stages respectively. There was no difference, however, between the 13.5 and 24.9 moles m^{-2} d^{-1} daily light integrals. Although numbers of leaf pairs on the main stems were the same in the 11.3 and 24.9 moles m^{-2} d^{-1} treatments, the differences in shoot numbers (Figure 6.8) and individual leaf sizes (Tables 6.2 & 6.3) resulted in differences in total leaf area between the 11.3 and 13.5 moles m^{-2} d^{-1} daily light integrals, especially when treatments were applied at the three leaf-pair growth stage.

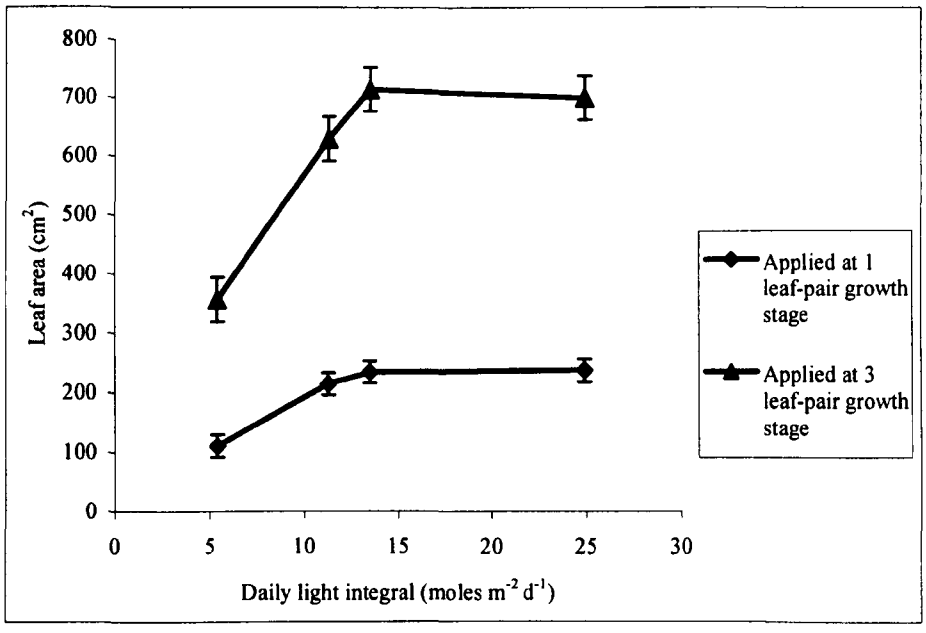

Figure 6.7 Total leaf areas after two weeks of shading treatments applied at the one and three leaf-pair growth stages

The areas of individual leaves were strongly affected by shading treatments, depending on when the treatments were applied (Table 6.2). For treatments applied at the one leaf-pair growth stage, significant differences were detected from the first pair of leaves to the fourth pair of leaves, because the first pair of leaves was not fully expanded when the treatment started.

Table 6.2 Effect of two weeks of shading treatments applied at the one leaf-pair growth stage on area of leaf pairs on the main stem

Position on the main stem	Daily light integral (moles m^{-2} d^{-1})				SED	P
	24.9	13.5	11.3	5.4		
1st leaf-pair (basal)	31.5	35.3	33.7	27.8	2.494	0.034*
2nd leaf-pair	62.3	63.1	60.2	43.1	4.430	<0.001***
3rd leaf-pair	55.1	52.2	44.4	22.4	4.450	<0.001***
4th leaf-pair (top)	27.2	27.8	23.8	2.7	3.03	<0.001***

When treatments were applied at the three leaf-pair growth stage, the third pair of leaves was not fully expanded so, as expected, differences between the third, fourth and fifth pairs of leaves were significant (Table 6.3).

Table 6.3 Effect of two weeks of shading treatments applied at the three leaf-pair growth stage on area of individual leaves on the main stem

Leaves on the main	Daily light integral (moles m^{-2} d^{-1})				SED	P
stem	24.9	13.5	11.3	5.4		
2nd leaf-pair (basal)	81.0	80.4	80.1	69.6	6.29	0.231
3rd leaf-pair	97.4	93.0	91.4	70.6	5.95	<0.001***
4th leaf-pair	77.5	78.9	73.6	44.8	6.61	<0.001***
5th leaf-pair (top)	40.4	43.0	33.2	14.3	5.43	<0.001***

6.3.1.5 Axillary shoots

The number of axillary shoots increased (P<0.001) with increases in the daily light integral (Figure 6.8). The number ranged from 0 to 2.5 when treatments were applied at the one leaf-pair growth stage and from 2.3 to 7.0 when treatments were applied at the three leaf-pair growth stage. After two weeks of shading treatments, shoot numbers were significantly lower in plants under 75% shade compared with plants without shade, and there was more than threefold difference when treatments were applied at the three leaf-pair growth stage. Differences between control, however, (no shade), 25 and 50% shading treatments were not significant. Overall, it appeared that the axillary shoots only decreased after the plants had three pair of leaves.

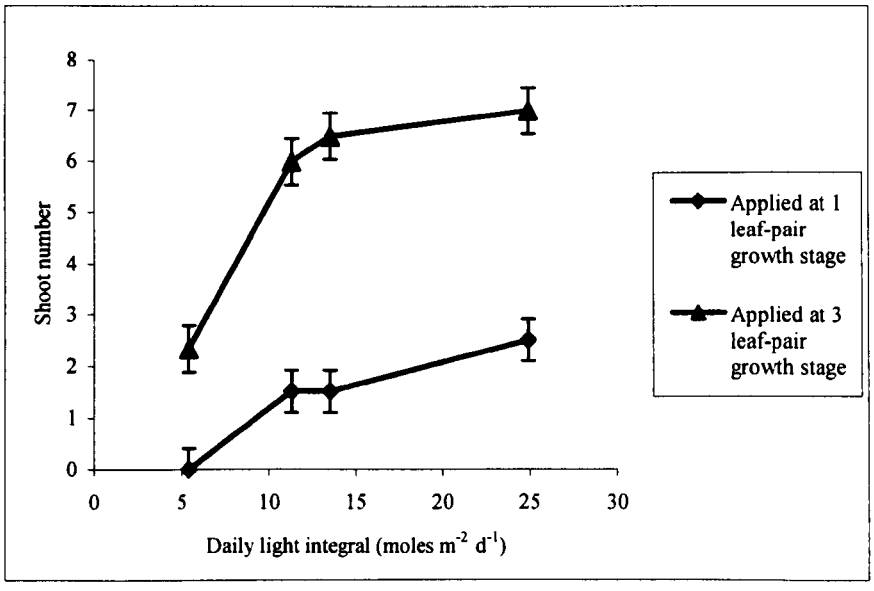

Figure 6.8 Numbers of axillary shoots after two weeks of shading treatments applied at the one and three leaf-pair growth stages

6.3.1.6 Specific leaf area

Specific leaf area was calculated by dividing total leaf area by total leaf dry weight. For treatments applied at the one leaf-pair growth stage, mean specific leaf area decreased from 731 cm^2 g^{-1} with 75% shading to 351 cm^2 g^{-1} with no shading ($p<0.001$) (Figure 6.9). However, there was no difference between 25% shading and no shading, and between 25% and 50% shading.

For treatments applied at the three leaf-pair growth stage, mean specific leaf area decreased from 533.7 cm^2 g^{-1} with 75% shading to 363.5 cm^2 g^{-1} with no shading ($p<0.001$). The trend was the same as with the treatments applied at the one leaf-pair growth stage, but the change was less. Although plants were at different growth stages, specific leaf area with no shading was very similar, however, there was a big difference under 75% shading. This suggests that, in terms of specific leaf area, young plants were more sensitive to daily light integral than older plants.

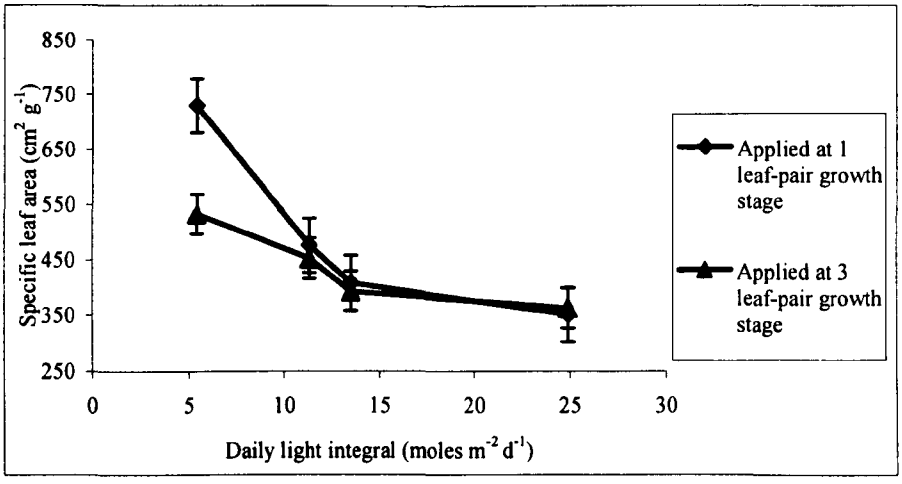

Figure 6.9 Specific leaf areas after two weeks of shading treatments applied at the one and three leaf-pair growth stages

6.3.2 Photosynthesis rate

Photosynthesis was strongly reduced by shading ($p<0.001$), with the CO_2 assimilation rate ranging from 15.1 to 37.7 μmoles m^{-2} s^{-1} between 75% shading and no shading respectively (Figure 6.10). There was no significant difference, however, between the 118.7 (25% shading) and 138.7 μmoles m^{-2} s^{-1} (no shading). The CO_2 assimilation rate increased greatly with increases in irradiance from the lowest level, but this increase rate declined at higher levels of irradiance.

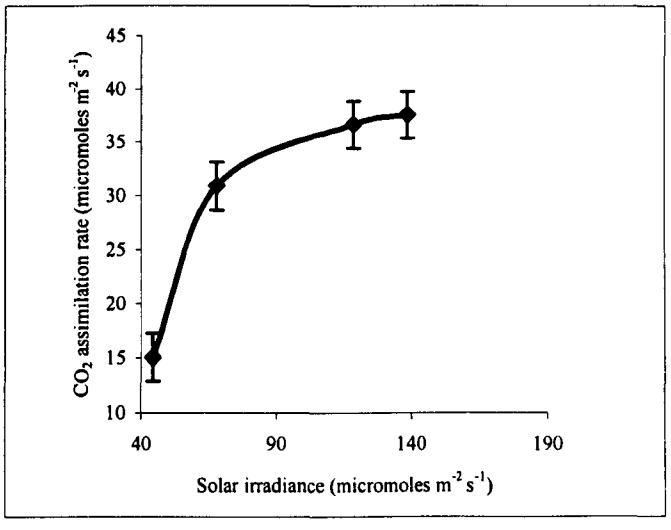

Figure 6.10 Photosynthesis rate under different solar irradiance

6.3.3 Effect on leaf temperature

With increase in irradiance and air temperature, leaf temperature was increased and was higher than the air temperature.

Since the highest air temperatures in the glasshouse on 16 and 22 April 2003 were less than 30°C, the trend in changes in leaf temperature under different shadings were the same as for the data collected on 15 July 2003 before 2.00pm. Likewise, the trend for leaf temperature at different times and under different shadings on 9 July 2003 was the same as that on 15 July 2003. Thus, the data from 15 July 2003 is presented here.

The mean air temperature was 19.4°C in the morning (from 9.00 to 10.00am) and leaf temperature was measured at 10.00am. There was no significant difference in leaf temperature between shading ($p>0.05$) (Figure 6.11).

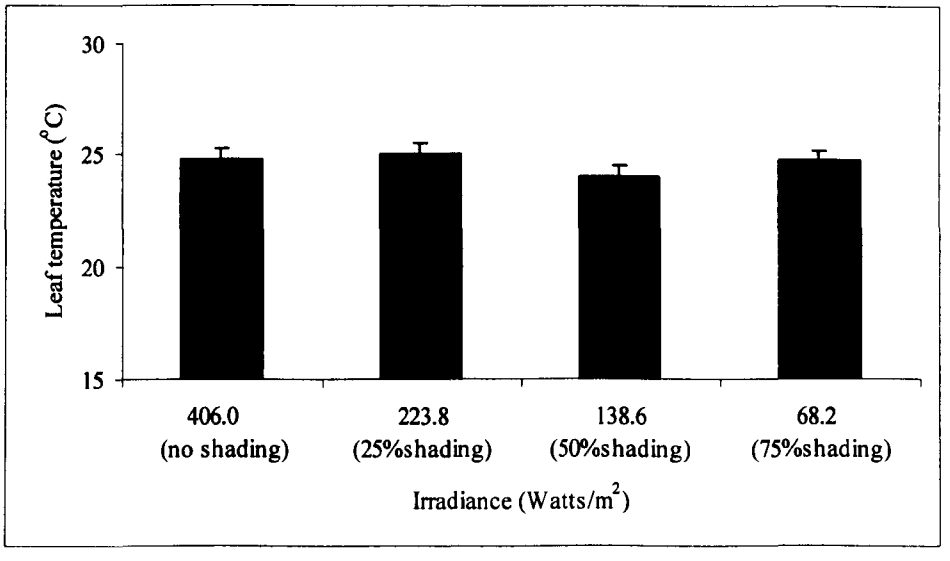

Figure 6.11 Leaf temperatures at 19.4°C air temperature (at 10.00am) under different shading conditions

When leaf temperature was measured at 2.00pm while the mean air temperature was 30.3°C (from 1.00 to 2.00pm), it was observed that, with the irradiance increasing, leaf temperature increased and there were differences between the shading treatments ($p<0.05$) (Figure 6.12).

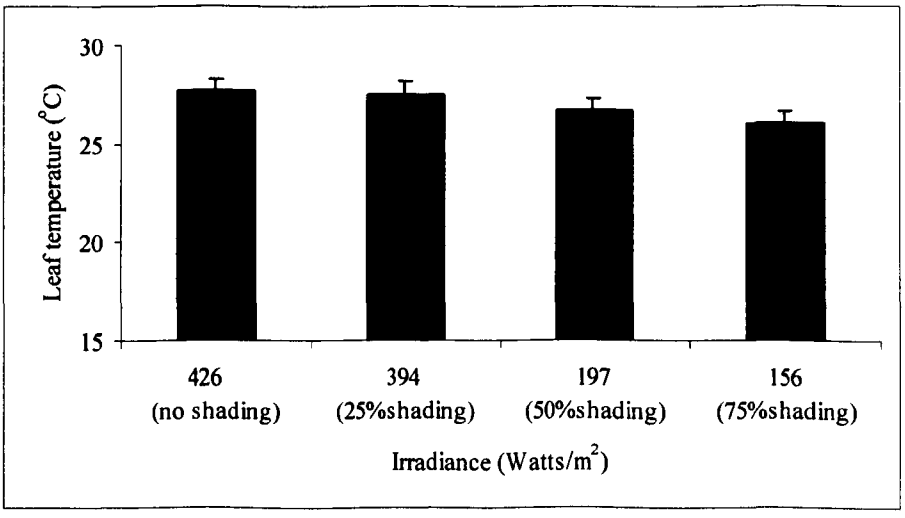

Figure 6.12 Leaf temperatures at 30.3°C air temperature (at 2.00pm) under the different shading treatments

At 4.00pm when the mean air temperature was 36.0°C (from 3.00 to 4.00pm), there was a highly significant difference ($p<0.001$) in leaf temperature under the different shading treatments (Figure 6.13).

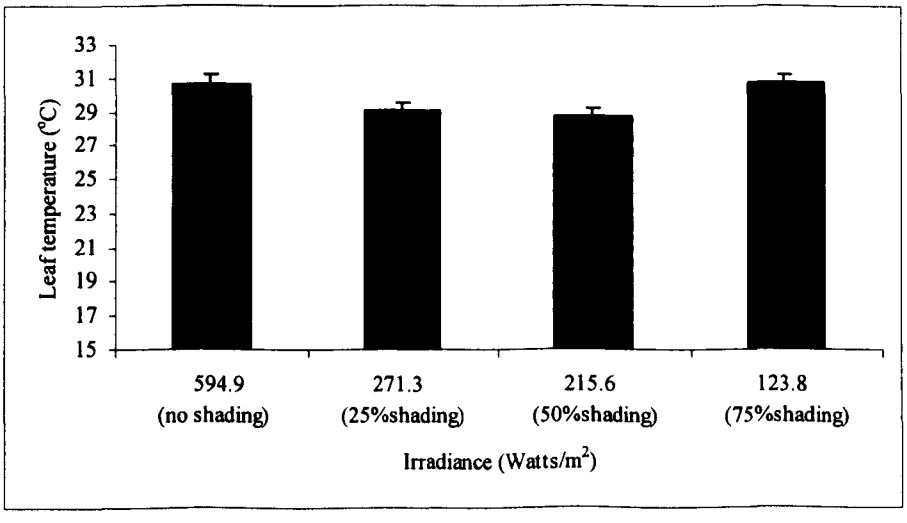

Figure 6.13 Leaf temperatures at 36°C air temperature (at 4.00pm) under the different shading treatments

Compared with leaf temperatures at the 30.3°C air temperature, the trend was different at 36°C air temperature under the different shading treatments. The former data showed that leaf temperature was decreased by shading, and the greater the shading, the more the temperature was decreased. But at 36°C air temperature, 75% shading increased leaf temperature with significant differences compared with 25% and 50% shading. There was no significant difference in leaf temperature between 75% shading and no shading.

At lower air temperature, leaf temperature was higher than air temperature but at higher air temperature, leaf temperature was lower than air temperature, although leaf temperature was increased with increase in air temperature. Under some higher air temperature conditions (< 30°C), shading effectively reduced leaf temperature, but at even higher air temperatures (36°C), heavy shading could not reduce leaf temperature.

6.3.4 Volatile compounds

Shading of basil plants led to reduction in the oil content and changes in oil composition in the leaves.

6.3.4.1 Volatile oil content

The content of total volatile oils in 5 g of fresh leaves was strongly affected by shading of the plants and differences between treatments applied at both the one and three leaf-pair growth stages were significant ($p<0.001$). In treatments applied at the three leaf-pair growth stage, there was a fivefold reduction in volatile oil content with the 5.4 moles m^{-2} d^{-1} daily light integral (75% shading) compared with the 24.9 moles m^{-2} d^{-1} daily light integral (no shading) (Figure 6.14). Treatments applied at the one leaf-pair growth stage showed no significant difference between 13.5 (25%

shading) and 24.9 moles m^{-2} d^{-1} daily light integrals (no shading). This suggests that there was no effect of slight shading on oil content in basil leaves during the youngest plant growth stage used with these daily light levels; in other words, the oil content in older plants was more sensitive to irradiance than in young plants.

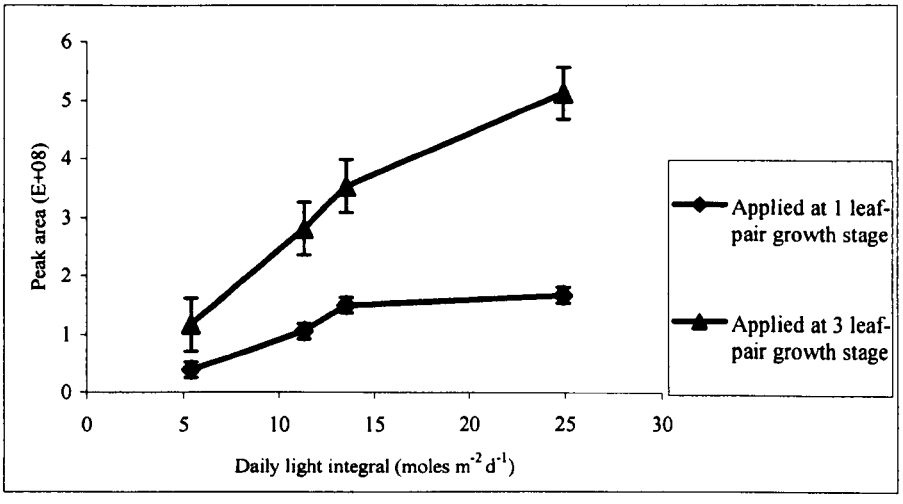

Figure 6.14 Total volatile oil content in 5 g fresh leaf samples after two weeks of shading treatments applied at the one and three leaf-pair growth stages

6.3.4.2 Volatile oil composition

There were thirty six individual chemical compounds (Appendix 3) identified in the basil leaf samples. The principal phenylpropanoids detected were eugenol and methyl eugenol, while the major terpenoids were 1,8-cineole, linalool, cis-ocimene and trans-α-bergamotene. Some small amounts of α- pinene, β-pinene, myrecene and camphor were also detected.

When plants were treated at the one leaf-pair growth stage, shading led to a reduction in the relative contents of linalool and eugenol but methyl eugenol was markedly increased. Linalool was reduced by a factor of four by 75% shading compared with no shading ($p=0.007$), and eugenol by a factor of three ($p=0.033$). Differences with 25% and 50% shading were not significant. The biggest treatment effect was observed with methyl eugenol ($p<0.001$), with sixfold enhancement by 75% shading compared with no shading (Figure 6.15).

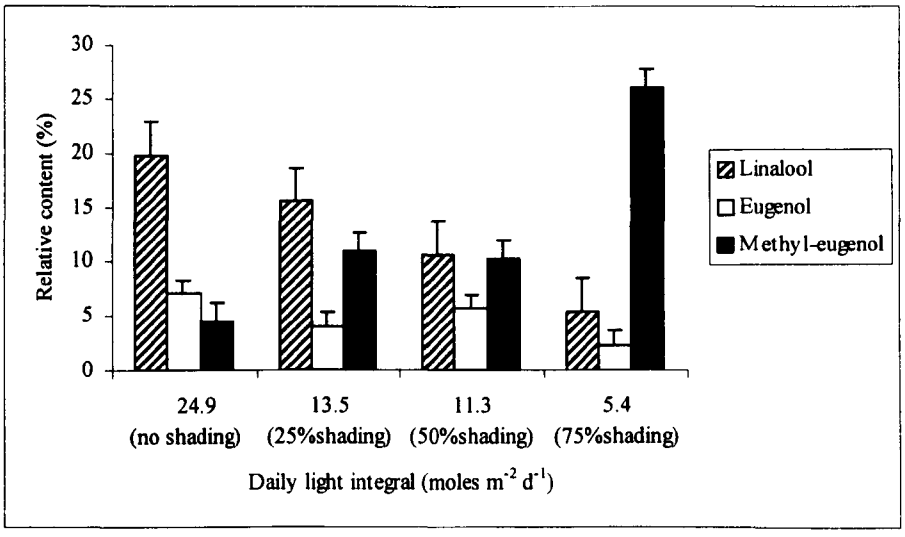

Figure 6.15 Relative content of selected volatile compounds after two weeks of shading treatments applied at the one leaf-pair growth stage

For treatments applied at the three leaf-pair growth stage, differences in the relative contents of both linalool and eugenol with 25%, 50% and 75% shading were not significant, however, with no shading these was an increase ($p<0.05$) (Figure 6.16). Methyl eugenol was again enhanced by shading ($p<0.001$), increasing more than fivefold from 0.34% with no shading to 1.89% with 75% shading. With plants at this growth stage, however, the relative content of methyl eugenol was significantly less compared to plants at the one leaf-pair growth stage.

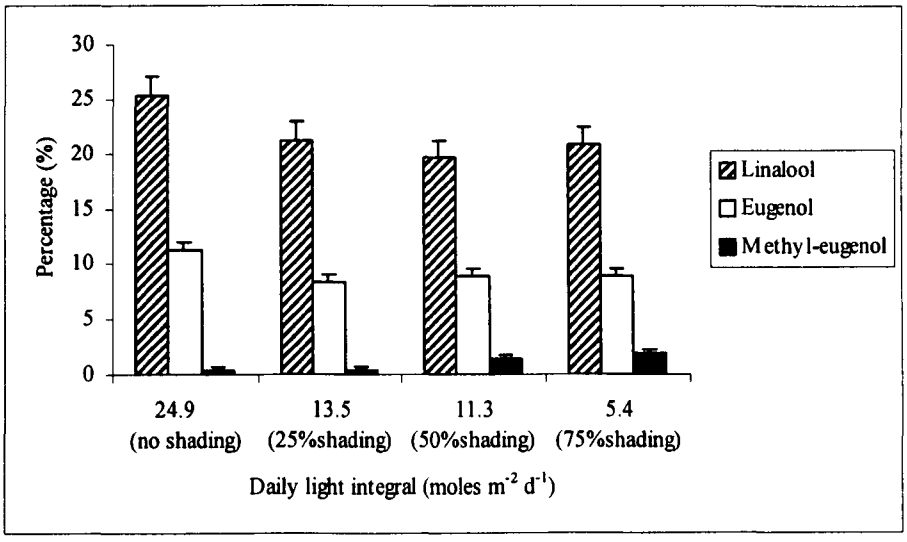

Figure 6.16 Relative content of selected volatile compounds after two weeks of shading treatments applied at the three leaf-pair growth stage

These results showed that shading of basil plants strongly affected the volatile oil composition and that young plants contained a higher relative content of methyl eugenol. In addition, plants at the younger of the two growth stages tested were more sensitive to different shading conditions.

6.3.4.2 Yield of selected chemical compounds

Using peak area and calibration curves for selected individual chemicals (described in Chapter 3), the yields of the three main chemicals (1,8-cineole, linalool and eugenol) were calculated.

Although differences in the relative content of 1,8-cineole in basil leaves when treatments were applied at the one leaf-pair growth stage were not significant, the quantity of 1,8-cineole in 5 g fresh leaves was strongly decreased by shading ($p<0.001$), ranging from 6.48 μg with 75% shading to 46.19 μg with no shading, i.e. a sevenfold difference (Figure 6.17).

For linalool, when treatments were applied at the one leaf-pair of growth stage, due to the differences in relative content combined with the differences in total content, differences between the shading treatments were significant ($p<0.001$), ranging from 1.24 μg with 75% shading to 40.53 μg with no shading, i.e. a factor of thirty-three times. Differences between 25% and 50% shading, however, were not significant (Figure 6.17).

The yield of eugenol from 5 g fresh leaves increased by a factor of nine from 0.93 μg, with 75% shading to 8.34 μg with no shading (figure 6.17). Differences between the shading treatments applied at the one leaf-pair growth stage were highly significant.

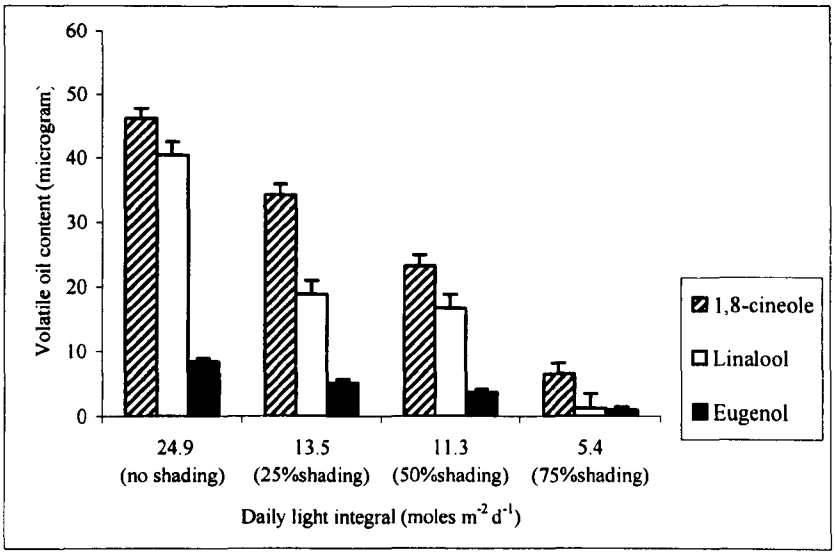

Figure 6.17 Selected volatile oil content after two weeks of shading treatments applied at the one leaf-pair growth stage

When shading treatments were applied at the three leaf-pair growth stage, a similar less marked trend was observed as when treatments were applied at the one leaf-pair growth stage, however the yields of the three oils were much higher (Figure 6.18).

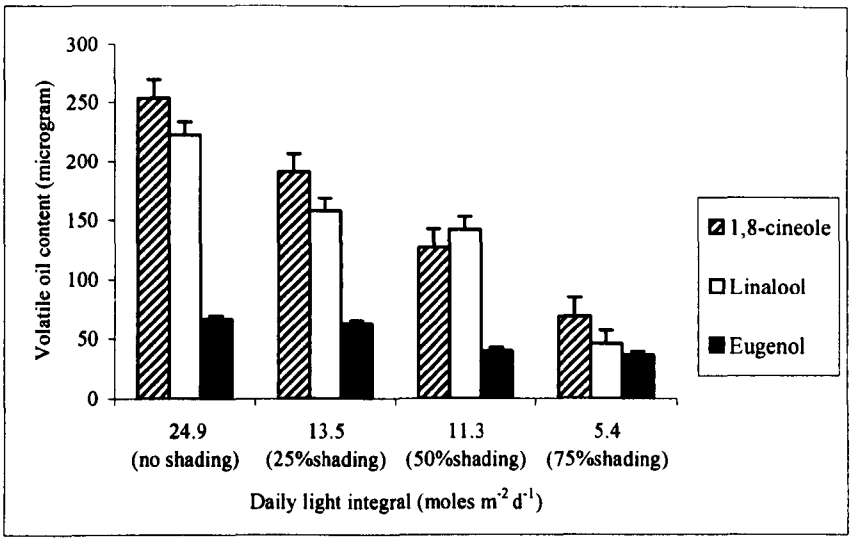

Figure 6.18 Selected volatile oil content after two weeks of shading treatments applied at the three leaf-pair growth stage

6.4 Discussion

6.4.1 Growth

Plants are affected in a complex manner by irradiance at all stages in their life, and the growth made by a plant is dependent on the quantity, duration and quality of light (Nilsen and Orcutt, 1996). Environmental factors influence photosynthesis and thereby inhibit or stimulate growth. Light is the driving force for production (Lawlor, 1993).

No significant differences in plant growth parameters were found in basil plants grown under daily light integrals from 13.5 (25% shading) to 24.9 moles m^{-2} d^{-1} (no shading). Although the former is only half of the latter, there were no significant differences between them in plant height, weight, shoot number, leaf weight and leaf area. This may be explained by the basil plants under slight shading (25% shading) possessing a greater conversion efficiency of light utilization for increasing plant size and dry matter. This is supported by previous reports for cineraria (Yeh, 1996) and strawberry (Wright and Sandrang, 1995). Commonly, dry matter accumulated by annual plants increases linearly with the amount of solar radiation intercepted, but shade-plants have a higher quantum efficiency due to a decrease in the ratio of the rates of respiration and photosynthesis (Yeh, 1996).

Shading at 50% and 75% strongly reduced plant size and weight, both in the young and older plants. As expected, plant height, dry matter and leaf area increased with increasing irradiance between 5.4 (75% shading), 11.3 (50% shading) and 13.5 (25% shading) moles m^{-2} d^{-1} daily light integrals. In general, plant responses to light include a variety of photosynthetic and biochemical adaptations that are translated into alterations of plant growth and architecture (Peralta *et al.*, 2002). Also, reductions in irradiance lead to a reduction in the rate of photosynthesis and therefore a reduction in the rate of growth (Corree, 1983).

This result suggested that, although basil grows well in sunny conditions (Putievsky and Galambosi, 1999), it can tolerate light but not heavy shading. Specific leaf area decreased as daily light integral increased up to 13.5 moles m^{-2} d^{-1}, and thereafter remained fairly constant. This could be characterised as a sun-adaptation feature. The reduced thickness of the leaves in shaded conditions may be associated with smaller cell volume. The present results support the finishing of Yeh (1996) who studied cineraria plants.

6.4.2 Photosynthesis rate

As expected, photosynthesis in the basil plants increased with increasing irradiance. The initial part of the light response curve is linear because light is the dominant limiting factor. On reaching light saturation, the curve becomes horizontal because resources other than light become limiting (Nilsen and Orcutt, 1996). In present study, there was no significant difference between the 118.7 (25% shading) and 138.7 μmoles m^{-2} s^{-1} (no shading), suggesting a saturated photosynthesis rate response at these light levels.

6.4.3 Leaf temperature

Leaf temperature is regulated primarily by the influence of net radiation, latent heat exchange, conduction and convection and air temperature (Nilsen and Orcutt, 1996). Any limitation of free convection reduces plant growth by limiting heat and gas exchanges between plant leaves and the ambient air (Kitaya *et al.*, 2001).

In this study, when the air temperature was 19.4°C at 10.00am, the leaf temperature exceeded the air temperature by as much as 4.6 to 5.7°C because, during periods of intense irradiance and/or high tissue irradiance absorption, tissue temperature can increase dramatically compared with the air temperature (Nilsen and Orcutt, 1996). With the air temperature increasing to 30.3°C by 2.00pm, leaf temperature was

effectively reduced by shading due to irradiance reduction.

When the air temperature was 36°C, 25% and 50% shading significantly reduced leaf temperature compared with no shading, but heavy shading (75%) resulted in increased leaf temperature. During this period, with the irradiance increasing, leaf temperature was increased, and light shading reduced leaf temperature through its effect on light reduction as stated above. Heavy shading (75%), however, restricted air movement due to the less porous shading net around the plants, with a consequent decrease in the conductance of heat and gases in the leaf boundary layer resulting in higher leaf temperature. Such critical air temperature conditions, however, are not common during the growing season in England. The plants under 75% shading made less growth due to the reduced irradiance and also due to the restricted free air convection retarding heat and gas exchanges between leaves and the ambient air. This is supported by the finding for sweet potato and barley (Kitaya *et al.*, 2001).

6.4.4 Volatile oils

Light is a key factor in the ultimate production of many compounds because it supplies the energy needed to fix carbon. Light intensity plays an important role in the biosynthesis of medicinally important metabolites, for example camptothecin (CPT), an anti-cancer agent in the tree of joy (*Camptotheca accuminata* L.), is produced at higher levels in shade conditons (Kaufman *et al.*, 1999). There is no publication, however, on how light intensity affects volatile oils in basil plants.

In the present study, total volatile oil content was strongly reduced by shading of the plants, especially heavy shading (75%). But the young plants were less sensitive to shading because there was no difference in the volatile oil content between 13.5 and 24.9 moles m^{-2} d^{-1} daily light integrals in treatments applied at the one leaf-pair growth stage. But there was a highly significant difference between treatments that were applied at the three leaf-pair growth stage.

The composition of volatile oils was strongly affected by light intensity. There were higher relative contents of linalool and eugenol under higher daily light integrals, and higher shading resulted in more methyleugenol. Phenylalanine is the precursor for both eugenol and methyleugenol and, under certain conditions, the methylation of eugenol to methyleugenol is catalysed by eugenol *O*-methyl transferase (EOMT) which has been successfully obtained from basil leaves (Gang *et al.*, 2001). In the present study, lower irradiance may have stimulated the activity of EOMT and resulted in the methylation of eugenol to produce more methyleugenol, but this requires further investigation.

Linalool is a common acyclic monoterpenoid compound, that can be synthesized from α-pinene or β-pinene (Bauer *et al.*, 2001). In plants, the enzyme linalool synthase (LIS), catalyzes geranyl pyrophosphate (GPP) directly to linalool (Kaufman *et al.*, 1999). In addition, GPP can be catalysed by 1,8-cineole synthase to 1,8-cineole (Gang *et al.*, 2001; Kaufman *et al.*, 1999) and α-terpineol (Gang *et al.*, 2001), catalysed by monoterpene cyclase to pinenes, 3-carene and limonene (Kaufman *et al.*, 1999). In the present study, there were no differences in the relative contents of α-pinene, β-pinene, 3-carene and 1,8-cineole under different shading treatments. Significant differences were only found for linalool, suggesting that higher light intensity may have stimulated the activity of linalool synthase thereby increasing the relative content of linalool. Some other chemicals maintained a relatively stable level under different conditions, because there were no differences observed in the relative contents of pinenes, 3-carene and limonene with different temperatures (Chapter 5) and supplementary UV-B or red light (Chapter 7).

The highest dry matter and volatile oil content were obtained under the highest daily light integrals, which suggests that, as secondary metabolites, volatile oil synthesis has a very close relationship with primary metabolism, i.e. the more the photosynthates produced, the more secondary metabolites accumulated. Precursors

usually derive from basic metabolic pathways (Wink, 1999). Both phenylalanine and GPP are derived from sucrose catalysed by a series of enzymes, such as sucrose synthase, glucose-6-phosphate dehydrogenase, geranyl pyrophosphate synthase and shikimate kinase (Gang *et al.*, 2001). (Also see the proposed network of metabolic pathways leading from D-glucose to three major volatile oil compounds in Appendix 4).

6.5 Conclusions

- ✧ Heavy shading (75%) resulted in shorter plants, lower weight, smaller leaf area, less shoots and higher specific leaf area. Light shading (25%) had no effect on plant size and weight.
- ✧ Heavy shading (75% and 50%) strongly reduced photosynthesis rate due to irradiance reduction. There was no difference in CO_2 assimilation rate between no shading and 25% shading.
- ✧ Shading effectively reduced leaf temperature due to solar irradiance reduction when air temperature was less than 30°C.
- ✧ Heavy shading (75%) could not reduce leaf temperature when air temperature was above 36°C due to limitation of free air convection. Consequently, leaf temperature increased.
- ✧ Heavy shading strongly reduced total volatile oil content in fresh leaves. The young plants were less sensitive to shading than the older plants.
- ✧ Thirty six chemical compounds were identified in basil leaves and, of these, linalool, eugenol and methyleugenol were present in significant relative amounts. The relative contents of linalool and eugenol were significantly increased by higher daily light integrals, whereas methyleugenol was increased by lower daily light integrals. No differences, however, in the relative content of 1,8-cineole were observed.

Chapter 7 SUPPLEMENTARY UV-B AND R/FR RATIO

Understanding the relationships between crop and environment has substantially improved during the last few decades of the 20th century (Kakani *et al.*, 2003), with light as a key factor in plant biosynthesis (Kaufman *et al.*, 1999) and controlling plant growth and development (Salisbury and Ross, 1992). Light quality, the ratio of red (R) to far-red (FR) light (i.e. R/FR), is believed to alter plant morphogenesis (Robin *et al.*, 1994), and shortwave irradiance (UV range) has a dramatic effect on the physiology, morphology and growth of plants (Nilsen and Orcutt, 1996). In this chapter, the effects of supplementary UV-B light and red light on basil plants are reported.

7.1 Supplementary UV-B light

Due to the decreasing trends in stratospheric ozone concentration, ultraviolet-B (UV-B) radiation has increased at the surface of the earth, which has led to much research on the effects of enhanced UV-B radiation on some changes in plants (Karousou *et al.*, 1998). In general, UV-B can reduce plant growth and yield (Barnes *et al.*, 1988), reduce plant height and leaf area, increase tillering, change plant geometry (Barnes *et al.*, 1988), reduce photosynthesis and increase plant secondary metabolites (Caldwell *et al.*, 1989; Renger *et al.*, 1989; Teramura and Sullivan, 1994).

There is little known on the effects of UV-B on the composition of volatile oils in sweet basil. Ioannidis *et al.* (2002) studied the effects of UV-B on the composition of essential oils and found no significant differences in the qualitative or quantitative composition. In contrast, Johnson *et al.* (1999) reported that UV-B light enhanced the levels of most of the major volatile oils in sweet basil, both phenyl-propanoids and terpenoids. In the two UV-B light experiments reported in the literature, UV-B dose was approximately equivalent to the normal daily dose on a summer's day in the Mediterranean for 1.0 h (Ioannidis *et al.*, 2002) and 1.5 h (Johnson *et al.*, 1999) per day for up to two weeks.

The present study determined the effects of UV-B light on the volatile compounds and plant growth in basil using the same supplementary UV-B light source as in the above two references, but supplemented 3 h each day for up to two weeks. It was hypothesized that supplementary UV-B light would reduce plant size and weight but increase the volatile oil content in leaves.

7.1.1 Methods and materials

7.1.1.1 Plant materials

Plants of basil cv Basil Sweet Genovese were raised from seeds and grown as described in chapter 3 prior to applying the UV-B treatment.

7.1.1.2 UV-B light treatments

Plants for supplementary UV-B irradiation and control plants were placed on benches in controlled environment rooms at constant temperature 25±1°C and 16 h daytime (between 04:00 and 20:00) photoperiod. The UV-B light was provided by two Philips 20 W/12 UV-B fluorescent tubes placed 1 m apart and 1 m above the bench for each treatment (Johnson *et al.*, 1999; Ioannidis *et al.*, 2002) (Plate 7.1).

Plate 7.1 Supplementary UV-B sources in a growth room

Two treatments were given, i.e. control (no supplementary UV-B light) and 3 h (between 04:00 and 07:00) supplementary UV-B light each day. Two weeks of treatments were applied to plants at the three leaf-pair and four leaf-pair growth

stages. The UV-B treatment commenced on 9 July 2003 and was repeated in a second experiment on 14 April 2004. Care was taken to ensure that there was no difference in PAR between treatments. PAR values were 444±25 and 450±25 μmol m^{-2} s^{-1} in the supplementary UV-B treatment room and control room respectively. The actual UV-B densities were 222.6 (supplementary UV-B) and 42.4 μW/m^2 (control).

7.1.1.3 Plant growth parameters

Plant height, number of leaf pairs on the main stem, number of axillary shoots, leaf area, plant fresh weight, leaf fresh weight, plant dry weight, leaf dry weight and specific leaf area were measured and calculated after two weeks of UV-B treatments as described in Chapter 3.

7.1.1.4 Sample preparation and compound identification

Extraction of volatile oils by Sorbent Tube Trapping and their analysis by TD-GC/MS was used. Volatile oil content and composition were analysed at one week and two weeks after supplementary UV-B treatments. Fresh leaf samples (5 g) were cut from the fifth pair of leaves immediately prior to being homogenized in a modified blender in order to avoid losses of the volatile oils. There were three replicate analyses carried out for each treatment.

7.1.2 Results

7.1.2.1 Plant growth parameters

Following two weeks of UV-B treatment, there were differences in plant height, specific leaf area, plant and leaf dry weights and number of axillary shoots. There were no significant differences, however, in plant leaf area and number of leaf pairs on the main stem between treatments.

Plant height was reduced by approximately 3 cm by supplementary UV-B light

applied at both the three and four leaf-pair growth stages ($p<0.05$) (Figure 7.1 & Plate 7.2).

Plate 7.2 Plant height after UV-B treatment for two weeks applied at the four leaf-pair growth stage; supplementary UV-B (left two) and control (right two)

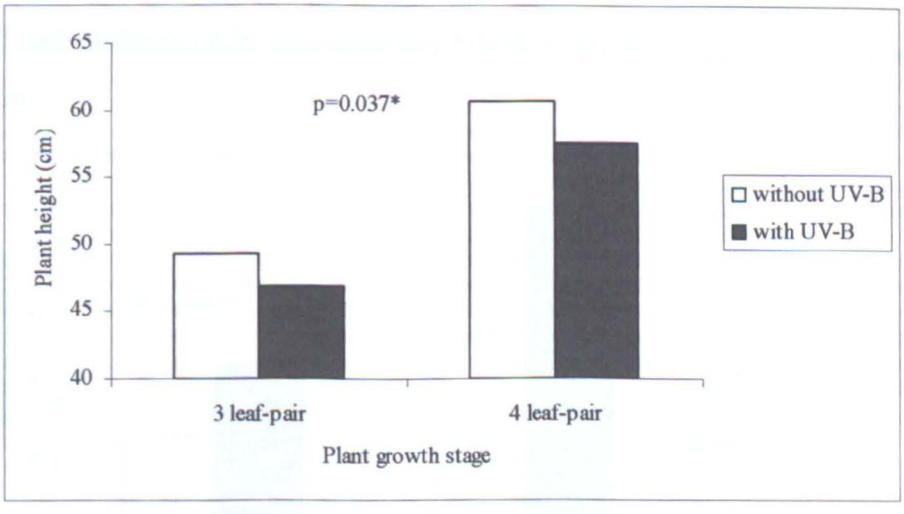

Figure 7.1 Plant height after two weeks of supplementary UV-B treatment applied at the three and four leaf-pair growth stages

Although differences in plant leaf area were not significant, specific leaf areas were significantly reduced ($p<0.005$) by UV-B treatment (Figure 7.2). In effect, the UV-B

treatment increased the thickness of basil leaves.

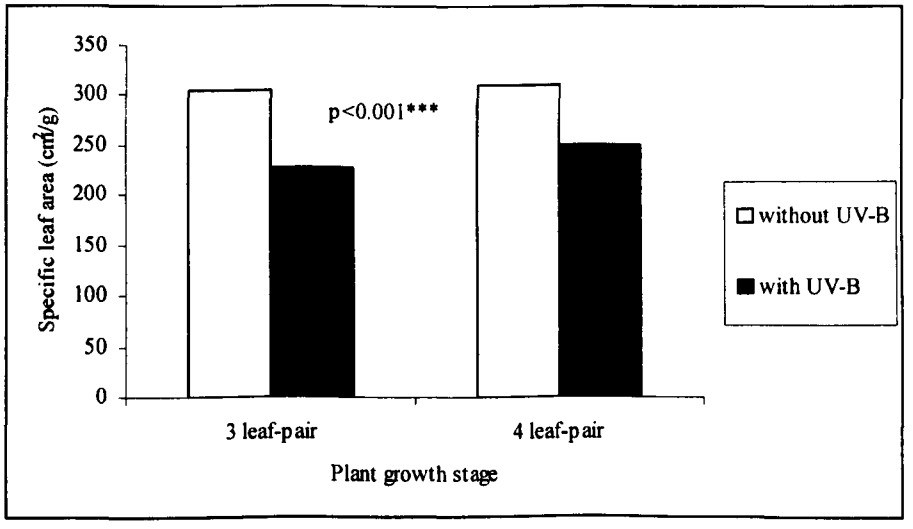

Figure 7.2 Specific leaf areas after two weeks of UV-B treatment applied at the three and four leaf-pair growth stages

Plant and leaf dry weights were significantly increased by two weeks of UV-B treatment applied at both the three and four leaf-pair growth stages (Figures 7.3 & 7.4). Although there was no significant difference in plant mean leaf area, the increased leaf thickness under supplementary UV-B conditions resulted in higher leaf dry weight.

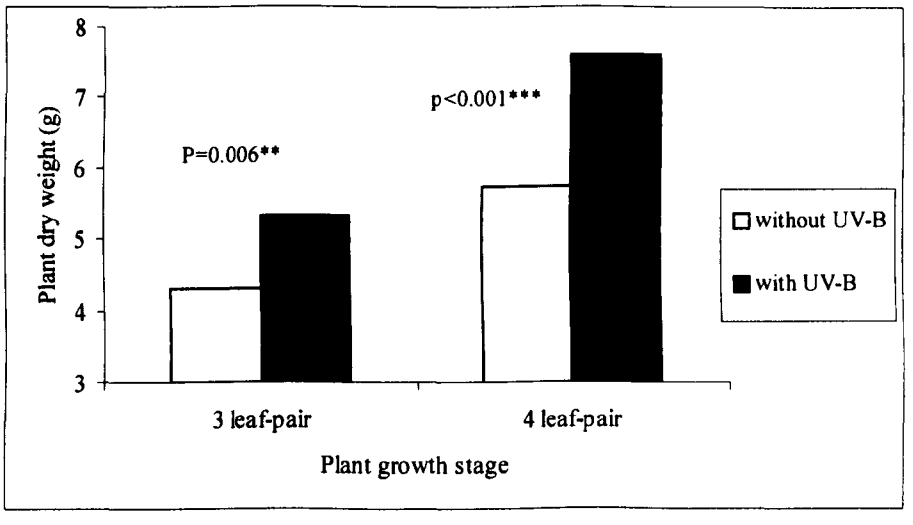

Figure 7.3 Plant dry weight after two weeks of UV-B treatment applied at the three and four leaf-pair growth stages

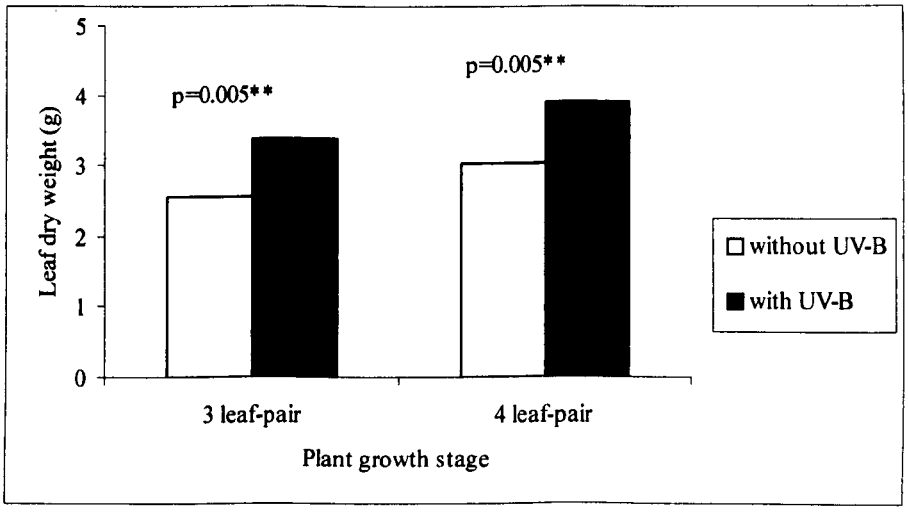

Figure 7.4 Leaf dry weight after two weeks of UV-B treatment applied at the three and four leaf-pair growth stages

When supplementary UV-B was applied at the four leaf-pair growth stage, the plants produced two more axillary shoots than the control plants (p=0.008). When applied at the three leaf-pair growth stage, in the first experiment there was no difference between the treatments (Figure 7.5), however, when the experiment was repeated, 1.2 more axillary shoots were produced by the UV-B treatment and this difference was significant.

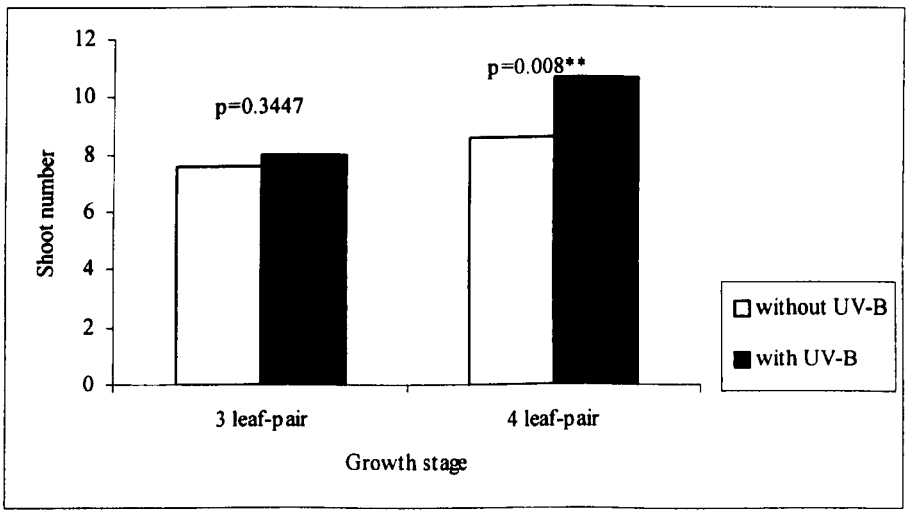

Figure 7.5 Shoot numbers after two weeks of UV-B treatment applied at the three and four leaf-pair growth stages

7.1.2.2 Volatile oils

Thirty six chemical compounds were identified and most of them were terpenoids, e.g. 1,8-cineole, linalool, pinenes and camphor, and the phenyl-propanoid, eugenol.

Comparing the integration peaks, there were significant differences in total volatile oil content between treatments when plants were applied at the three leaf-pair and four leaf-pair growth stages (Figures 7.6 & 7.7). Overall, supplementary UV-B light effectively increased the total volatile oil content of basil leaves.

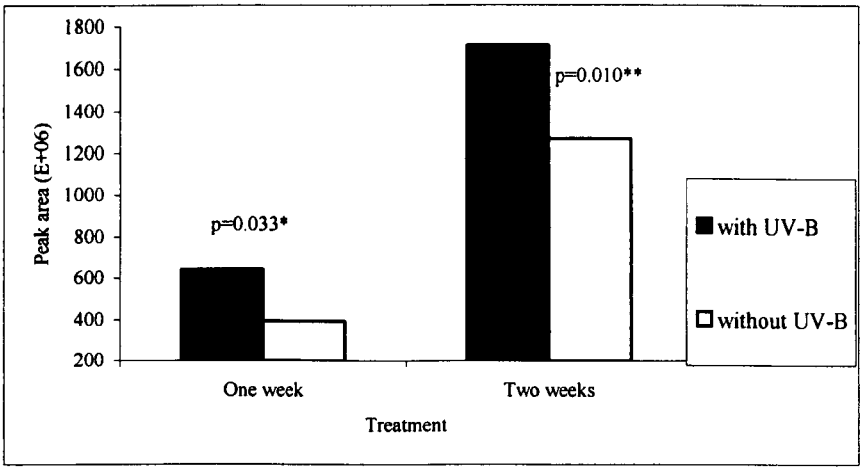

Figure 7.6 Total peak area after one and two weeks of UV-B treatment applied at the three leaf-pair growth stage

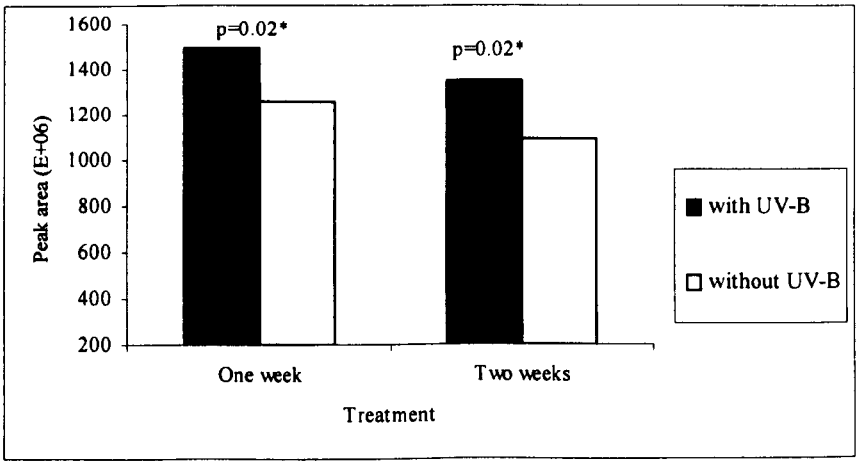

Figure 7.7 Total peak area after one and two weeks of UV-B treatment applied at the four leaf-pair growth stage

Differences in the relative content of the thirty six individual chemical compounds between the supplementary UV-B light and control treatments, both in plants treated at the three and four leaf-pair growth stages, were not significant. There were differences, however, in the yield of three major volatile oils, i.e. 1,8-cineole, linalool and eugenol, with the same trend observed in plants treated at both growth stages and for both durations. Supplementary UV-B enhanced the levels of these major volatiles significantly (Figures 7.8 & 7.9), in line with the increase in the total content of volatile oils.

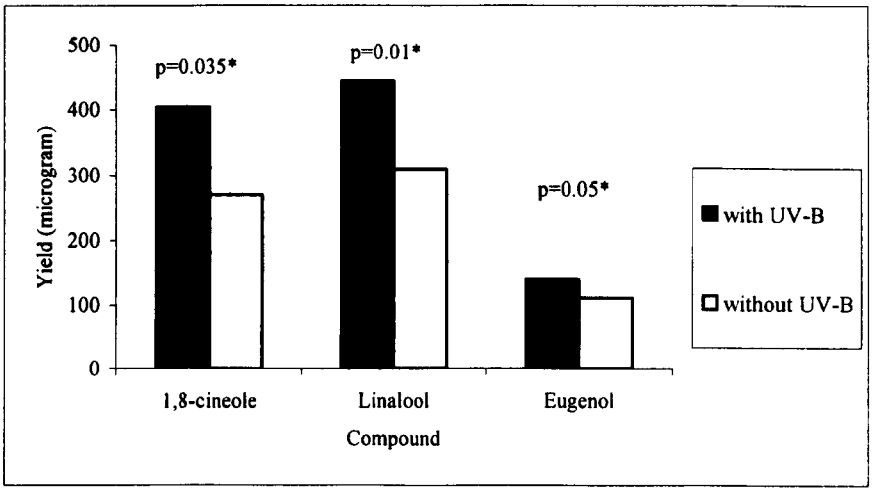

Figure 7.8 Yield of selected volatile compounds in 5 g fresh leaves following two weeks of treatment applied at the three leaf-pair growth stage

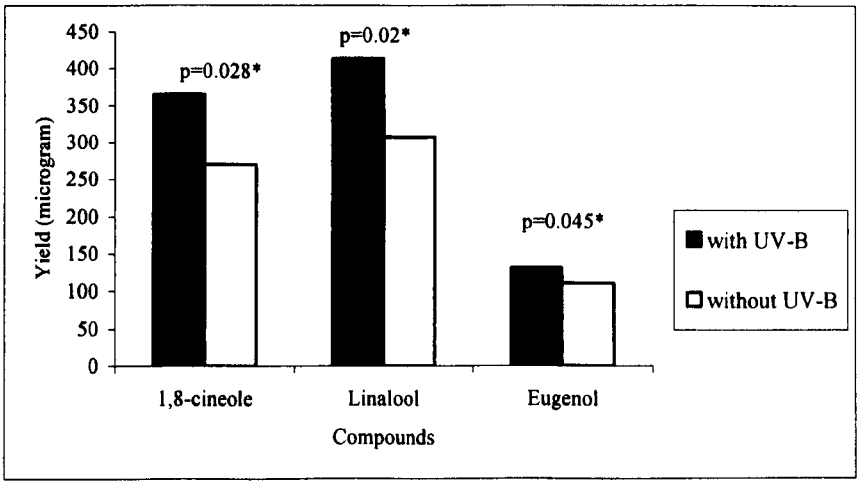

Figure 7.9 Yield of selected volatile compounds in 5 g fresh leaves following two weeks of treatment applied at the four leaf-pair growth stage

7.1.3 Discussion

Enhanced UV-B radiation reduced elongation of the main stem, resulting in more compact plants, possibly due to changes in the phytohormones, especially IAA that plays a role in stem elongation in sunflower (Mark and Tevini, 1996). In the present study, mean plant height was effectively reduced by supplementary UV-B light and this supports previous reports for other plants, such as peppermint (Maffei and Scannerini, 2000), corn, cotton, pea, and rice (Kakani *et al.*, 2003).

There is no published information to confirm the present finding that UV-B causes an increase in number of shoots. This could be explained by the elimination of apical dominance due to the decrease in IAA concentration in the apex of the main stem, since some studies have indicated a breakdown of IAA on exposure to UV-B radiation (Ros and Tevini, 1995; Huang *et al.*, 1993). This could result in a shorter main stem (Kakani *et al.*, 2003), and stimulation of the growth of lateral shoots.

During the past 18 years, there have been 40 studies using 23 crop species to reveal the effect of UV-B light on plant dry weight (Kakani *et al.*, 2003), but there is no information for basil. Only 5% of the studies demonstrated an increase in dry matter accumulation under supplementary UV-B. Approximately one third of studies reported no effect on dry weight and more than 50% reported a reduction in dry matter. The differences were probably associated with crop species, genotypes and UV-B doses (Kakani *et al.*, 2003). The present study has shown that supplementary UV-B light can increase dry matter of basil following two weeks of treatment applied at the three and four leaf-pair growth stages. This was probably because the basil plants were more tolerant to UV-B damage than other species, or the UV-B dose used was not strong enough to reduce dry matter accumulation. Kakani *et al.* (2003) showed that crop biomass production in response to UV-B radiation was highly dependent on UV-B dose and, in the present study, the increase in shoot number would be one of the reasons for the increase in plant dry weight.

Although differences in plant mean leaf area were not significant, highly significant differences were found in leaf dry matter and specific leaf area. This resulted from the increase in leaf thickness due to UV-B radiation, possibly due to the addition of spongy mesopyll cells as Weston *et al.* (2002) stated when they studied the effect of light quality on leaf cell and chloroplast development in *Arabidopsis thaliana.*

One of the mechanisms that plants possess to adapt to enhanced UV-B radiation is to increase production of secondary metabolites in leaf tissues, and most of these metabolites accumulate in the epidermal layer to absorb or screen UV-B radiation and protect the underlying tissues against this harmful radiation (Kakani *et al.*, 2003). To date, there have been two reports on the effects of UV-B on volatile oils in basil plants. Johnson *et al.* (1999) reported that UV-B not only increased total content but also changed the oil composition. In contrast, Ioannidis *et al.* (2002) stated that neither qualitative nor quantitative composition of the volatiles was affected by UV-B. The present study achieved a compromise between these two previous reports, i.e. the total content of volatiles was significantly increased by UV-B but there was no effect on composition. It is possible that the effect of UV-B on volatiles in basil depends very much on genotype, because these three studies used three different genotypes. In the present research, seeds of basil cv Basil Sweet Genovese were obtained from Nicherson-Zwaan Ltd (UK). In the research of Johnson *et al.* (1999), seeds were obtained from Vilmorin (La Verpilliere Cedex, France) and, in the research of Ioannidis *et al.* (2002), seeds were obtained from Franchi Sementi S. P. A. (Bergamo Italy). In all of these studies, UV-B light was supplied by two Philips 20 W/12 UV-B fluorescent tubes placed 1 m apart and 1 m above the bench.

Comparing the three studies, besides using different genotypes, the differences in the time of year and in the duration of the UV-B treatment also could have led to different results. In addition, the two previous studies did not mention temperature

conditions. This could be important because Mark and Tevini (1996) found that temperature alleviates the effects of UV-B when they studied sunflower and corn. Thus, future studies should report genotypes, UV-B dose, as well as abiotic factors, such as water stress, CO_2 concentration, nutrients, light density and temperature.

7.1.4 Conclusions

- ✧ Supplementary UV-B significantly reduced plant height, but increased the number of shoots.
- ✧ Supplementary UV-B did not affect plant leaf area, or number of leaf-pairs on the main stem. Specific leaf area, however, was significantly decreased due to the increase in leaf thickness.
- ✧ Supplementary UV-B strongly increased plant dry matter and leaf dry matter.
- ✧ Supplementary UV-B treatment increased total volatile oil content in fresh leaves, however, there was no effect on the composition of the volatiles.
- ✧ The content of 1,8-cineole, linalool and eugenol in fresh leaves was significantly increased by supplementary UV-B.

7.2 R/FR ratio

Plants depend on light as their key source of energy (Genick and Chory, 2000), and there is evidence that morphological changes in green plants induced by the presence of tall neighbours are triggered by alterations in light quality through a reduction in the red (660nm) to far-red (730nm) ratio of the incident light (Robin *et al.*, 1994). As a plant canopy grows and fills the available space, a reduction in the ratio of R/FR light occurs because FR light is filtered through or reflected by neighbouring vegetation (Morelli and Ruberti, 2002).

Starting in the late 1960s, studies showed that the R/FR ratio could modify the size of shoots and leaf size in tobacco and soybean (Loughrin and Kasperbauer, 2003). To date, there have been no publications on the effect of supplementary red light on basil, however, Loughrin and Kasperbauer (2001, 2003) observed changes in the volatile oils in basil when different coloured mulches (black, red, green, blue, yellow and white) were used. This use of mulches strongly influenced the temperature of the soil surface ranging between 32.3°C (white mulch) and 64.1°C (black mulch). So, it is not possible to determine whether the colour of the mulches or temperature was the main factor affecting the plant growth or volatile compounds.

It was hypothesized that supplementary red light influences the concentration of volatile compounds in basil leaves.

7.2.1 Materials and methods

7.2.1.1 Plant materials

Plants of basil cv Basil Sweet Genovese were raised from seeds and grown as described in chapter 3 prior to applying red light treatments.

7.2.1.2 Red light treatments

Plants for supplementary red light irradiation and control plants were placed on benches in two controlled environment rooms at 25±1°C with 16 h daytime (between 04:00 and 20:00) photoperiod. The red light was provided by two Sylvania GRO-LUX, F36W/GRO-T18 tubes (Germany) (Plate 7.3) placed 1m apart and 1m above the bench. Two treatments were given, i.e. control (no supplementary red light) and 3h (between 04:00 and 07:00) supplementary red light each day. Two weeks of treatments were applied to plants at the two and four leaf-pair growth stages. The red light treatments commenced on 16 May 2003 and were repeated in a second experiment on 3 June 2003.

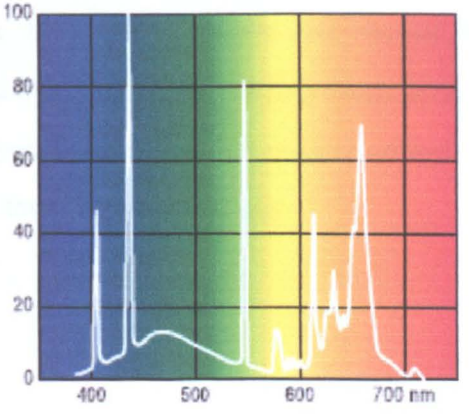

Plate 7.3 Light spectrum of Sylvania GRO-LUX/F36W/GRO-T18 (http://www.sylvania.com)

Care was taken to ensure that there was no difference in PAR between the treatments. PAR values were 444±25 μmol m^{-2} s^{-1} and 450±25 μmol m^{-2} s^{-1} in supplementary red light treatment room and control room respectively.

R/FR ratio was measured using a Skye 660/730 Sensor (Skye light, Skye Instruments LTD, UK). Data was collected at ten replicate points 50 cm above each bench. Although higher than in natural daylight (R/FR = 1.19), the ratios for the red light treatment (2.177) and the control treatment (1.833) were significantly different ($p<0.001$).

7.2.1.3 Plant growth parameters

Plant height, number of leaf-pairs, number of shoots, leaf area, plant fresh weight, leaf fresh weight, plant dry weight, leaf dry weight and specific leaf area were measured and calculated after two weeks of treatments. There were six replicates for each treatment.

7.2.1.4 Sample preparation and compound identification

Extraction of volatile oils by Sorbent Tube Trapping and their analysis by TD-GC/MS was as described in Chapter 3. Volatile oil content and composition were analysed after two weeks of supplementary red light treatments. Fresh leaf samples (5 g) were cut from the fifth pair of leaves and immediately prior to being homogenized in a modified blender in order to avoid losses of the volatile oils. There were three replicate analyses carried out for each treatment, and each replicate sample was collected from three individual plants.

7.2.2 Results

7.2.2.1 Plant growth parameters

When treatments were applied at the four leaf-pair growth stage, two weeks of supplementary red light had no effect on all of the plant growth parameters compared with the control (Table 7.1).

Table 7.1 Plant growth parameters following two weeks of supplementary red light treatment applied at the four leaf-pair growth stage

Plant growth parameters	Control	Red light	SED	p
Plant height (cm)	44.5	44.4	2.42	0.689
Number of leaf-pairs	6	6	0.258	1.000
Leaf area (cm^2)	504	495	66.4	0.896
Number of shoots	7.3	7.0	0.803	0.687
Plant fresh weight (g)	30.6	29.4	3.51	0.689
Leaf fresh weight (g)	16.69	15.63	1.952	0.600
Plant dry weight (g)	5.05	4.83	0.609	0.718
Leaf dry weight (g)	3.41	3.27	0.505	0.785

In contrast, when treatments were applied at the two leaf-pair growth stage, there were highly significant differences in plant height, leaf area, specific leaf area and weights. Differences in leaf number and number of shoots, however, were not significant. The red light treatment effectively reduced the elongation of the main stem of the plants (Figure 7.10).

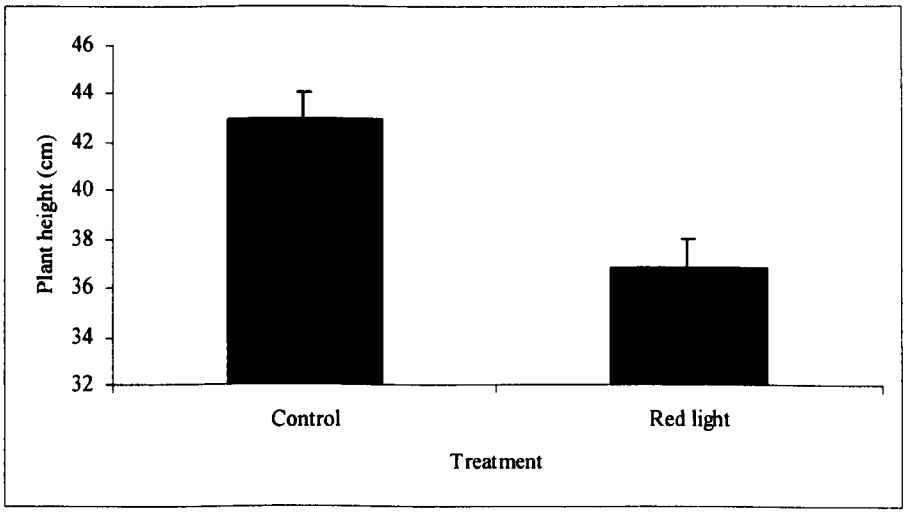

Figure 7.10 Plant mean height after two weeks of supplementary red light treatment applied at the two leaf-pair growth stage

The fresh weight and dry weight of whole plants and leaves were reduced by red light treatment (plant fresh weight: p=0.002, leaf fresh weight: p=0.024, plant dry weight: p=0.05, leaf dry weight: p=0.05) (Figures 7.11, 7.12, 7.13 & 7.14).

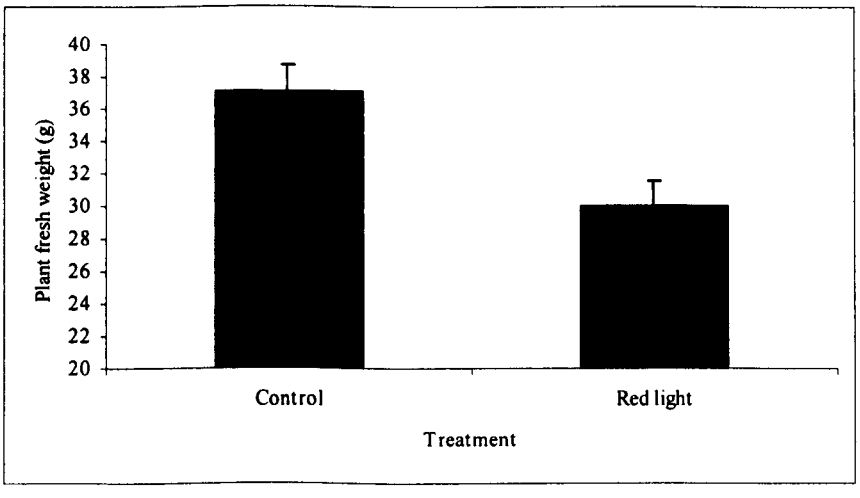

Figure 7.11 Plant fresh weight after two weeks of supplementary red light treatment applied at the two leaf-pair growth stage

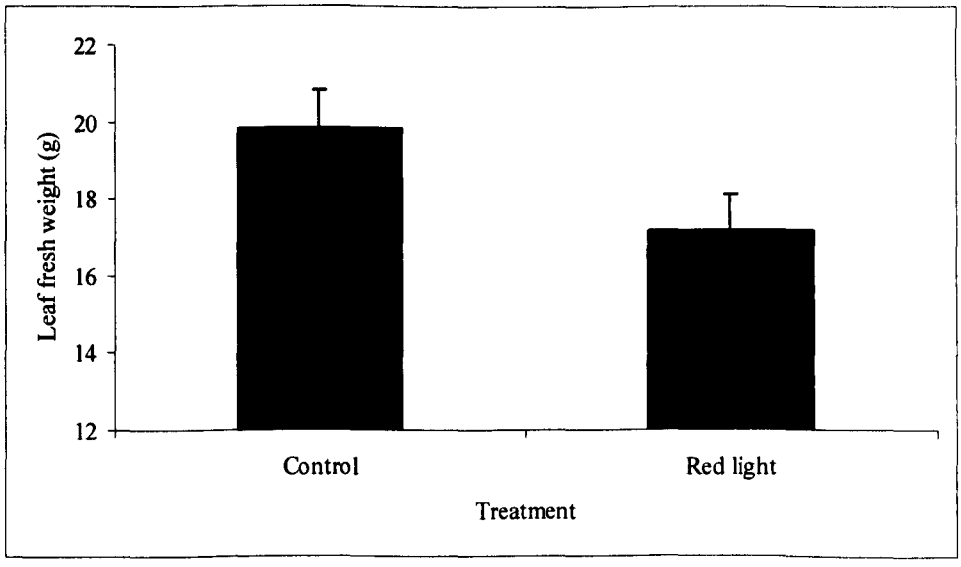

Figure 7.12 Leaf fresh weight after two weeks of supplementary red light treatment applied at the two leaf-pair growth stage

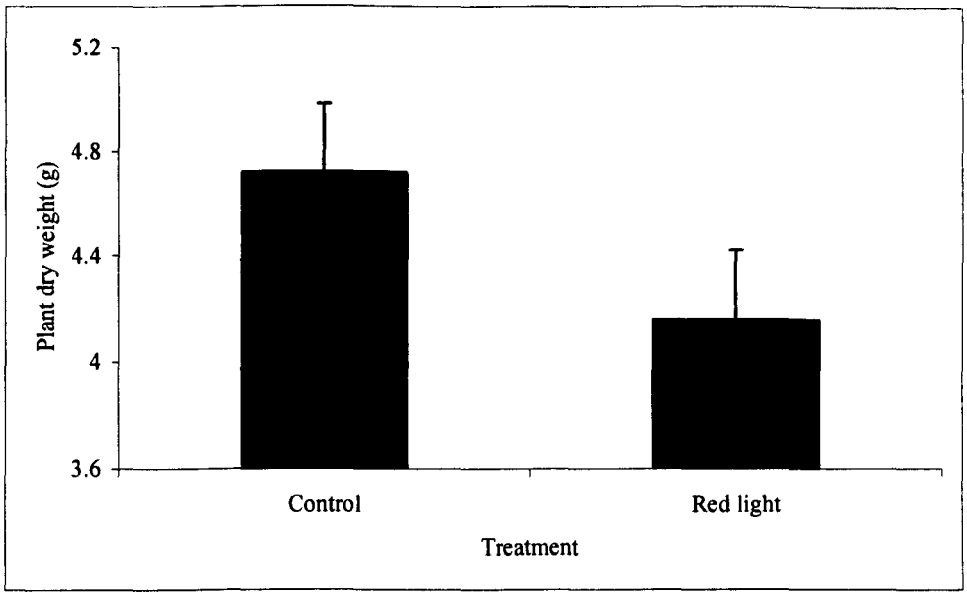

Figure 7.13 Plant dry weight after two weeks of supplementary red light treatment applied at the two leaf-pair growth stage

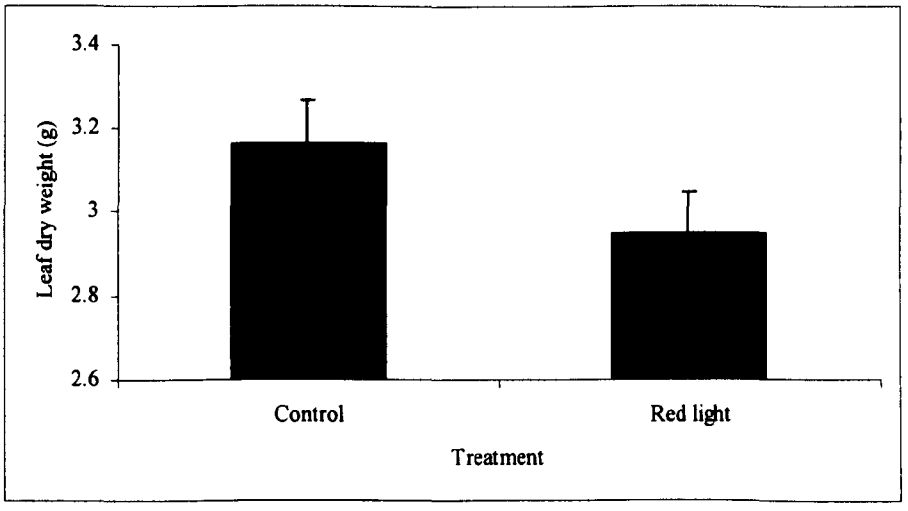

Figure 7.14 Leaf dry weight after two weeks of supplementary red light treatment applied at the two leaf-pair growth stage

Likewise, leaf area and specific leaf area were both significantly reduced by supplementary red light ($p=0.003$ and $p<0.001$ respectively) (Figures 7.15 & 7.16).

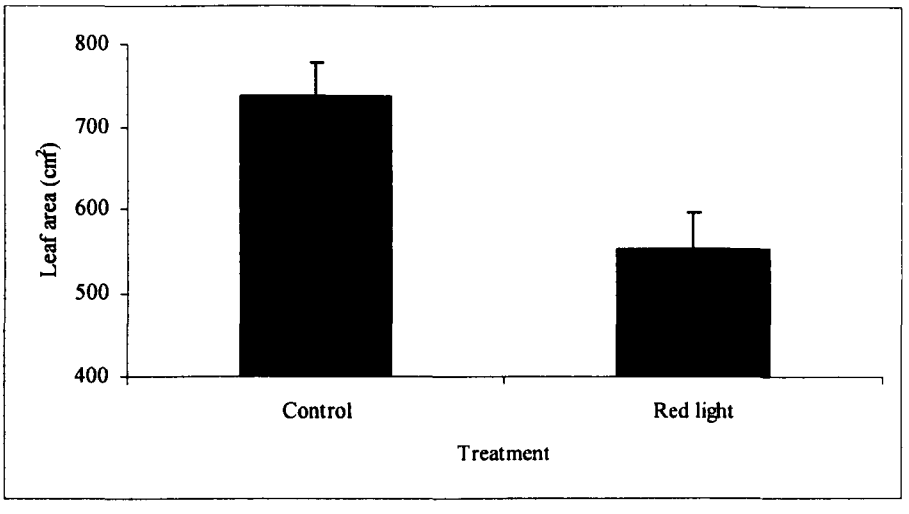

Figure 7.15 Leaf area after two weeks of supplementary red light treatment applied at the two leaf-pair growth stage

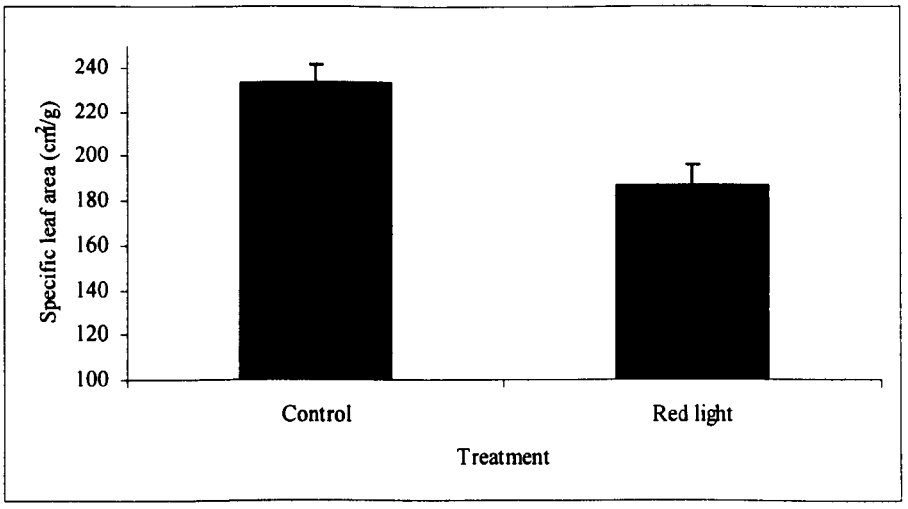

Figure 7.16 Specific leaf area after two weeks of supplementary red light treatment applied at the two leaf-pair growth stage

7.2.2.2 Volatile compounds

There were clear effects of supplementary red light on the quantity and composition of volatile oils in plants at both growth stages. In total, thirty six compounds were identified and, when compared by peak area, eleven of these compounds showed significant differences between the treatments.

In the leaves of plants treated at the two and four leaf-pair growth stages, the total volatiles were enhanced by supplementary red light ($p<0.05$). Eugenol and ten terpenoid derivatives were significantly enhanced ($p<0.05$) (Figure 7.17). Comparing plants at the two growth stages, only trans-α-bergamotene and β-myrcene showed differences with treatments at the two leaf-pair growth stage; there was 130% (2.3 times) enhancement of β-myrcene but no effect on trans-α-bergamotene. With treatments applied at the four leaf-pair growth stage, both β-myrcene and trans-α-bergamotene were enhanced by more than 50%.

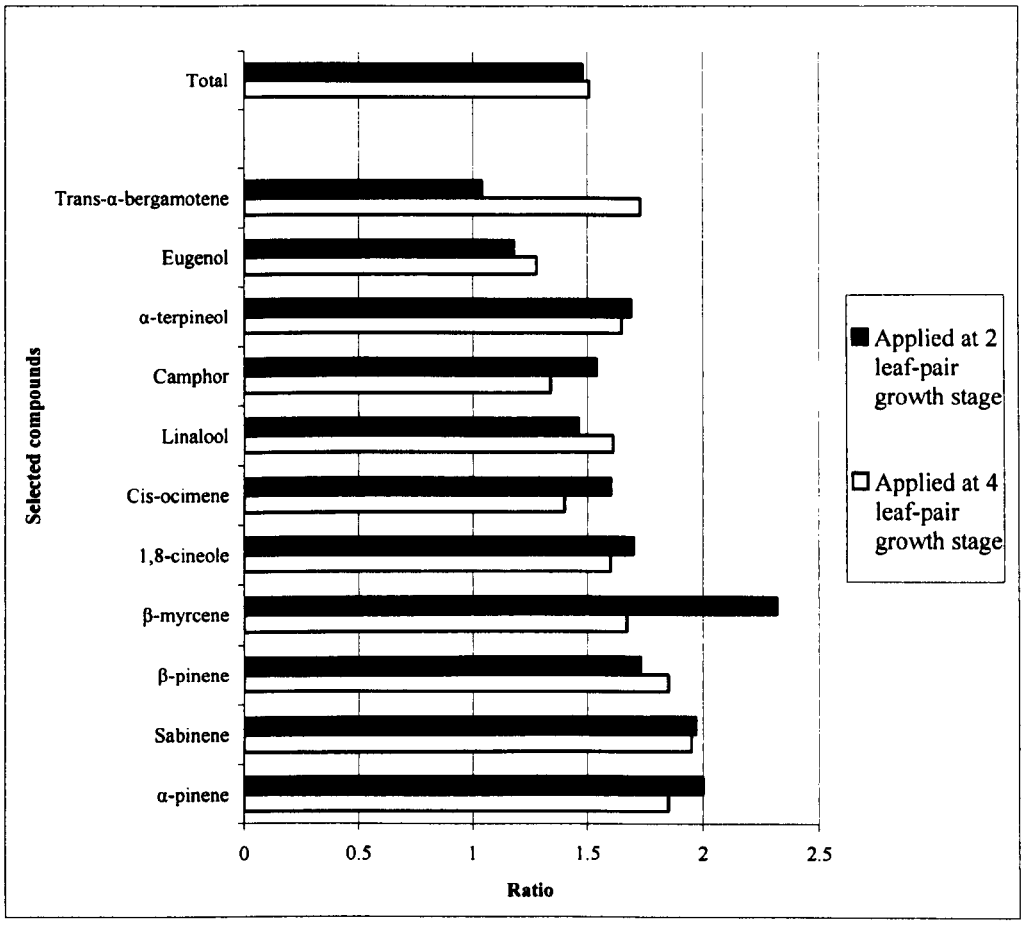

Figure 7.17 Ratio of content of selected volatile oils and total content in supplementary red light treated compared with control plants ***(A value of 1 indicates equal level in both control and red light treatments, and a value of 2 indicates double the level, etc.)***

Ideally, if there was no change in volatile composition, the enhancement of all compounds should be similarly around 50% (x 1.5). The differences in the degree to which individual chemical compounds were enhanced suggest that the volatile oil composition is changed by different factors.

Using relative content to compare the volatile oil composition, there were no significant differences in most of the individual compounds, including the two main compounds, 1,8-cineole and linalool (Figure 7.18 & 7.19). The relative content of eugenol, however, was significantly reduced by the red light treatment and the content of β-myrcene was significantly increased.

It was clear that when treatments were applied at the four leaf-pair growth stage there was no difference in the relative content of 1,8-cineole and linalool between the control and supplementary red light treatments (Figure 7.18).

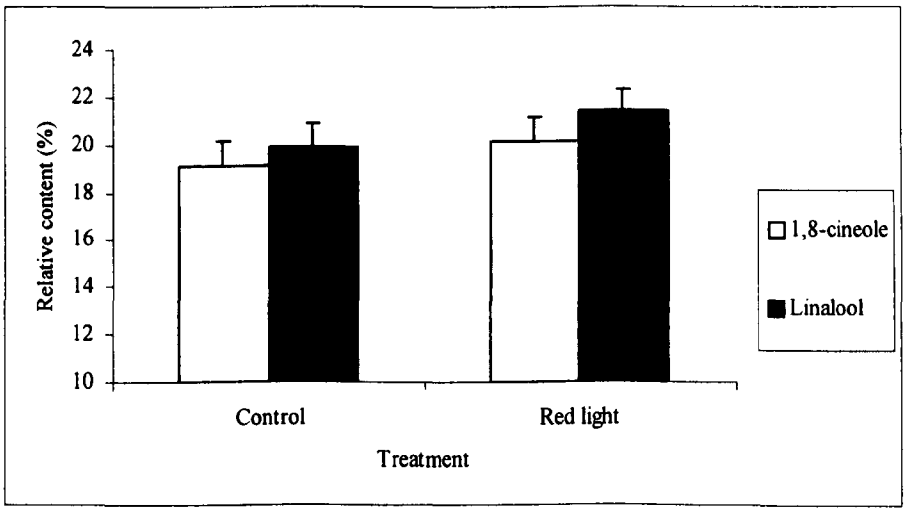

Figure 7.18 Relative content of selected compounds following two weeks of supplementary red light treatment applied at the four leaf-pair growth stage

When treatments were applied at the two leaf-pair growth stage, although the relative content of 1,8-cineole was increased slightly by red light, the difference was not significant (p=0.073) (Figure 7.19). There was no difference in linalool.

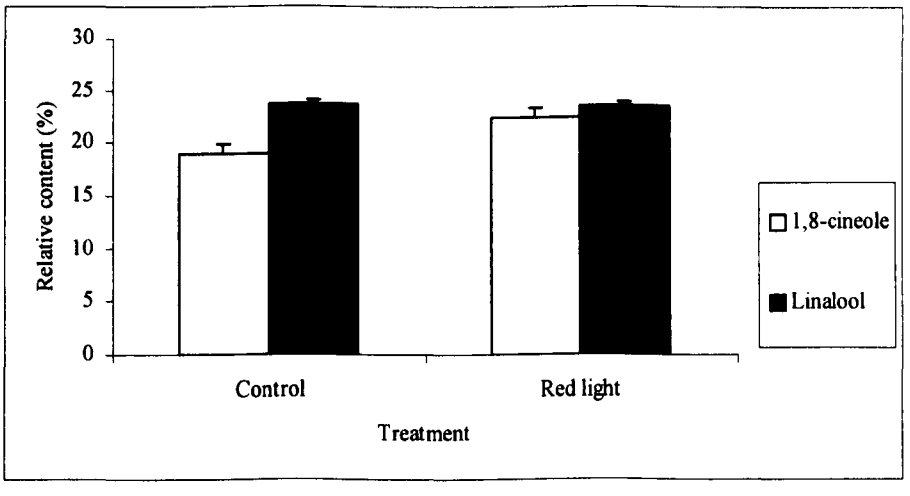

Figure 7.19 Relative content of selected compounds following two weeks of supplementary red light treatment applied at the two leaf-pair growth stage

The relative content of eugenol was strongly decreased by supplementary red light, and differences between treatments were significant for both of the growth stages (Figure 7.20).

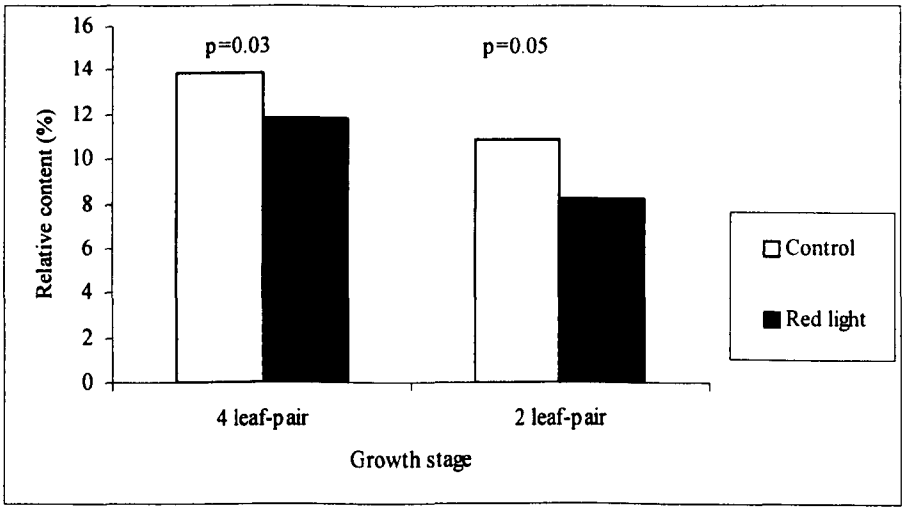

Figure 7.20 Relative content of eugenol following two weeks of supplementary red light treatment applied at the two and four leaf-pair growth stages

The relative content of β-myrcene was strongly increased under supplementary red light when treatments were applied at the two leaf-pair growth stage. Although there was no significant difference when treatments were applied at the four leaf-pair growth stage, the trend was the same (Figure 7.21).

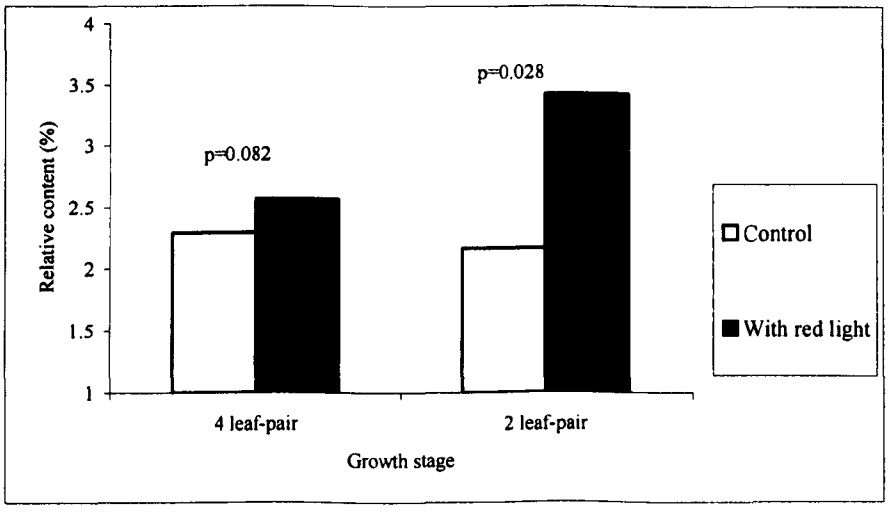

Figure 7.21 Relative content of β-myrcene following two weeks of supplementary red light treatment applied at the two and four leaf-pair growth stages

The yield of the three main volatile oils, 1,8-cineole, linalool and eugenol, was significantly enhanced by the red light treatment (Figures 7.22 & 7.23).

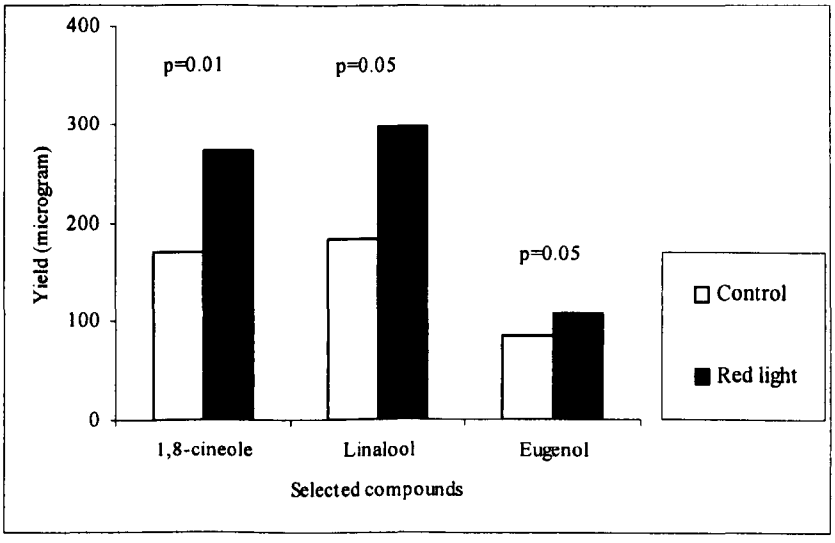

Figure 7.22 Yield of selected volatile compounds in 5 g fresh leaves following two weeks of supplementary red light treatment applied at the four leaf-pair growth stage

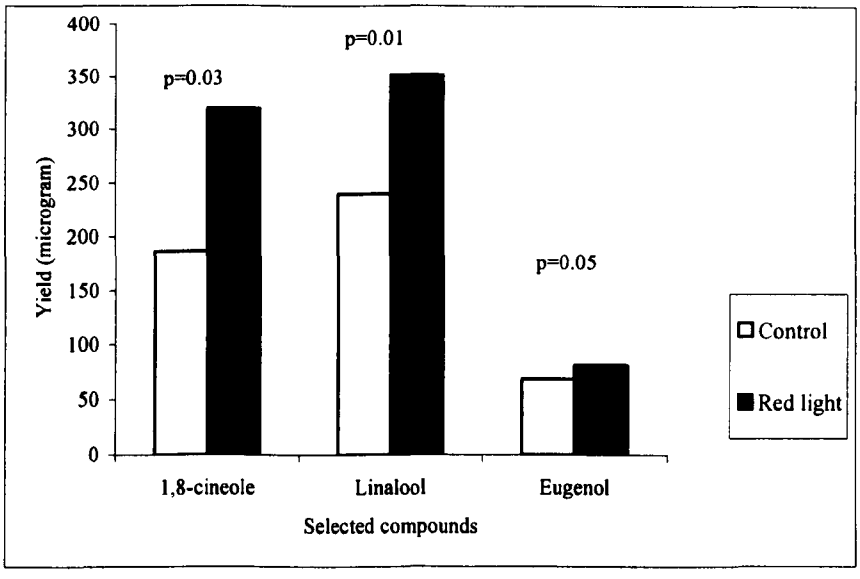

Figure 7.23 Yield of selected volatile compounds in 5 g fresh leaves following two weeks of supplementary red light treatment applied at the two leaf-pair growth stage

7.2.3 Discussion

The absence of any difference in plant growth parameters following two weeks of supplementary red light treatment applied at the four leaf-pair growth stage, compared with the effects at the two leaf-pair growth stage, suggests that the younger plant material was more sensitive to the different R/FR ratios.

Phytochromes mediate responses during the entire life span of a plant, and respond to light intensities. Phytochromes can exist in two stable states, one of them is the red light absorbing form ($\mathbf{P_R}$) with absorption maximum at around 665nm wavelength, the other is the far-red light form ($\mathbf{P_{FR}}$) with absorption maximum at around 730 nm wavelength (Genick and Chory, 2000). Plants perceive the R/FR ratio through a phytochrome photoreception system to trigger plant morphological changes. The most dramatic response is the stimulation of elongation growth under low R/FR ratios (Plate 7.4) (Morelli and Ruberti, 2002).

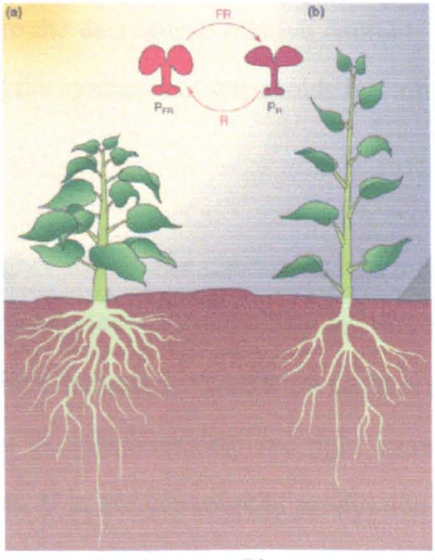

Plate 7.4 Effect of R/FR on plant development. Plants grown under high (a) or low (b) R/FR ratios. $\mathbf{P_R}$, the R-light-absorbing form is converted by R light to the FR-light-absorbing form called $\mathbf{P_{FR}}$. The $\mathbf{P_{FR}}$ form, in turn, can be converted back to PR by FR light (Morelli and Ruberti, 2002)

The auxin/indole-3-acetic acid (Aux/IAA) genes encode short-lived transcription factors that are regulated by auxin or IAA and phosphorylated by phytochrome (Carmona, Chen, Yeh and Abel, 2000), indicating that photocontrol of stem growth may involve close association between phytochrome and hormone signalling pathways (Parks *et al.*, 2001). In the present study, plant height was effectively reduced by the higher R/FR ratio and supports this theory. Supplementary red light also produced basil plants with lower weight, lower leaf area and thicker leaves.

Even though the plant growth parameters were not affected by two weeks of supplementary red light applied to plants at the four leaf-pair growth stage, the effect on volatile oil content was the same regardless of whether the plants were at the two or four leaf-pair growth stage.

Supplementary red light highly increased the total volatile oil content in basil leaves. Of the thirty six compounds identified, most of the terpenoids were increased around or above the mean level of enhancement, but the phenyl-propanoid eugenol was lower than the average due to the decrease in relative content. This suggests that the higher R/FR ratio stimulated the synthesis of terpenoids but inhibited the synthesis of phenyl-propanoids.

Basil plants grown under higher R/FR ratio conditions had a higher volatile content on a per weight basis but a lower yield of leaves. So with this 15% decrease in fresh leaf weight per plant, but a 50% increase in volatile oil content on a per weight basis, there was a positive effect of the supplementary red light treatment when applied at the two leaf-pair growth stage. If in the commercial production of basil the objective is to maximize vegetative yield as well as volatile oil yield, using supplementary red light when plants are relatively mature may be more acceptable. At the four leaf-pair growth stage, there were no differences in plant height and weight but there was a significant increase in volatile oil content on a per weight basis.

7.2.4 Conclusions

- ✧ There were no significant differences observed in plant growth parameters following two weeks of supplementary red light treatment applied at the four leaf-pair growth stage. However, all parameters were slightly reduced by supplementary red light.
- ✧ Supplementary red light strongly reduced plant height, weight and leaf area when applied for two weeks at the two leaf-pair growth stage.
- ✧ Leaf thickness was significantly enhanced by supplementary red light.
- ✧ Supplementary red light applied for two weeks effectively enhanced the total content of volatile oils in basil leaves.
- ✧ Supplementary red light significantly decreased the relative content of eugenol and increased the relative content of β-myrcene, however, there was no effect on the relative content of 1,8-cineole and linalool.
- ✧ Although there were no significant differences in the relative contents of 1,8-cineole and linalool, their content in 5 g fresh leaves was highly increased due to the enhancement of total volatile oil content.
- ✧ Use of supplementary red light after the four leaf-pair growth stage could be applied in production systems to maximize vegetative yield as well as yield of volatile oils.

Chapter 8 SENSORY ANALYSIS

As an aromatic plant, basil is cultivated mainly for the food, cosmetic and pharmaceutical industries, and for fresh leaf consumption. Thus, the taste and aroma of fresh leaves are important. So far, there are no publications on the relationship between aroma and volatile oil content.

The present study has shown that basil plants grown under different environmental conditions contain different concentrations of volatile oils as determined by GC/MS. The volatile oils in the plant are responsible for its characteristic aroma, so it is appropriate to determine if humans can detect any difference in aroma in relation to the method of cultivation. The results could provide guidance for basil cultivation and breeding in the future. Sensory analysis was carried out to determine the following:

1. If the increase in volatile oil content due to growth conditions is sufficient to produce a perceptible increase in basil aroma.
2. If a perceptible increase exists, is it desirable or not?

8.1 Materials and Methods:

8.1.1 Preparation of leaf samples

Plants of basil cv Basil Sweet Genovese were raised from seeds and grown as described in chapter 3. Plants at the 3 leaf-pair growth stage were grown for two weeks at 15 and 25°C (120 plants at each temperature) in controlled environment rooms. The experiments was stated on 9 July 2003 and repeated in a second experiment on 21 September 2003. After two weeks of treatment, fresh leaf samples (5 g per sample) were collected from the fifth pair of leaves and immediately stored in airtight glass bottles for sensory testing.

8.1.2 Preparation of reference samples

In this study, 1,8-cineole, linalool and eugenol have been determined to be the major compounds present in basil leaves, present in the ratio approximately 1 : 1.5 : 1.5 for eugenol, 1,8-cineole and linalool respectively. Therefore, three standard solutions at different concentrations were prepared as reference samples (Table 8.1).

Table 8.1. Concentrations of standards in reference samples for sensory analysis

Reference	Eugenol		1,8 - cineole		Linalool	
samples	Volume	Concentration.	Volume	Concentration	Volume	Concentration
1	1µl	0.001%	1.5µl	0.0015%	1.5µl	0.0015%
2	10µl	0.01%	15µl	0.015%	15µl	0.015%
3	100µl	0.1%	150µl	0.15%	150µl	0.15%

Solvent: deionised water; volume: 100ml

8.1.3 Assessing the intensity of basil aroma

To assess the intensity of basil aroma in the different leaf samples, a trained panel of expert assessors was used. These assessors possessed a high degree of sensory sensitivity, experience of sensory methodology and were able to make consistent and repeatable sensory assessments of various products (Shepherd, 1999).

8.1.3.1 Panel training

Before the samples of leaves were assessed, in order to check that the trained panel could identify differences in aroma intensity of the volatile compounds present in basil, the three reference samples were presented in a random balanced order (to reduce sample order effects and bias). The assessors were asked to assess the aroma of each sample with gentle sniffing and to place them in order of aroma intensity.

8.1.3.2 Assessment of leaf samples

A paired comparison test was used to determine if a perceivable difference in aroma intensity existed between 2 samples of basil leaves.

Five g fresh basil leaves and 25 g odour free fine sand were placed into clean dry sample bottles (250 ml) and sealed immediately. The basil leaves had been cut from the plant no longer than 2 hours prior to the assessment. Bottles were labeled with a random 3 digit code to hide the identity of the 2 samples (Table 8.2). A sensory panel of 21 trained assessors aged between 30 and 60 was presented with the two samples. The order of presentation was randomised and balanced so that the same sample was not always presented first.

Table 8.2 Source of samples for trained panel

Sample	Sample code	Plant growth conditions from 3 leaf-pair growth stage
1	262	15°C for 2 weeks
2	519	25°C for 2 weeks

Basil leaves possess on their surface two types of glandular trichomes, termed peltate and capitate glands, and most of these glands (>75%) can be removed by cotton swabs or small glass beads rubbing against both leaf surfaces without any further damage to the epidermis (Gang *et al.*, 2001). In the present study, fine sand was used to break the glands on the leaf surface and to release volatile oils. In a preliminary investigation, 50% and 90% of volatile oils in fresh leaves were released by shaking the leaves with sand for 30 s and 2 min respectively. Shaking for 2 min, however, resulted in the samples smelling like 'cut grass' and, consequently, shaking for 30 s was considered to be more appropriate for generating the typical basil aroma.

Assessors were instructed to shake the 2 samples gently for 30 s and leave them for 30 s to equilibrate, after which they removed the lids and gently sniffed the aroma from the leaves. The samples were sniffed in the order provided (left hand sample first) and the lids were replaced between samples. Assessors selected the sample with the stronger aroma and were then encouraged to provide comments describing the aroma characteristics for each sample.

8.1.4 Consumer assessment of preference

Consumer testing is one of the important activities in product development. The primary purpose is to assess the personal response by current and potential customers to a product, product ideas or specific product characteristics (Resurreccion, 1998).

In a separate experiment, a paired preference test was used to determine if a

significant preference existed for one of two samples of basil leaves. Using the procedure described in section 8.1.3.2, samples of fresh basil leaves and fine sand were placed into clean dry sample bottles and sealed (Table 8.3).

Table 8.3 Source of samples for non-trained panel

Sample	Sample codes	Plant growth conditions from 3 leaf-pair growth stage
1	374	25°C for 2 weeks
2	141	15°C for 2 weeks

A consumer panel of 64 people who use basil was selected from the employees and students at Nottingham University. They answered the following questions regarding their use of basil:

- How frequently do you use basil (often, sometimes, never)?
- Which type of basil do you use (fresh, dried, both)?

They were then presented with the two samples of leaves. The order of presentation was randomized and balanced so that the same sample was not always presented first. They were instructed to shake the 2 samples gently for 30 s, after which they removed the lid and gently sniffed the aroma of the leaves. The samples were sniffed in the order provided (left hand sample first) and the lids were replaced between samples. The consumers were asked to select which sample they preferred and then encouraged to make comments about why this was their preferred choice.

8.1.5 TD-GC/MS analysis

On the same day of carrying out the sensory testing, the content and composition of volatile oils in the leaf samples were analysed by TD-GC/MS. Extraction of the volatile oils by Sorbent Tube Trapping and analysis by TD-GC/MS were as described in Chapter 3. All samples of fresh leaves were collected from the fifth pair of leaves,

and there were three replicate analyses of the volatile oils for each temperature treatment.

8.2 Results

8.2.1 Assessing the intensity of basil aroma

8.2.1.1 Panel training

Sixteen out of the 21 assessors (Table 8.4) placed reference sample 3 as the strongest, whereas there was no clear outcome as to which was the stronger between reference samples 1 and 2. The data was analysed using Friedman's analysis of variance for ranked data (this is a non-parametric version of analysis of variance). The Friedman test showed a significant difference between samples ($p=0.0003$). The multiple comparison test (significance level 5%) indicated that reference sample 3 was significantly stronger than reference samples 1 and 2 but that 1 and 2 were not significantly different ($p>0.05$).

Table 8.4 Number of assessors ranking reference samples in order of strength

Order of strength (rank)	Reference sample 1	Reference sample 2	Reference sample3
1	10	10	1
2	7	10	4
3	4	1	16
Total	21	21	21
Rank sum	36.0	33.0	57.0

Sum of squares of sum of ranks: 5634.0

F: 16.2857

Significance (F) (probability of achieving F): 0.0003***

In conclusion, the trained panel was able to detect differences in intensity of aroma for the combination of volatile oils at the highest concentrations (reference sample 3).

8.2.1.2 Assessment of leaf samples

Eighteen out of the 21 assessors selected basil sample 2 as the stronger sample (Table 8.5). The data were analysed using a binomial test such that the probability of the result occurring is calculated against a null hypothesis of no difference between samples. The binomial test was two tailed as it was assumed that a direction of response could not be predicted (it would not be acceptable to calculate a one tailed test assuming the direction of assessor response matched analytical results).

In hypothesis testing it is typical to reject the null hypothesis (no difference between samples) in favour of an alternative hypothesis (that the samples are different) if the probability of the result is lower than p=0.05.

Table 8.5 Assessment of intensity of basil aroma. Data analysed by two-tailed paired test

Answers taken	Product	Answer	Significance
21	No answer	0	
	Sample 1 (262)	3	
	Sample 2 (519)	18	0.0015**

The binomial test gave a probability of 0.0015, therefore it can be concluded that a significant difference existed between the two samples with regard to perceived intensity of basil aroma, i.e. sample 2 from plants grown at 25°C was significantly stronger than sample 1 from plants grown at 15°C.

8.2.1.3 Assessors comments

After assessment, some of the assessors described the differences between sample 2 with the higher volatile oil concentration and sample 1 with the lower volatile oil concentration. These comments were based on the taste and visual quality of the samples. Most of the assessors considered that sample 2 was typically basil with clove like aroma and stronger than sample 1. Two panelists, however, felt that sample 2 was too sharp and cloying. Some assessors considered that the leaves of sample 2 were greener and healthier than sample 1.

8.2.2 Consumer assessment of preference

8.2.2.1 Assessment of leaf samples

Forty one out of the 64 assessors selected basil sample 1 (374) as the preferred sample (Table 8.6). The data were analysed using a binomial test as stated above.

Table 8.6 Consumer preference of leaf samples, analysed by two-tailed paired test

Answers taken	Product	Answer	Significance
64	No answer	0	
	Sample 1 (374)	41	0.0328*
	Sample 2 (141)	23	

The binomial test gave a probability for this result of 0.0328, therefore it can be concluded that a significant preference existed between the two samples, i.e. sample 1 (374) was significantly preferred to sample 2 (141).

8.2.2.2 Use of basil by the assessors

Most of the assessors had experience of consuming basil (Figure 8.1). Possibly due to the low number in this group, the group of people who were 'often' using basil did not show a significant preference between the leaf samples, whereas the group using basil 'sometimes' showed a highly significant preference (p=0.0064).

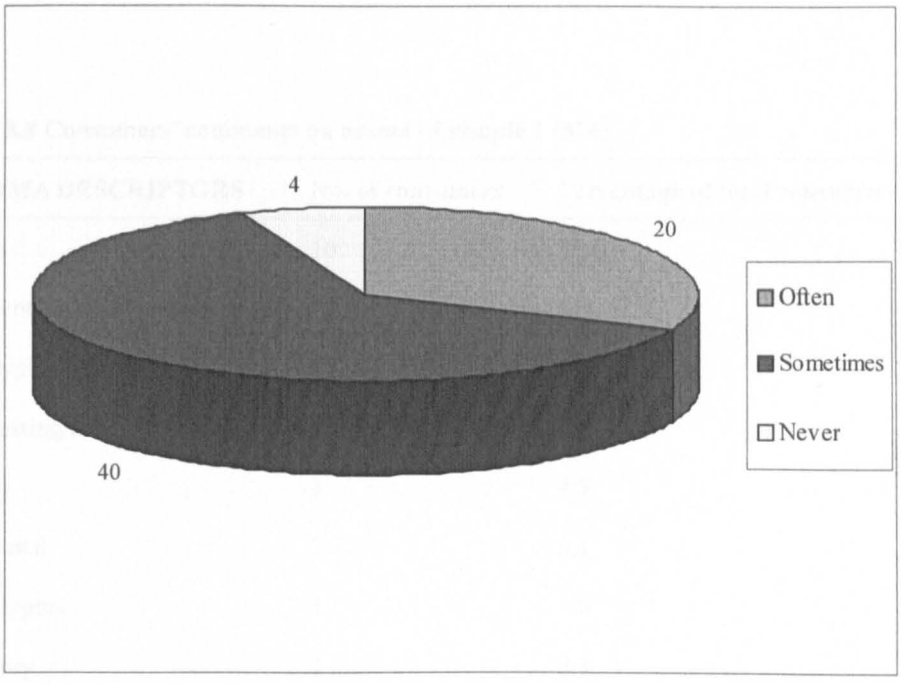

Figure 8.1 Use of basil by the assessors

8.2.2.3 Consumers' comments

The consumers provided comments on why they preferred one of the samples (Tables 8.7, 8.8 & 8.9). This was a very subjective questionnaire, and the responses depended on the consumers' habits and olfactory senses, as they were non-trained assessors.

Most of the consumers thought that sample 1 (374) was stronger than sample 2 (141), however, five consumers regarded sample 2 to be stronger than sample 1 (Table 8.7).

Table 8.7 Consumers' comments on aroma strength of two leaf samples

Sample	Aroma strength	Number of consumers	Percentage of total consumers
1 (374)	Pungent/sharp/strong/powerful	24	37.5
	Milder than 141	1	1.6
	Too strong	1	1.6
2 (141)	Weaker than 374	9	14.1
	Stronger than 374	5	7.8

Table 8.8 Consumers' comments on aroma of sample 1 (374)

AROMA DESCRIPTORS	No. of consumers	Percentage of total consumers
Fresh	10	15.6
Authentic/typically basil	7	11
Not typically basil	2	3.1
Disgusting/unpleasant	4	6.3
Minty	3	4.7
Off smell	2	3.1
Eucalyptus	1	1.6
Peppery	2	3.1
Sweeter (than 141)	2	3.1
Clovey	1	1.6
Herby	1	1.6
Strange smell	1	1.6
Bitter	1	1.6
Better for food	1	1.6
Fragrant	1	1.6
Aniseed	1	1.6
Chemical/antiseptic/medicinal	1	1.6

Table 8.9 Consumers' comments on aroma of sample 2 (141)

AROMA DESCRIPTORS	No. of consumers	Percentage of total consumers
Not typically basil/aniseed aroma	4	6.3
More typical than 374	3	4.7
Fresh	4	6.3
Sharper	2	3.1
Sweeter	2	3.1
Floral	2	3.1
Aromatic	2	3.1
Pesto-like	2	3.1
Musty/fusty	2	3.1
Spicy	1	1.6
Appetizing	1	1.6
Wild	1	1.6
Unusual	1	1.6
Less perfumed than 374	1	1.6
Nutty	1	1.6
Cooked potato	1	1.6
Sickly	1	1.6
Medicinal	1	1.6
Gentle	1	1.6
Minty	1	1.6
Fruity	1	1.6

8.2.2.4 Type of basil used by consumer panel

Regarding the type of basil used, it was difficult to draw any conclusions about preference because of the numbers in each group (Figure 8.2).

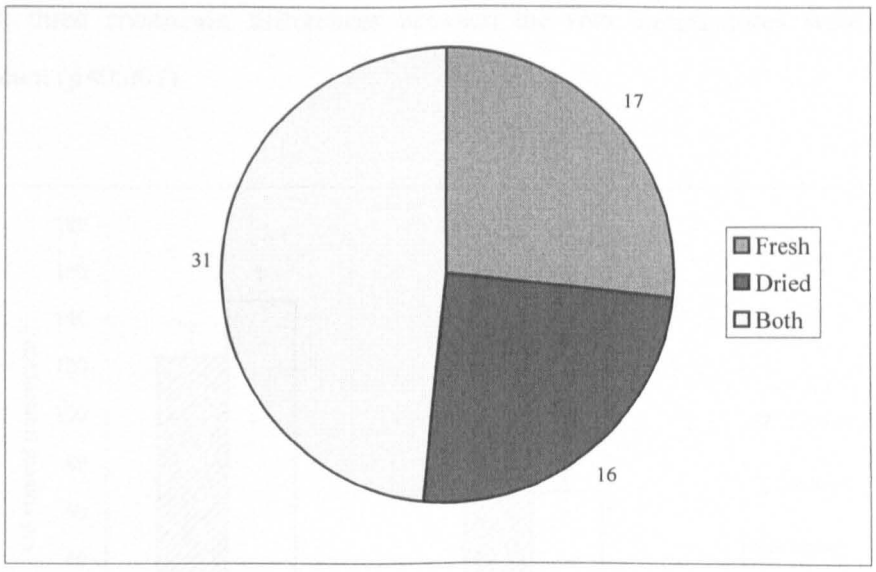

Figure 8.2 Type of basil used by consumers

8.2.3 TD-GC/MS analysis

The total oil content of plants grown at 25 °C was 70% higher than in plants grown at 15 °C ($p<0.001$) (Figure 8.3).

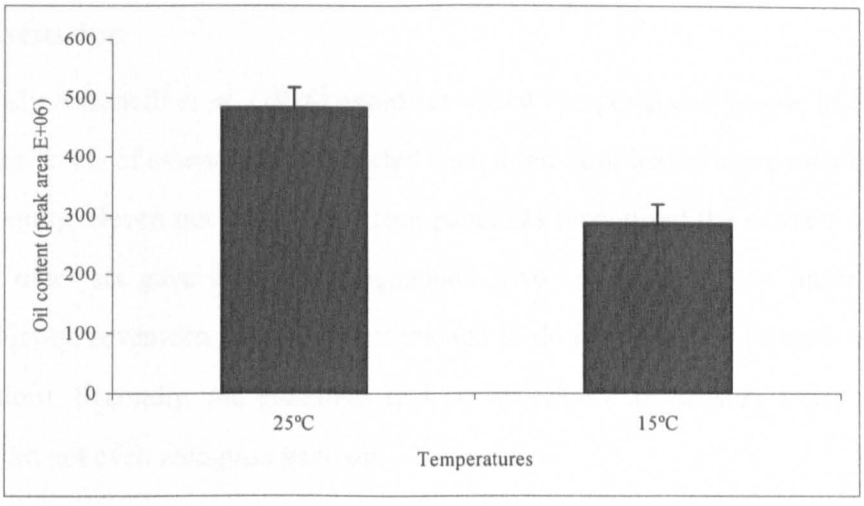

Figure 8.3 Total oil content after two weeks of temperature treatments

Using the peak area and calibration curves as described in chapter 3, the content of 1,8-cineole, linalool and eugenol in 5 g of fresh leaves was calculated (Figure 8.4).

For all three chemicals, differences between the two temperatures were highly significant ($p<0.001$).

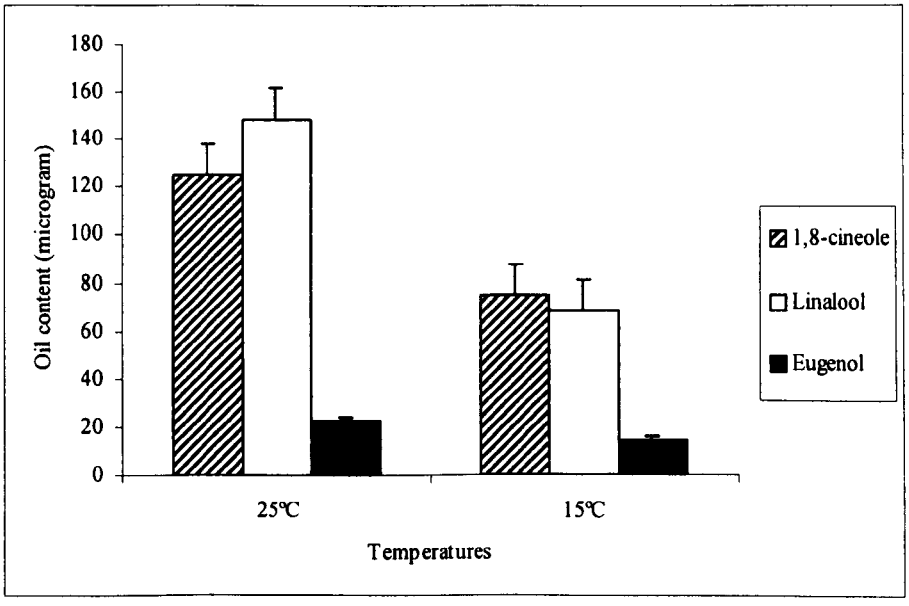

Figure 8.4 Content of selected volatile oils in 5g of fresh leaves after two weeks of temperature treatments

8.3 Discussion

Previously Antonelli *et al.* (1998) asked seventeen inexperienced people to evaluate their four levels of essential oils extracted from dried basil leaves using ranking tests. In that study, eleven out of the seventeen panellists recognised the extracts as basil, but the other six gave incorrect assignations. Two criticisms can be made of this study. Firstly, seventeen people are not enough to do this test (low representative of population). Secondly, the panellists had no experience of sensory tests, and six people did not even recognise basil oil.

In the present study, it was novel to analyse the link between aroma and volatile oil content in fresh basil leaves. Since the 21 trained panellists were able to detect differences in the intensity of the aroma of the mixture of eugenol, 1,8-cineole and

linalool, they were able to carry out the assessment of the fresh basil leaves. However, if the volatile oil content in the basil leaves was very low, it would be not be detected by the assessors.

The selection of the sample from plants grown at 25°C by 18 out of 21 assessors to be the stronger sample was supported by the result from the GC/MS. Likewise, 41 out of 64 consumers selected the stronger sample as their significant preference ($p=0.0328$). Based on the consumers' habits and olfactory senses, comments were provided for explaining why 23 consumers preferred the leaf sample containing the lower volatile oil content. Three consumers thought that it was more typical than the stronger sample, four consumers believed it was fresh, two consumers deemed it was sweeter and one consumer deemed it was appetizing. In contrast, two consumers thought that the stronger sample was not typically basil, two consumers thought it possessed on off smell and four consumers even deemed it was disgusting.

After separating the 64 consumers into different basil user groups, the low numbers (less than 30) present within each group made it difficult to draw conclusions almost the preferences of those user groups. Of the user groups with more than 30 people, the group using basil 'often' did not show a significant preference, whereas the group using basil 'sometimes' showed a highly significant preference ($p=0.0064$) and selected the sample with the higher content of volatile oils. This needs further investigation. Using more than 100 people would increase the chances of there being more than 30 consumers in each basil using group.

In this study, differences were found in the total volatile oil content and composition in basil leaves grown at 15°C and 25°C. Therefore, further investigation is needed to determine if the preference is related to the absolute intensity or to the quality of basil aroma. Furthermore, this test only looked at three main chemicals, i.e. 1,8-cineole, linalool and eugenol, but there are many others, which may also impact on the basil aroma. A further study should also consider an increased sample size, i.e.

three or more levels, and look at more chemicals and different volatile oil compositions to determine customers' preferences.

8.4 Conclusions

- ✧ Trained sensory panellists can perceive different intensities of volatile oils in fresh basil leaves.
- ✧ A consumer preference test suggested that most of the people, i.e. current and potential customers, preferred the stronger intensity.

Chapter 9 GENERAL DISCUSSION

The effects of constant and alternating temperature, irradiance, supplementary UV-B light and red light on plant growth parameters and chemical compounds in basil plants have been discussed in previous chapters. The purpose of this chapter is to analyse the effects of environmental factors, and to provide some suggestions for production practice and further academic studies.

There is no doubt that temperature and light affected both plant growth and volatile compounds in basil. In the literature, the growth and volatile compounds in basil have been less studied in relation to light than in relation to temperature.

9.1 Plant growth parameters

The basil plants grew faster and produced the tallest plants and highest dry matter under warm conditions, i.e. 25°C, and less at 30°C. This was supported by a previous report that the fastest growth rate occurred at 27°C (Putievsky and Galambosi, 1999). When plants were treated with one-week alternating temperature treatments over a period of three weeks, the tallest plants and the highest dry weights were achieved under the highest accumulated day degrees. After three weeks of treatment, all plants had received the same accumulated day degrees but in a different sequence of temperatures, and there was no difference between the treatments. This suggests that the plant growth rate was strongly affected by accumulated day degrees, and that temperature order had less effect on plant growth rate.

Heavy shading reduced both plant height and dry matter accumulation, however there were no differences between slight shading and the control. This suggested that basil can tolerate slight shading. The reduced plant height and weight with heavy shading were due to the reduction in photosynthetic rate (Chapter 6).

Supplementary UV-B light reduced plant height in this study probably due to the breakdown of IAA (Ros and Tevini, 1995; Huang *et al.*, 1993), but this requires confirmation. Because more axillary shoots were produced, overall dry matter was increased under supplementary UV-B light. Regarding supplementary red light, a high R/FR ratio results in the FR-light-absorbing form phytochrome (called P_{FR}) being produced, which up-regulates IAA proteins (Parks *et al.*, 2001) and results in shorter plants, as well as less dry matter accumulation (Chapter 7). This study also showed that the growth of young plants, compared with other plants, was more sensitive to changes in the R/FR ratio.

In the present study, low temperature, low irradiance (heavy shading) and supplementary red light led to small leaf area due to the slow growth of leaves, but the leaves became thicker. It also revealed that supplementary UV-B light had no effect on leaf area, but increased leaf thickness and therefore resulted in the increase in leaf dry matter.

In the commercial production of basil, the yield of leaves is important because it is the material harvested either for fresh and dry leaf uses or for volatile oil extraction. The concentration of oil in the leaves is also important when the objective is to produce volatile oils. Therefore, in basil production, conditions resulting in faster growth and higher volatile oil content are ideal. As discussed above, these conditions are warm temperature, supplementary UV-B light and sufficient irradiance to benefit plant vegetative growth.

9.2 Volatile oils

Out of the many compounds identified from fresh basil leaves, in terms of yield, the terpenoids were the greatest component, with 1,8-cineole and linalool comprising approximately 50%. Another major compound was eugenol, and its relative content

varied between 2% and 15% with the different treatments. Although normally only a trace of methyl eugenol was present, it was more than 25% in the young plants under heavy shading. But, overall, the study showed that warm temperature, sufficient irradiance, supplementary UV-B light and red light (high R/FR ratio) effectively increased the concentration of volatile oils in fresh basil leaves.

Under warm temperature conditions, both leaf fresh weight per plant and the volatile oil concentration in fresh leaves were significantly increased, resulting in a higher total yield of volatile compounds per plant. The relative content of eugenol was increased under warm conditions, probably because eugenol synthase is more sensitive to temperature than linalool synthase, 1,8-cineole synthase and other enzymes. Although alternating temperature did not affect most of the plant growth parameters, volatile oil concentration was strongly affected by the last two weeks of temperature experienced and, the higher the accumulated day degrees, the higher the concentration of volatile oil produced. Likewise, the higher the temperature before harvest, the higher the relative content of eugenol produced (Chapter 5). Since the findings from the two temperature experiments supported each other, it could be concluded that the activity of eugenol synthase, a proposed enzyme in the phenylpropene pathway (Gang *et al.*, 2001) may have been stimulated by warm temperature. It has been reported that the optimum temperature for activity of CST (a *p*-coumaroyl-CoA: shikimic acid *p*-coumaroyl transferase) was 30-37°C and the activity was much higher in tissues that were actively producing eugenol (Gang *et al.*, 2002a; Lavid *et al.*, 2002).

Heavy shading reduced not only leaf fresh weight but also volatile oil concentration in fresh basil leaves. This could have resulted in the critical decrease of total volatile oil yield per plant. Since there was a fivefold difference in the concentration of volatile oils in fresh leaves and a threefold difference in fresh leaf weight, there was a fifteen times increase in total volatile oil yield without shading (control) compared to heavy shading (75% shade) (Chapter 6). This suggests that light is the key

environmental factor regulating both plant growth (Kaufman *et al.*, 1999) and the accumulation of volatile oils.

The present study revealed that irradiance affected the concentration of volatile compounds as well as their composition. Under heavy shade (75% shading), the relative content of methyl eugenol was high, whereas the relative contents of linalool and eugenol were high without shading. When plants were treated at a relatively young age (one leaf-pair growth stage), the relative contents of methyl eugenol were between 26.09% and 4.54% of the total under heavy shading (75%) and no shading respectively; the relative contents of eugenol were between 2.31% and 7.04% of the total under heavy shading and no shading respectively; the relative contents of linalool were between 5.4% and 19.8% of the total under heavy shading and no shading respectively. When treatments were applied to the older plants (three leaf-pair growth stage), although the same trend was obtained, the amount of volatiles was different compared with treatments applied to the younger plants. This may have occurred due to high expression of eugenol *O*-methyl transferase (EOMT) in the peltate glandular trichomes on the surface of the young basil leaves (Gang *et al.*, 2002b). No literature was found to explain why shade conditions stimulated the accumulation of methyleugenol. This requires further investigation.

It has been shown that one of the adaptive mechanisms of plants to enhanced UV-B irradiance is to increase the production of secondary metabolites in the leaf epidermal layer to shield the underlying tissues against harmful UV-B radiation (Kakani *et al.*, 2003).

In the present study, supplementary UV-B light increased the concentration of volatile oils in basil leaves, but there was no effect on oil composition. This supports the findings of Maffei and Scannerini (2000) for peppermint where the total oil content was significantly increased in plants irradiated with UV-B but no qualitative differences were observed between irradiated and control plants. For basil, however,

Johnson *et al.* (1999) reported that UV-B increased volatile oil concentration and also changed the oil composition and Ioannidis *et al.* (2002) reported that neither the concentration nor composition of oils in fresh basil leaves was affected by supplementary UV-B light. They suggested that plants treated with supplementary UV-B for 3 or 4 days would result in increased essential oils in the glands, with further increases in dose leading to bursting of some glands. This interpretation seemed to be reasonable, but it could not explain the previous and present results. Comparing the methods used in the two previous studies and the present study, it is possible that, besides different genotypes, temperatures and UV-B doses, different light intensities could have contributed to the conflicting results since, with a decrease in light intensity, the deleterious effects of UV-B on growth increases (Nilsen and Orcutt, 1996).

Since the discovery and purification of phytochrome in the early 1960s, the ratio of red to far-red light has been widely studied in the responses of plants to light quality. But most of the studies have focused on plant growth, with limited studies on the effects of R/FR ratio on plant secondary metabolites. A high FR/R ratio serves as a signal for the allocation of photosynthate to above ground portions of plants (Loughrin and Kasperbauer, 2003), and the converse, i.e. a high R/FR ratio, results in the FR-light-absorbing form phytochrome ($\mathbf{P_{FR}}$), which up-regulates IAA proteins (Parks *et al.*, 2001). Meanwhile, it probably stimulates the activities of some enzymes and regulates the pathway of metabolism to enhance the accumulation of volatile compounds in basil leaves. The present study revealed that the R/FR ratio affects not only plant growth but also volatile oils. The higher R/FR ratio significantly increased volatile oil concentration in basil leaves, reduced the relative content of eugenol and enhanced the relative content of β-myrcene.

Maffei and Scannerini (2000) stated that the roles of secondary metabolites under supplementary UV-B conditions include protection against harmful UV-B radiation as well as against potential pathogen attack. The reason, however, for the increases in

volatile compounds under a high R/FR ratio is not clear.

This study was the first attempt to analyse the link between aroma and volatile oil content in fresh basil leaves. The trained panellists were able to detect differences in intensity of aroma in the basil leaves and 41 out 64 consumer assessors preferred the stronger sample. Although a very interesting result, further investigation is required because, when 64 consumers were split into different basil user groups, there were less than 30 people in each group.

9.3 Suggestions for further studies

Due to the limited availability of space in controlled environment rooms, there were only three temperatures (15, 25 and 30°C) in the temperature experiment. This temperature range seems insufficient to analyse the relationships between temperature and basil plants. Therefore, more temperatures should be used in further studies for evaluating temperature effects on the growth and volatile oil compounds in basil plants.

The shading experiment was carried out in a glasshouse, so the light intensity and quality was changed after penetrating the glass. Measurements taken on 15 August 2003, which was a very clear day, showed the PAR to be 1600 and 900 μmol m^{-2} s^{-1}, outside and inside the glasshouse respectively. The UV-B light density was significantly reduced after penetrating the glass, as shown by a measurement taken on 15 March 2004 when densities of 308.0 and 21.2 μW m^{-2} were recorded outside and inside the glasshouse respectively. Since basil plants are reputed to grow well in full sun conditions, a future irradiance experiment should include a treatment with full sunlight.

As methyleugenol was strongly increased under shading conditions, it is desirable to isolate and test the activity of EOMT, and to determine if low daily light integral or

low irradiance stimulates the activity of EOMT and results in the methylation of eugenol to produce more methyl eugenol.

Kakani *et al.* (2003) reported that ambient PAR (1000-1600 μmol m^{-2} s^{-1}) is able to ameliorate the effects of UV-B radiation, however the present UV-B research was carried out in controlled environment growth rooms, with relatively low PAR (450 μmol m^{-2} s^{-1}). Therefore, in future studies, for fresh basil production all year round in glasshouses, experiments should be carried out under glasshouse PAR levels. Likewise, for field production, experiments should be carried out under ambient PAR levels at that location.

UV-A is not thought to have damaging photochemical activity, however Maffei and Scannerini (2000) found that in peppermint UV-A (360nm) stimulates the biosynthesis of some monoterpenes (i.e. menthol), which are suppressed by UV-B (280-320nm) and blue light (450nm). Therefore, further studies evaluating the effects of UV on basil plants should consider both UV-B and UV-A as well as account for spectral differences of UV-B.

In the present and two previous studies, between 1 and 3 h of supplementary UV-B light were used which was equivalent to a normal daily dose on a summer day in the Mediterranean. Future studies should include several daily doses of UV-B and a range of intensities.

The effects of natural UV-B on plants will be influenced by factors such as water stress, mineral deficiency, increased concentration of carbon dioxide and temperature (Maffei and Scannerini, 2000; Kakani *et al.*, 2003), therefore the interactions of UV-B radiation with these factors should be studied.

The increase in the volatile oil content and the changes in oil composition associated with the use of supplementary red light require further investigation. Such a

manipulation of the light environment could be used easily and safely by growers to produce fresh basil for a regular supply of material all the year round.

Traditionally, basil leaves are used as an herbs, however a major use for basil is the production of volatile oils for use in the food and fragrance industries, as well as traditional herbal medications and aromatherapy. In recent years, the market for fresh aromatic herbs has expanded, with a significant shift away from traditional use of dried herbs towards fresh material, either grown by home gardeners or commercial companies. The sensory analysis showed that consumers preferred stronger intensity of volatile oils in fresh basil leaves. This suggests that research on basil should focus on both the maximisation of vegetative output and the maximisation of volatile oil content in fresh leaves to meet the requirements of the market.

The novel sensory analysis carried out should be improved. More consumer assessors should be used. Using more than 100 people would increase the chances of there being more than 30 assessors in each basil using group.

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Appendix 1: Cultivars of basil available in the UK

- **Basil Anise:** Decorative purple tinged foliage and pale pink flowers, and an aroma of liquorice. The seed stalks can be used for decoration, the leaves are excellent in sauces with fruit, juices, teas and salads adding their own flavour.
- **Basil Ararat:** Striking dark green foliage with purple markings. Sweet flavour, with a hint of liquorice. Ideal in salads, tomato dishes, as a spice, and as a garnish.
- **Basil Bush:** Needs warmth and shelter, grows well under glass/polytunnels. Small leaved cultivar, much used in Italian cooking. The flavour increases when cooked. It is soothing to the stomach. Good to grow alongside tomatoes to repel insects and aid growth.
- **Basil Cinnamon:** Vigorous plant from Mexico with a distinct flavour and a strong cinnamon aroma. Superb in salads, with vegetables and in soups, and also highly ornamental.
- **Basil Greek:** Improved bush basil, compact with tiny leaves and excellent as pot plant.
- **Basil Green Globe:** Much improved bush basil type, producing compact small, round bushes approx 70 cm across and height 40 cm. Good strong flavour. Excellent as an ornamental in pots and borders.
- **Basil Holy Green:** Low growing bush with musky aroma used in salads, cold dishes, in Thai cooking for stir fry with red hot chillies and meat dishes including pork, chicken and beef.
- **Basil Holy Red:** Stems reddish purple tinge and deep purple. Culinary herb.
- **Basil Lemon:** Bushy habit with citrus-scented leaves, ideal in tea and for flavouring meat, fish dishes and potpourri.
- **Basil Lettuce Leaved:** Large leaf cultivar, excellent for pasta sauce with a distinctive flavour. Ideal for lining salad cups.
- **Basil Lime:** Similar in habit to Lemon basil but with a zesty lime aroma, and

ideal in salads and fish dishes.

- **Basil Neopolitana:** Large, crinkled lettuce type leaf with a distinctive flavour.
- **Basil Nufar F1:** Possesses hybrid vigour and resistance to fusarium. Sweet Genovese type with broader leaves and superior flavour.
- **Basil Red Rubin:** Highly ornamental deep purple bronze leaves of outstanding taste and aroma.
- **Basil Ruffles Purple:** Serrated leaves, strong scent, very decorative with pinkish-purple flowers. Ideal in herb garden or border.
- **Basil Ruffles Green:** Serrated leaves, ideal for the herb garden or decorative border.
- **Basil Siam Queen:** Strain of Thai Basil with dark green leaves, deep purple flower stems and excellent spicy, anise/liquorice aroma and flavour. Excellent ornamental pot and border plant.
- **Basil Spice:** Very distinctive with dark green slightly hairy leaves, which are very aromatic. Long stems of pink flowers, which are very decorative when dried.
- **Basil Sweet Genovese:** Most commonly grown variety, tall with large leaves. Used in salads especially with tomatoes, pasta sauces, combines well with garlic. Requires a sunny sheltered position. By removing flowering shoots the plant will produce more shoots, which will increase the harvest of leaves. Excellent as a companion plant for tomatoes.
- **Basil Thai:** Dwarf anise type, used as a vegetable and in curries, mainly for Vietnamese and Thai cooking.
- **East Indian:** Large, robust variety with felt-like grey-green leaves. Strong clove scent and spicy flavour. Antiseptic, aromatic herb that repels insects, used medicinally for headache, fever and rheumatism. Culinary, a tea can be made from the infused leaves and used for flavouring.
- **Magical Michael:** Compact and very floriferous ornamental cultivar. Green foliage with attractive purple bracts and small creamy white flowers. Excellent as a foliage plant in mixed containers.

Appendix 2: Selection of basil genotypes

Plants of genotypes Basil Sweet Genovese (Sweet), Basil Bush (Bush), Basil Ruffles Purple (Ruffles Purple) and Basil Ruffles Green (Ruffle Green) were grown in Levington compost M2 in 12 cm pots in a glasshouse at 25°C (day) and 18°C (night). There were three replicates of 5 plants with one plant per pot. The experiment was set up on 5 November 2001. After 8 weeks, plant height, number of leaves, number of shoots, fresh weight and four major volatile oil compounds in leaves were measured.

Volatile oils were extracted by distillation using a reflux apparatus equipped with a 500 ml flask. Fresh leaf sample (20 g) was added to 150 ml deionised water and the mixture was hydrodistilled for 3 h. The distillate was extracted three times with freshly distilled ethyl ether. The solvent was then removed at room temperature, and the essential oil was diluted with 5 ml ethyl acetate, and stored in an airtight container in the dark at 4°C (Miele, *et al.*, 2001).

The content of volatile oils was measured by GCMS using a DB-5MS (0.25mm x 30m) column coupled directly to a quadrupole MS. The conditions were as follows: carrier gas, He; splitless; injection point, 240°C; oven programme, initial temperature of 40°C for 1 min, ramped at 20°C min^{-1} to 120°C, and ramped at 5°C min^{-1} to final temperature 210°C (Miele, *et al.*, 2001).

Plants of genotypes Sweet and Bush were taller (Plate A2.1 & Table A2.1), heavier and possessed more leaves than plants of Ruffles Green and Ruffles Purple. Ruffles Green and Ruffles Purple did not produce any lateral shoots (Table A2.1); leaves of Sweet contained more of the major volatile oils than the others, especially eugenol (Figure A1.1).

Plate A2.1 *Four cultivars, Bush, Sweet, Ruffles Green and Ruffles Purple (from left to right) 8 weeks after sowing.*

Table A2.1 Plant growth parameters 8 weeks after sowing

Genotypes	Plant height (cm)	Fresh weight(g)	Auxiliary Shoots	Leaves (pairs)
Sweet	41.4	115	8	7.1
Bush	41.5	121	13.7	7.9
Ruffles Purple	5.9	5.5	0	4.5
Ruffles Green	10.3	20	0	2.3
SED	1.750	2.110	0.475	0.351
p	0.001***	0.001***	0.001***	0.001***

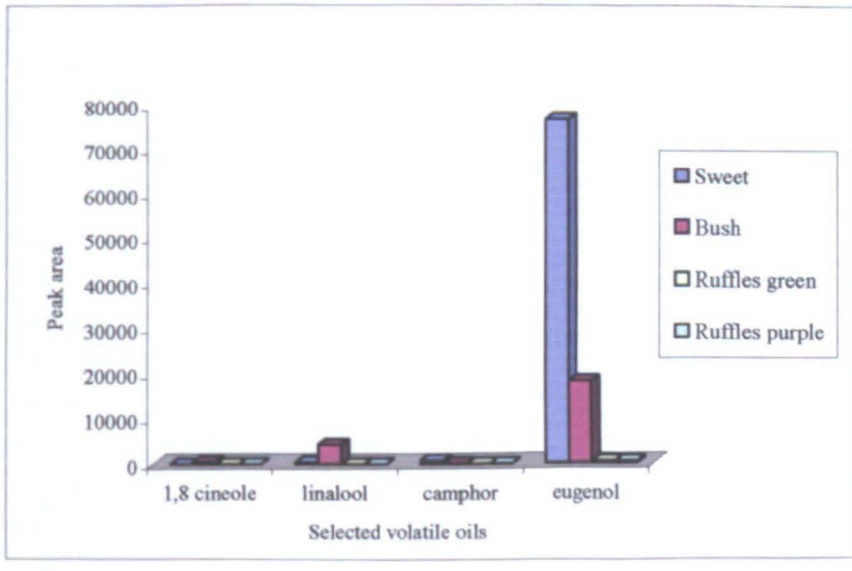


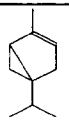
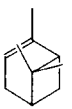
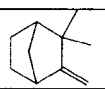
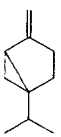
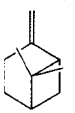
Figure A2.1 Peak area of selected volatile oils from leaves of four genotypes

Although differences in growth parameters between Bush and Sweet were not significant, plant height, leaf size and leaf shape of Bush were not uniform. So, Sweet was selected for use in the present study.

Appendix 3. Volatile compounds identified in this study

According to relative retention times, using standard samples and comparing peaks with library wiley7n.L, there were 36 volatile oil compounds identified in this study.

Table A3.1. Volatile compounds that were identified in present research

Common name	IUPAC name	CAS number	Retention time (min)	Chemical family	Molecular weight	Molecular formula	Molecular structure
α - thujene	Bicylo [3.1.0] hex-2-ene, 2-methyl- 5-[1-methylethyl]-	003387-41-5	4.165	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
α - pinene	Bicyclo [3.1.1] hept-2-ene, 2,6,6-trimethyl-	000080-56-8	4.275	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
Camphene	Bicyclo [2.2.1] heptane, 2,2-dimethyl-3-methylene-	000079-92-5	4.481	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
Sabinene	Bicycle [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-	003387-41-5	4.822	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
β-pinene	Bicycle [3.1.1] heptane, 6,6-dimethyl-2 methylene-	000127-91-3	4.872	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	

IUPAC: International Union of Pure and Applied Chemistry; CAS: Chemical Abstracts Service

Table A3.1 (Continued). Volatile compounds that were identified in present research

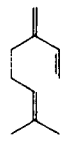
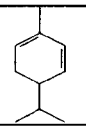
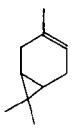
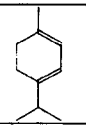
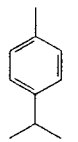
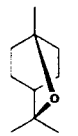
Common name	IUPAC name	CAS number	Retention time	Chemical family	Molecular weight	Molecular formula	Molecular structure
β-myrcene	1,6-octadiene, 7-methyl-3methylene-	000123-35-3	5.053	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
α - Phellandrene	1,3-cyclohexadiene, 2-methyl-5- (1-methylethyl)-	000099-83-2	5.254	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
δ.3-carene	Bicycle [4.1.0] hept-3-ene, 3,7,7-trimethyl-	013466-78-9	5.334	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
α -terpinene	1,3-cyclohexadiene, 2-methyl-4- (1-methylehtyl)-	000099-86-5	5.434	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
β-cymene	Benzene, 1-methyl-4- (1-methylehtyl)-	000099-87-6	5.530	*Monterpene hydrocarbon*	134.22	$C_{10}H_{14}$	
1,8-cineole	1,3,3-trimethyl-2 -oxabicyclo[2.2.2] -0ctane	000470-82-6	5.660	*Oxygenated monoterpene*	154.14	$C_{10}H_{18}O$	

Table A3.1 (Continued). Volatile compounds that were identified in present research

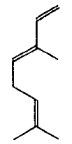
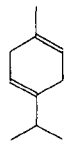
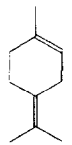
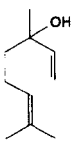
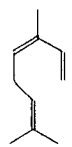
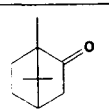
Common name	IUPAC name	CAS number	Retention time	Chemical family	Molecular weight	Molecular formula	Molecular structure
Cis-ocimene	1,3,7-octatriene, 3,7-dimethyl-	006874-10-8	5.725	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
γ-terpinene	1,4-cyclohexadiene, 2-methyl-4-(1-methylehtyl)-	000099-85-4	6.051	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
α -terpinolene	Cyclohexene, 1-methyl-4-(1-methylethylidene)	000586-62-9	6.377	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
Linalool	3,7-Dimethyl-1, 6 -Octadien-3-ol	000078-70-6	6.704	*Oxygenated monoterpene*	154.14	$C_{10}H_{18}O$	
β-ocimene	1,3,6-octatriene, 3,7-dimethyl-	013877-91-3	7.095	*Monterpene hydrocarbon*	136.13	$C_{10}H_{16}$	
Camphor	Bicyclo [2.2.1] heptan –2-one, 1,7,7-trimethyl-	000076-22-2	7.396	*Oxygenated monoterpene*	152.12	$C_{10}H_{16}O$	

Table A3.1 (Continued). Volatile compounds that were identified in present research

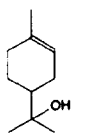
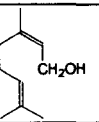
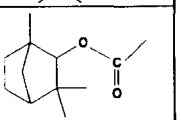
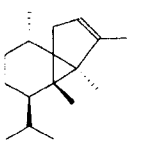
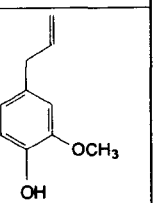
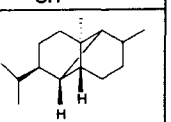
Common name	IUPAC name	CAS number	Retention time	Chemical family	Molecular weight	Molecular formula	Molecular structure
α –terpineol	3-cyclohexene-1- methanol, α, α, 4- trimethyl	000098-55-5	8.053	*Oxygenated monoterpene*	154.14	$C_{10}H_{18}O$	
Nerol	2,6-octadiene-1-ol, 3,7-dimethyl-(Z)	000106-25-2	8.966	*Oxygenated monoterpene*	154.14	$C_{10}H_{18}O$	
Fenchyl acetate	Bicyclo [2.2.1] heptan-2-ol, 1,3,3 – trimethyl-, acetate, endo-	004057-31-2	9.463	*Oxygenated monoterpene*	196.15	$C_{12}H_{20}O_2$	
α –cubebene	1H-cyclopenta [1,3] cyclopropa [1,2] benzene, 3a, 3b, 4, 5, 6, 7-hexahydro - 3, 7-dimethyl-4-(1-methylethyl)-	017699-14-8	10.376	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
Eugenol	2-methoxy-4-allylphenol	000097-53-0	10.486	*Phenyl-propanoid*	164.08	$C_{10}H_{12}O_2$	
α -copaene	Tricyclo [4.4.0.0.2.7] dec-3-ene, 1,3 -dimethyl- 8 - (1-methylethyl)-	003856-25-5	10.767	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	

Table A3.1 (Continued). Volatile compounds that were identified in present research

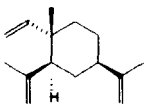
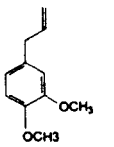
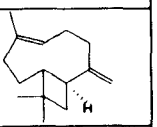
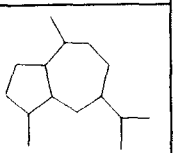
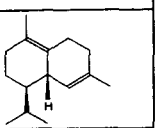
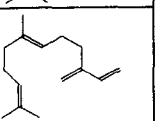
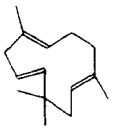
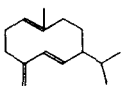
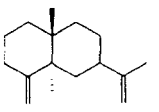
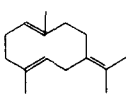
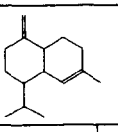
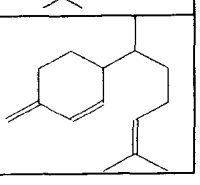
Common name	IUPAC name	CAS number	Retention time	Chemical family	Molecular weight	Molecular formula	Molecular structure
β-elemene	Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis (1-methylethenyl)-	000515-13-9	10.978	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
Methyleugenol	Benzene, 1,2-dimethoxy-4-(2-propenyl)	000093-15-2	11.103	*Phenyl-propanoid*	178.10	$C_{11}H_{14}O_2$	
Trans- caryophyllene	Bicyclo [7.2.0] undec-4-ene, 4,11,11 –trimethyl – 8 – methylene-, [1R-(1R*, 4E, 9S*)]-	000087-44-5	11.384	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
Trans-α-bergamotene	biocyclo[3.1.1] hept-2-ene, 2, 6-dimethyl –6- (4-methyl-3-pentenyl)-, [1S-(1.α., 5.α., 6.α.)]-	013474-59-4	11.570	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
α-guaiene	Azulene, 1, 2, 3, 4, 5, 6, 7, 8 –octahydro -1,4-dimethyl-7-(1-methylethenyl)-, [1S- (1. α., 4. α., 7. α.)]-	003691-12-1	11.625	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
δ-cadinene	Naphthalene, 1, 2, 3, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	000483-76-1	11.735	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
Trans-β- farnesene	1, 6, 10, - dodecatriene, 7, 11-dimethyl-3-methyl-3-methylene-	000502-60-3	11.769	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	

Table A3.1 (Continued). Volatile compounds that were identified in present research

Common name	IUPAC name	CAS number	Retention time	Chemical family	Molecular weight	Molecular formula	Molecular structure
α-humulene	1, 4, 8- cycloundecatriene, 2, 6, 6, 9-tetramethyl-, (E, E, E)-	006753-98-6	11.851	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
Germacrene-D	1,6-cyclodecadiene, 1-methyl-5-methylene-8- [1-methylethyl]-	023986-74-5	12.212	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
β-selinene	Naphthalene, decahydro-4a-methyl-1-methylene-7- (1-methylethyl)-	017066-67-0	12.297	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
Germacrene-B	1,5-cyclodecadinene, 1,5-dimethyl-8-[1-methylethylidene]-	015423-57-1	12.413	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
γ-cadinene	Naphthalene, 1,2,3,4, 4a, 5, 6, 8a- octahydro -7-methyl -4-methyl-	039029-41-9	12.614	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	
β- sesquiphellandrene	2-methyl-6-(4-methylenecyclohex-2-enyl)-2 - heptene	020307-83-9	12.714	*Sesquiterpene Hydrocarbons*	204.19	$C_{15}H_{24}$	

Appendix 4 Proposed network of metabolic pathways leading from D-glucose to 1,8-cineole, linalool and eugenol