FACTORS AFFECTING RIND OIL CONTENT OF LEMON

[*Citrus limon* (L.) Burm. f.]

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

SUMMARY

Essential oils are derived from volatile natural oils in plants and have been used by mankind for millennia. Citrus essential oils are widely used in various applications and lemon [*Citrus limon* (L.) Burm. f.] rind oil is the most important citrus oil in commerce. Rind oil glands are located in the exocarp, or flavedo, of the fruit and are formed schizogenously. The purpose of this study was to quantify the factors affecting rind oil content of lemons. The factors studied were light exposure and canopy position, growing region in South Africa, genotype, i.e. scion and rootstock, as well as the relationship between seedless clones of cultivars and the cultivars from which the seedless clones were derived, and various plant growth regulators were screened to determine whether they influenced rind oil content.

Following the sampling of fruit from different positions in the tree's canopy, light exposure was found to affect rind oil content of 'Eureka' lemon fruit. Fruit borne on the outside of trees, higher in the tree, north-facing or not within the hedgerow had the highest rind oil content. Photosynthetically active radiation data supports the hypothesis that rind oil content is correlated with light exposure. To optimise rind oil content of lemons, trees should not be too dense or too high as to overshadow the lower parts of adjacent trees.

South Africa has a diverse climate, and rind oil content from fruit produced in different growing regions was compared. 'Eureka' lemon fruit from Upington had the highest rind oil content in all seasons sampled. Fruit from Malelane and Marble Hall ranked second to Upington and rind oil content for fruit from Karino was intermediate. Rind oil content for fruit from Vaalharts was the lowest at each sampling time. When rind oil content was regressed against cumulative heat units there was a positive linear relationship in 2003, but in

2004 the relationship was weak. However, in general, rind oil content increased with increasing heat unit accumulation.

A large variation exists among citrus cultivars and rootstocks and their effect on fruit growth and quality. 'Limoneira 8A', followed by 'Cicily', 'Lisbon' and 'Genoa' had the highest rind oil content. 'Villafranca', 'Messina' and 'Yen Ben Lisbon' had the lowest rind oil content. Rind oil content from 'Eureka' lemon fruit was disappointingly low. Seedless cultivars, 'Eureka SL' and 'Lisbon SL', had ~18.0% higher rind oil content than the seeded cultivars from which they were derived. With regards to rootstock, fruit from lemon trees budded on non-invigorating rootstocks, e.g. X639 [*C. reshni* Hort. ex Tan. × *P. trifoliata* (L.) Raf.], had the highest rind oil content, whereas rind oil content was low on invigorating rootstocks such as rough lemon (*C. jambhiri* Lush.).

Synthetic gibberellins, cytokinins, ethylene and auxins were applied on lemon trees at different times and concentrations to screen their ability to enhance rind oil content. Of all the gibberellins and cytokinins applied, Promalin®, a combination of gibberellic acid 4/7 and benzyl adenine-phosphate, a cytokinin, had a small, but nonsignificant effect on rind oil content. Ethephon, which induces ethylene synthesis, affected rind oil content in 2004, when applied 8 weeks before harvest. However, ethephon and aminoethoxyvinylglycine (AVG, an ethylene biosynthesis inhibitor) had an inconsistent effect on lemon rind oil content. Auxins did not affect rind oil content. Further experiments should be conducted, especially on the timing and concentration of applied gibberellins, e.g. Promalin®, and ethephon.

OPSOMMING

Essensiële olies is vlugtige, natuurlike plantolies wat al vir eeue deur die mens gebruik word. Sitrus essensiële olies het verskeie toepassings en van hierdie sitrus olies is dié verkry uit suurlemoenskil [*Citrus limon* (L.) Burm. f.] die belangrikste. Skiloliekliere is in die eksokarp, of flavedo, van die vrug geleë en vorm skisogenies. Die doelwit van hierdie navorsing was om faktore wat die olie-inhoud van suurlemoenskil affekteer te kwantifiseer. Faktore wat bestudeer is sluit ligblootstelling en posisie in die boom, produksiearea in Suid-Afrika, en genotipe (bo- en onderstam) in. Ook is saadlose klone vergelyk met die kultivars waaruit dit ontwikkel is. Verskeie plantgroeireguleerders se effek op skilolie-inhoud is ook geëvalueer.

Ligblootstelling het skilolie-inhoud van 'Eureka' suurlemoene affekteer toe monsters van verskillende posisies in die boomtop vergelyk is. Vrugte aan die noorde- en buitekant, of hoër in die boom het die hoogste skilolie-inhoud gehad. Vrugte binne die plantry het minder skilolie bevat. Fotosinteties-aktiewe ligvlakmetings ondersteun die hipotese dat skilolie-inhoud korreleer met ligblootstelling. Vir opitmale skilolie-inhoud in suurlemoene is dit dus belangrik dat bome nie te dig of te hoog moet wees nie, sodat dit nie die onderste dele van aangrensende bome oorskadu nie.

Sitrus word in diverse klimaatstreke in Suid-Afrika verbou. Gevolglik is die skilolie-inhoud van vrugte uit verskillende produksieareas vergelyk. 'Eureka' suurlemoenvrugte uit Upington het met elke monsterneming die hoogste skilolie-inhoud gehad, gevolg deur vrugte uit Malelane en Marble Hall. Skilolie-inhoud van vrugte uit Karino was gemiddeld, terwyl vrugte van Vaalhaarts met elke monsterneming die laagste skilolie-inhoud gehad het. Regressie van skilolie-inhoud op kumulatiewe hitte-eenhede het 'n positiewe lineêre

verwantskap in 2003 getoon. Hoewel die verwantskap swakker was in 2004, neem skilolie-inhoud oor die algemeen toe met toenemende akkumulasie van hitte-eenhede.

Sitruskultivars en -onderstamme varieer aansienlik in groeikrag en vrugkwaliteit. 'Limoneira 8A', gevolg deur 'Cicily', 'Lisbon' en 'Genoa' het die hoogste skilolie-inhoud gehad, terwyl 'Villafranca', 'Messina' en 'Yen Ben Lisbon' die laagste skilolie-inhoud gehad het. Die skilolie-inhoud van 'Eureka' suurlemoene was teleurstellend laag. Die skilolie-inhoud van die saadlose kultivars, 'Eureka SL' en 'Lisbon SL', was ~18% hoër as die skilolie-inhoud van die kultivars waaruit dit ontwikkel is. Vrugte van bome wat op minder groeikragtige onderstamme geënt is, bv. X639 [*C. reshni* Hort. ex Tan. × *P. trifoliata* (L.) Raf.], het 'n hoë skilolie-inhoud gehad, terwyl vrugte van bome op groeikragtige onderstamme, bv. growweskilsuurlemoen (*C. jambhiri* Lush.), minder skilolie bevat het.

Sintetiese gibberelliene, sitokiniene, etileen en ouksiene is op verskillende tye en teen verskillende dosisse op suurlemoenbome toegedien om die effek daarvan op skilolie-inhoud te bepaal. Promalin® (GA_{4+7} en bensieladenienfosfaat) het 'n klein effek op skilolie-inhoud gehad, maar die effek was nie statisties beduidend nie. Ethephon, wat etileensintese induseer, het skilolie-inhoud in 2004 geaffekteer toe dit 8 weke voor oes toegedien is. Ethephon en aminoetoksievinielglisien (AVG, 'n etileenbiosintese inhibeerder) het egter nie 'n konstante effek op suurlemoen skilolie-inhoud gehad nie. Ouksiene het nie skilolie-inhoud geaffekteer nie. Verdere eksperimente is veral nodig op die toedieningstyd en konsentrasie van toegediende gibberelliene, bv. Promalin®, en ethephon.

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Language and style used in this thesis are in accordance with the requirements of the scientific journals of the *American Society for Horticultural Science*. This thesis presents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

Essential oils are derived from volatile natural oils in plants. The essential oil industry is one of the oldest industries, dating from 2000 BC (Weiss, 1997). Lemon [*Citrus limon* (L.) Burm. f.] essential oil has a wide range of uses and is mostly (60%) used as flavouring in soft drinks (Misitano, 2000; Morton, 1987), as well as flavouring in confectionary, perfumes, soaps and shampoos and for furniture polish and detergents. *d*-Limonene, the major component in citrus rind oils, is extracted from the oil and used as a biodegradable solvent in the electronic industry (Teeter, 1996).

Rind oil glands are located in the exocarp, or flavedo, of the fruit. These glands consist of a cavity, filled with oil, and a stalk, connecting the gland with the rind surface (Schneider, 1968; Spiegel-Roy and Goldschmidt, 1996; Ting and Attaway, 1971). Oil gland formation starts from two meristimatic cells in the ovary which differentiate into the gland and stalk (Bosabalidis and Tsekos, 1982a). The glandular cavity is formed schizogenously, i.e. cell initials separate to create an intercellular cavity lined with epithelial cells (Turner, 1999; Turner et al., 1998).

Rind oil is synthesised in plastids in the secreting cells via the terpene pathway. This pathway starts with three acetyl coenzyme A molecules which form mevalonic acid (MVA). MVA is converted to isopenthyl pyrophosphate (IPP), which is then isomerised to dimethylallyl pyrophosphate (DMAPP). From the IPP and DMAPP geranyl pyrophosphate (GPP) is formed, which is the basis from which most terpenes are synthesised, e.g. *d*-limonene (Bramley, 1997; McGarvey and Croteau, 1995). The plastids, in which the oil is synthesised, are closely arranged with the endoplasmic reticulum (ER). Oil moves along the ER to the plasmalemma and is transported through the apoplast to the central cavity of the oil gland (Bosabalidis and Tsekos, 1982b; Bosabalidis and Tsekos, 1986).

Rind oil has an anti-fungal and anti-bacterial protection mechanism in citrus fruit (Ben-Yehoshua et al., 1992; Klieber et al., 2000). Lemon fruit also show resistance to fruit flies (*Ceratitis* spp.), due to toxic effects on the eggs and larvae (Greany et al., 1983).

Lemon rind yields 4 to 10 kg of oil per ton of fruit (Sinclair, 1984). Various factors affect rind oil content of lemons. However, relatively little is known about factors affecting lemon rind oil content, and the factors known to influence citrus oil are not always constant. Within a fruit, oil content increases from the stem- to the stylar-end (Hendrickson et al., 1970; Sinclair, 1984). Fruit with thin peels yield more oil than fruit with thick peels (Quaggio et al. 2002; Sinclair, 1984). Maturity also plays a role in rind oil content. Fruit at colour-break have the highest oil content, after which oil content decreases (Combariza et al., 1994; Kesterson and Hendrickson, 1962). Oil content decreases during the winter, increases through the spring and is the highest in summer fruit (Crescimanno et al., 1988).

'Limoneira 8A' is the cultivar with the highest rind oil content (Foguet et al., 1996; Saunt, 2000). 'Bearss', also known as 'Sicilian' (Kesterson and Braddock 1977; Kesterson et al., 1974), and 'Feminello St Teresa' also have high rind oil content (Crescimanno et al., 1988; Saunt, 2000). Not much research has been done on the effect of rootstock on rind oil content, but 'Valencia' sweet orange [*C. sinensis* (L.) Osb.] on Savage and Troyer citrange rootstocks [*Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osb.] had the highest oil yield when compared to other rootstocks, e.g. rough lemon (*C. jambhiri* Lush.) (Bitters and Scora, 1970).

Within a cultivar, climate has the biggest influence on rind oil content (Misitano, 2000; Staroscik and Wilson, 1982). Inland fruit, grown in a desert-like environment have more oil than fruit grown in a cooler, coastal environment (Bartholomew and Sinclair, 1946). Nutrition has both direct and indirect effects on rind oil content. Applied N increased rind oil content, whereas K decreased rind oil content (Kesterson and Braddock, 1977; Kesterson et al., 1974; Quaggio et al., 2002). Indirectly, fertilisation affects fruit yield and fruit size (Du Plessis et al., 1975; Embleton et al., 1973; Embleton et al., 1967; Koo et al., 1974; Quaggio et al., 2002), which will influence rind oil yield.

Auxins have an effect on fruit size, but no known effect on rind oil content (El-Otmani and Ait-Oubahou, 1999; Hield et al., 1964; Monselise, 1979; Moss, 1975). Ethylene also has no known effect on citrus rind oil content but improves fruit colouring and size (El-Otmani and Ait-Oubahou, 1999). Gibberellins are the only plant growth regulator known to influence citrus rind oil (McDonald et al., 1997). Grapefruit treated with gibberellic acid had higher rind oil content (Wilson et al., 1990). However, this was not a consistent reaction.

Thus, the objective of this study was to quantify the effects of various factors that possibly affect lemon rind oil content. Factors investigated included different canopy positions and choice of scion and rootstock, lemon rind oil content from different climatic regions was compared, and various plant growth regulators were screened to determine whether they influenced rind oil content.

CHAPTER 2

REVIEW OF LITERATURE

Essential oils, volatile natural oil in plants, have been used by mankind for millennia. Literature from India, written about 2000 BC, describes aromatic substances which were used for religious and medical purposes. Dynastic Egypt is also well known for use of aromatic material in embalming and cosmetics (Weiss, 1997). The ancient Greeks and Romans extracted plant oils by placing flowers into glass bottles containing a high quality, fatty oil which were then placed in the sun, and later separated the fragrant oil from the solids (Urdang, 1948). Since these ancient times, the essential oil industry has shown many changes.

Citrus oil is widely used. Lemon [*Citrus limon* (L.) Burm. f.] rind oil is the most important citrus oil in commerce. Similar to other citrus oils, lemon rind oil is rich in *d*-limonene and has a unique character which makes it a commercially important essential oil, especially in the soft drink industry.

Almost the whole lemon fruit can be used commercially, either fresh, or processed (Morton, 1987; Sinclair, 1984). The juice is extracted for fresh or concentrated use. Seeds are used to produce seed oils or in animal feed. Lemon rind is used in products such as marmalade, candied peel and dehydrated peel. The albedo is rich in pectin, used in jams and jellies. The flavedo contains the oil glands from which essential oils are extracted. Essential oils are used as flavouring in beverages (60%) and confectionary (29%), in perfumes, soaps and shampoos and for furniture polish and detergents (Misitano, 2000; Morton, 1987). *d*-Limonene is extracted from lemon rind oil and used as a biodegradable solvent, replacing chlorofluorocarbons (CFCs) to clean circuit boards in the electronic industry (Teeter, 1996). World lemon oil production is ~5600 to 5800 tons per year (Misitano, 2000).

2.1 Rind anatomy and ontogeny of oil glands

Citrus fruit is botanically classified as a hesperidium berry, arising from the ovary. The fruit can be divided into endocarp, mesocarp and exocarp (Fig. 2.1). The endocarp, or carpel segments, is the edible portion of the fruit. The mesocarp, also called the albedo, is the white spongy part directly under the exocarp or flavedo (Schneider, 1968; Spiegel-Roy and Goldschmidt, 1996; Ting and Attaway, 1971). The outermost layer of the flavedo is the epidermis. Under the epidermis are layers of collenchyma and parenchyma cells in which oblate, spherical oil glands are located (Fig. 2.1). The glands vary in size from 0.2 to 1.0 mm in diameter.

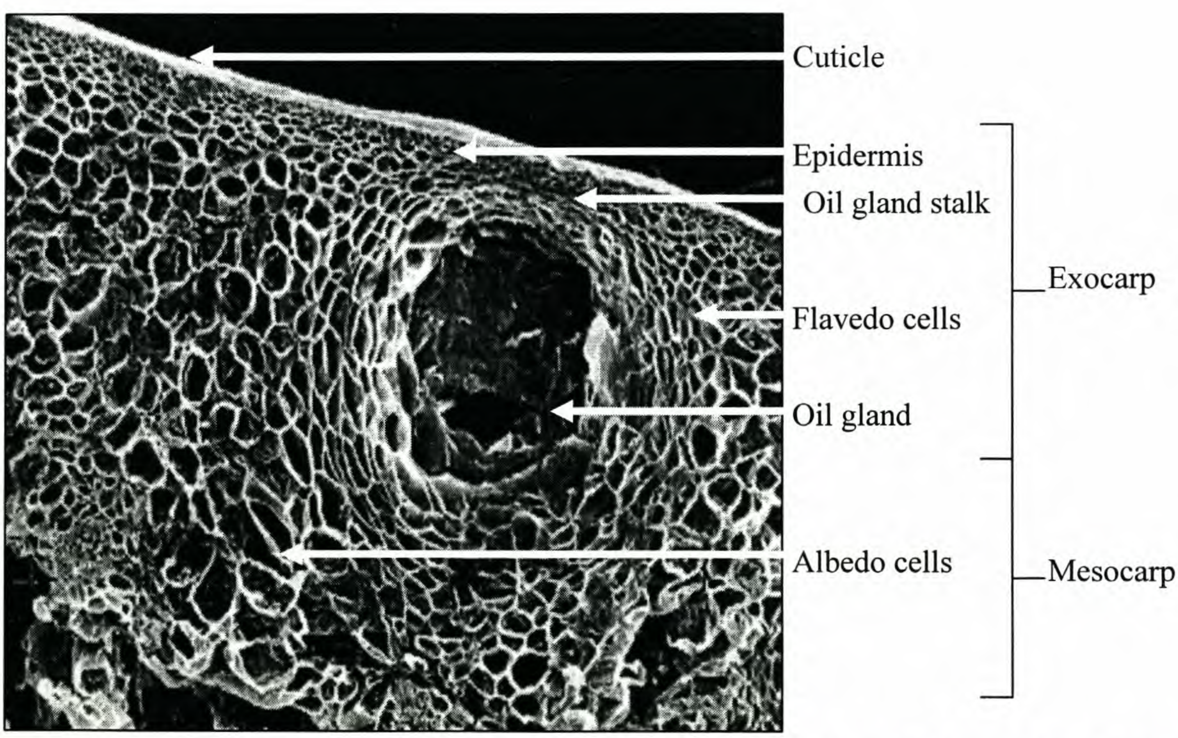

Fig. 2.1. SEM (x66) cross-section of *Citrus* rind from mature 'Murcott' tangor fruit (Spiegel-Roy and Goldschmidt, 1996). Oil glands are embedded within the flavedo and connected to the epidermis through a conical stalk (Bosabalidis and Tsekos, 1982a).

Oil glands are initiated before flowering in 'Navel' oranges [*C. sinensis* (L.) Osbeck] (Klieber et al., 2000), and are already observed in the ovary stage of lemon flower development (Ford, 1942). Oil gland ontogeny starts with two meristimatic cells in the ovary, an epidermal cell and the cell beneath it (Bosabalidis and Tsekos, 1982a). These cells differentiate into a conical stalk and a globular gland. The stalk connects the oil gland with the surface of the rind.

Knight and co-workers (2001) studied oil gland development in relation to fruit development in 'Washington Navel' oranges. They divided oil gland development into six phases (Fig. 2.2). Phase 1 is a cluster of up to ten cells adjacent to the epidermis which lack starch. Phase 2 is a larger cluster of cells (up to 30 cells). In phase 3 the cells begin to differentiate into boundary cells and inner cells. Phase 4 is a differentiated gland with flattened boundary cells enclosing polyhedral inner cells. Phase 5 is when the polyhedral cells' walls start to form a cavity, and Phase 6 is a fully mature oil gland with an expanded central cavity.

Oil glands are present in pre-anthesis floral ovaries and at various phases, up to phase 4. As fruit develop, more oil glands are initiated until ≈20 mm fruit diameter, when oil gland initiation ceased. When fruit are 32 to 52 mm in diameter all oil glands are fully developed. Between 52 and 88 mm fruit diameter, rapid oil gland enlargement was observed, which coincides with the cell growth period of fruit development. These observations confirm previous observations made by Bain (1958); oil glands enlarged and new ones were formed during the stage I of fruit development (cell division period). In stage II of fruit development (cell enlargement), oil glands increased in size and this continued through stage III (maturation period). Therefore, oil gland initiation occurs during the early stages of fruit

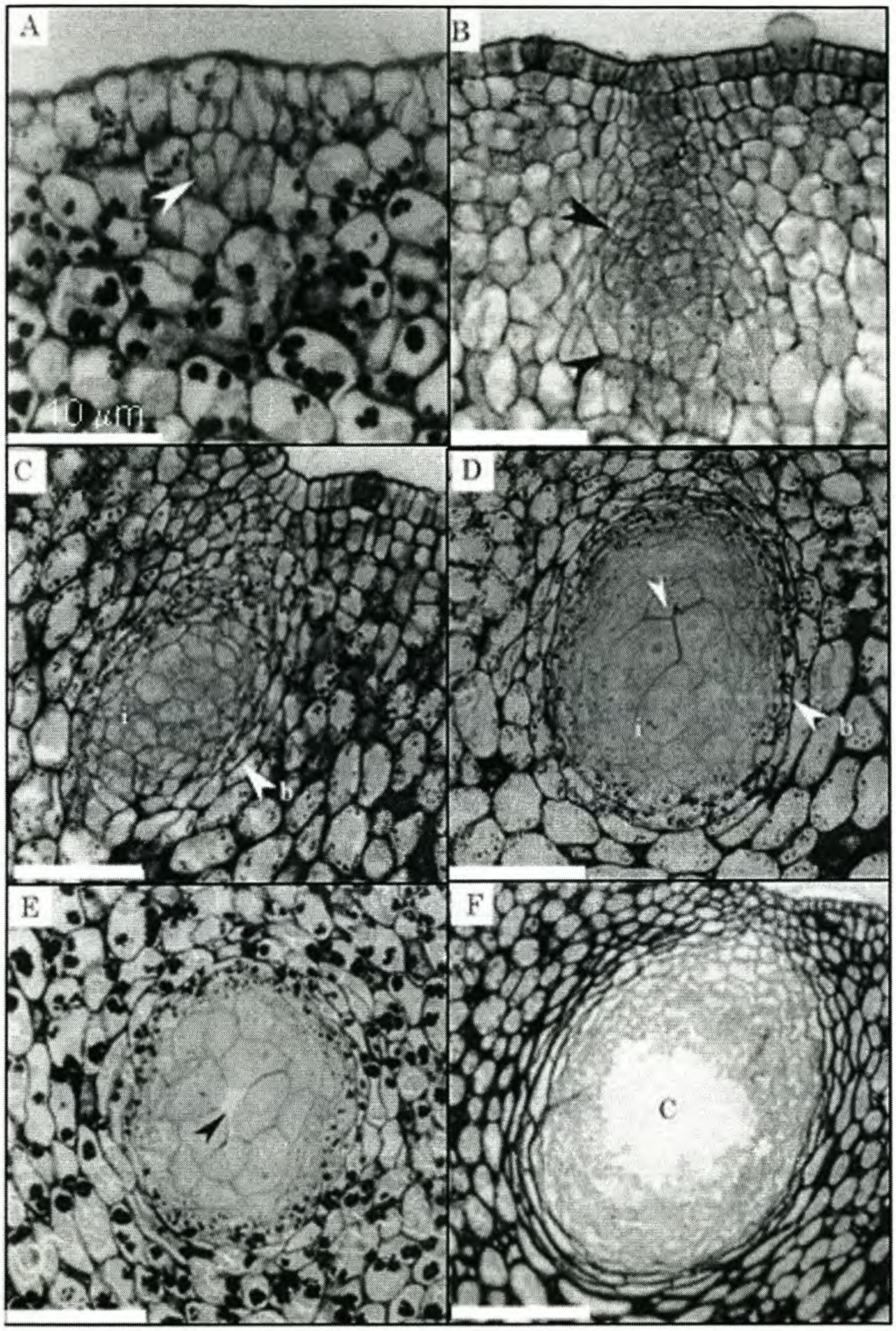

Fig. 2.2. Phases in oil gland development in 'Washington Navel' orange rind. A: Phase 1, Cluster of 10 cells; B: Phase 2, Larger cluster of cells (up to 30 cells); C: Phase 3, Cells differentiate into boundary and inner cells; D: Phase 4, Differentiated gland with flattened boundary cells enclosing polyhedral inner cells; E: Phase 5, Polyhedral cells' walls start to form the cavity; F: Phase 5, Fully mature oil gland with an expanded central cavity and short stalk that connects the oil gland with the surface of the rind (Knight et al., 2001).

development and all oil gland were mature when fruit were still immature, but continued to enlarge throughout fruit growth.

There are three ways in which oil gland cavities develop (Turner, 1999; Turner et al., 1998). Firstly, a cavity can form following the separation of cell initials to create an intercellular space, lined with epithelial cells, i.e. schizogenously. Secondly, a group of mature, secretion-filled cells undergoes autolysis to release the secretion as they degenerate, i.e. lysigenously. Thirdly, a combination of the first two, i.e. schizolysigenously; the cavity starts to form schizogenously and increases in size through autolysis of glandular calls. However, there is much controversy on the subject. After further investigation, Turner (1999) and Turner et al., (1998) claimed that lysigeny in citrus (especially in lemon) is found to be a fixation artifact caused by osmotic swelling of glandular cells immersed in hypotonic fixation solutions, and that cavities in citrus rind oil glands form shizogenously.

When the oil gland is fully developed, but before the cavity opens, the number of plastids in glandular cells increases dramatically (Bosabalidis and Tsekos, 1982a). The endoplasmic reticula (ER) and mitochondria are also highly developed. The stage when oil secretion starts coincides with the time when the cavity starts to form. Oil droplets are produced in the plastids. ER-cisternae arrange closely around the plastids. ER-membranes fuse with the plasmalemma and the oil droplets are transported via the apoplast to the central cavity of the oil gland (Bosabalidis and Tsekos, 1982b; Bosabalidis and Tsekos, 1986; Shomer, 1980).

2.2 Composition of citrus rind oil

Citrus oils are composed of monoterpene hydrocarbons, sesquiterpene hydrocarbons, aldehydes, ketones, oxides, esters and alcohols (Usai et al., 1996). Up to 100 constituents can be identified in citrus oils (Boelens and Jimenez, 1989). However, monoterpenes are the major components of citrus oils and constitute 92 to 99% of the total volume of oil (Boelens and Jimenez, 1989). The dominant monoterpenes in citrus are *d*-limonene (60% to 95.6%), β-pinene (1% to 15%), α-pinene (0.4% to 2.5%), and sabinene (Boelens, 1991; Boelens and Jimenez, 1989).

The composition of citrus oil differs among species resulting in the characteristic flavour of each fruit type. Shaw (1979) reported values for the all components in cold-pressed citrus oils (Table 2.1) for sweet orange, lemon, grapefruit (*C. paradisi* Macf.), mandarin (*C. reticulata* Blanco) and Mexican lime [*C. aurantifolia* (Christn.) Swing.]. Ayedoun et al. (1996) found that in lemon rind oil, limonene was the major constituent (50% to 85%), with γ-terpinene (≈12%) and β-pinene (≈8%) being the other two main components.

Table 2.1. Comparison of cold-pressed rind oil components (%) for different *Citrus* species (Shaw, 1979).

Citrus type	*d*-Limonene	Citral	α-Pinene	β-Pinene	γ-Terpinene
Sweet orange	83 – 97	0.05	0.1 – 0.6	–	0.1
Lemon	54 – 76	2.0 – 2.6	0.4 – 2.5	2.2 – 9.8	2.9 – 11.8
Grapefruit	86 – 95	0.05	0.2 – 0.6	–	0.5 – 0.8
Mandarin	65 – 90	–	0.3 – 2.5	0.9 – 2.1	2.1 – 4.0
Lime	47	3.1 +	1.2 – 2.4	10.1 – 11.9	7.3 – 16.3

Climate, which implicates growing region, has a large influence on rind oil composition (Misitano, 2000). Staroscik and Wilson (1982) concluded that "despite the uncontrolled variables, the distinct differences in geographical, and hence climatic, origin of the fruit seems to correlate well with the distinct differences in chemical composition of the expressed oils for lemons". They showed that β-pinene content were higher in lemons from coastal areas (California) than desert areas (Arizona), whereas limonene content were higher in desert-grown lemons than lemons from coastal areas. Boelens and Jimenez (1989) compared citrus oils from Italy and Spain. Italy (Sicily) is a coastal area, whereas Spain (Andalucia) is a desert-like area. They found Italian (coastal) oils had a higher concentration of α- and β-pinene than the corresponding oils from Spain, whereas oil from Spain (desert-like) had more limonene.

As fruit mature, the composition of the rind oil changes. In 'Navel' oranges, *d*-limonene content decreased over the season, α-pinene increased and β-myrcene also decreased (Scora et al., 1969). Attaway et al. (1967) showed that *d*-limonene increased early in the season and then stayed fairly constant in rind oil of mandarins, grapefruit and oranges. α-Pinene in grapefruit and oranges increased, and β-pinene and γ-terpinene in grapefruit decreased through the season.

Oil composition also varies among cultivars. Usia et al. (1996) showed limonene varied between 70.1% and 76.5% for various lemon cultivars grown in Sardinia, Italy. For example, oil from 'Santa Teresa' lemon had 8.7% more monoterpenes than oil from 'Interdonato' lemon. Usia et al. (1996) further stated that fruit from one bloom were highly variable and that fruit from different blooms had a greater difference in rind oil composition than fruit from different cultivars for the same bloom.

2.3 Terpenoid biosynthesis

As mentioned above, citrus rind oil contains mostly monoterpenoids. The monoterpenoids can be divided into four structural groups, namely acyclic, cyclopentanoid, cyclohexanoid and irregular monoterpenoids (Bramley, 1997), and are built from C_5 isoprene units. Limonene, the largest component of citrus rind oil, is an example of a cyclohexanoid monoterpene. Monoterpenes are exclusively synthesised in plastids. The terpene pathway starts with the fusion of three acetyl coenzyme A molecules (acetyl-CoA) to form hydroxymethylglutaryl-CoA (HMG-CoA) (Bramley, 1997; McGarvey and Croteau, 1995). This fusion occurs in two steps and is catalysed by two separate enzymes; acetoacetyl CoA thiolase and HMG-CoA synthase (Fig. 2.3). HMG-CoA is reduced by HMG-CoA reductase in two steps, each requiring nicotinamide adenine dinucleotide phosphate (NADPH) and mevalonic acid (MVA) is formed. MVA is converted to isopentenyl pyrophosphate (IPP) in three steps, each requiring an adenosine triphosphate (ATP) molecule. In the first two steps, MVA is phosphorylated by MVA kinase and mevalonate-5-phosphate kinase. The third step also requires a bivalent metal ion (Mg^{2+} or Mn^{2+}) to form IPP through decarboxylation. The enzyme in the third step is mevalonate-5-diphosphate decarboxylase. Several enzymes in monoterpene synthesis require divalent metal ions as a cofactor, and Mn^{2+} is preferred to Mg^{2+} (Lücker et al., 2002). It was also found that K^+ inhibited lemon enzymes (Lücker et al., 2002). IPP is isomerised to dimethylallyl pyrophosphate (DMAPP) by IPP isomerase. Isoprene, the simplest terpenoid, is synthesised directly from DMAPP. To form limonene, DMAPP and IPP condense to form geranyl pyrophosphate (GPP), catalysed by the enzyme prenyl transferase. GPP can combine with further IPP molecules to form higher terpenoids (Bramley, 1997; McGarvey and Croteau, 1995). When GPP loses the pyrophosphate group and is further oxidated, citral is formed. However, GPP is ionised and isomerised to form

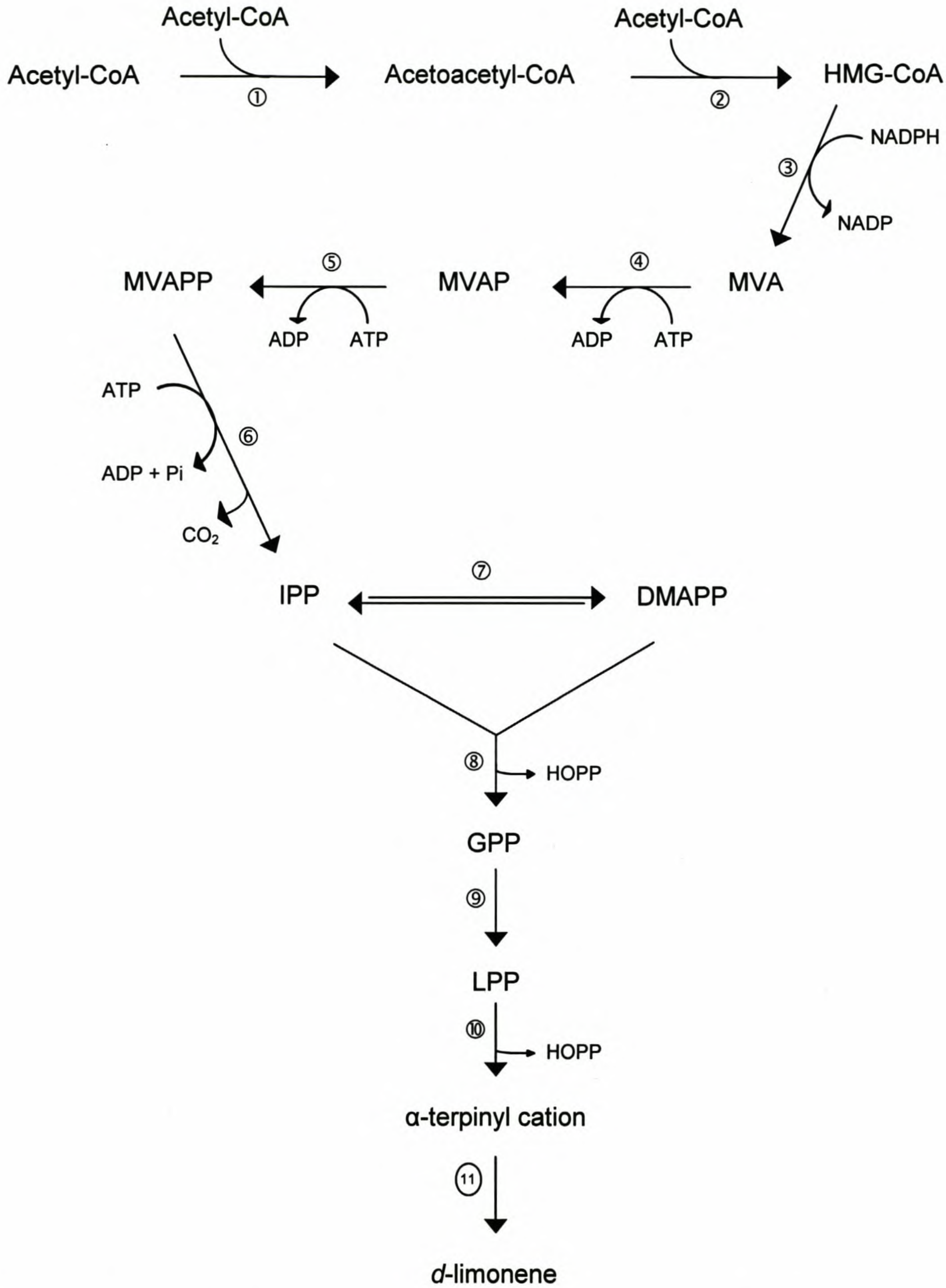

Fig. 2.3. Biosynthetic pathway of *d*-limonene synthesis. HMG-CoA = hydroxymethylglutaryl-CoA; MVA = mevalonic acid; MVAP = mevalonate-5-phosphate; MVAPP = mevalonate-5-pyrophosphate; IPP = isopentenyl pyrophosphate; DMAPP = dimethylallyl pyrophosphate; HOPP = pyrophosphate; GPP = geranyl pyrophosphate; LPP = linalyl pyrophosphate. Enzymes are: 1 = acetoacetyl CoA thiolase; 2 = HMG-CoA synthase; 3 = HMG-CoA reductase; 4 = MVA kinase; 5 = MVAP kinase; 6 = mevalonate-5-diphosphate decarboxylase; 7 = IPP isomerase; 8 = prenyl transferase; 9 = GPP isomerase; 10 = prenyl cyclase; 11 = limonene synthase (after Bramley, 1997; McGarvey and Croteau, 1995; Waterman, 1993).

linalyl pyrophosphate (LPP) (McGarvey and Croteau, 1995). LPP is ionised to undergo cyclisation, by a prenyl cyclase enzyme (Waterman, 1993) to yield the enzyme-bound α-terpinyl cation, a highly reactive universal intermediate of the cyclisation reactions. When limonene synthase catalyses the next reaction, limonene is the principle product and in addition, smaller amounts of myrcene and α- and β-pinene are also produced (McGarvey and Croteau, 1995).

2.4 Function of rind oil in citrus

Rind oil does not seem to have a physiological function within the rest of citrus plants, as it is stored in extra-cellular glands. However, there is an apparent defence mechanism role. Braverman (1949) suggested that rind oil might act as protection against insect attack, but was more satisfied to regard it as a waste product. Ben-Yehoshua et al. (1992) and Klieber et al. (2000) found citrus rind oil contained anti-fungal compounds, such as citral. The essential oils are known to prevent microbial diseases in the fruit and the oils have a toxic effect on fungi and phytopathogenic bacteria (Arras, 1999). When citrus fruit are mechanically damaged, rind oil and its anti-fungal compounds are released to the surface and underlying tissue. These cells collapse, observed as oleocellosis, and form a barrier against fungal attack (Klieber et al., 2000). Citrus, especially lemons, also show some resistance to fruit flies (*Ceratitis* spp.). Rind oil is toxic to fruit fly eggs and larvae (Greany et al., 1983). Greany et al. (1983) found lemons were virtually immune to fruit fly attack compared to other citrus, such as grapefruit, which are highly susceptible to fruit fly attack. Lemons have a thicker flavedo layer, more oil per unit area of rind, and, most important, lemons have a higher concentration of oxygenated terpenoids in the oil (limonene: linalool ratio), than, for example, grapefruit.

As fruit mature, the anti-fungal, anti-bacterial and insect-repellent properties of rind oil decline, because the concentration of the compounds conferring these properties decline. Therefore, once the initial basic ecological requirement to ensure preservation of the species has been met in terms of fully developed seeds, the required defence mechanism apparently becomes less important.

2.5 Factors affecting rind oil content

Rind oil yield of lemons varies from 4 to 10 kg·ton^{-1} of fruit (Sinclair, 1984), but usually falls within the 5.5 to 7.5 kg·ton^{-1} range. Numerous factors affect rind oil content, some of which are controllable, but others are not. A summary of these factors and their affects on rind oil content is presented.

2.5.1 Physical fruit characteristics

2.5.1.1 *Fruit size*

Large fruit yield more rind oil than small fruit when oil content is expressed on a fruit surface area basis. But when expressed on a percentage by fruit weight basis, small fruit yield more oil because they have greater fruit surface area than large fruit (Bartholomew and Sinclair, 1946; Sinclair, 1984).

2.5.1.2 *Within-fruit variation*

Within individual lemon fruit, there is less oil in rind from the stem-end than from the stylar-end of the fruit, and oil content progressively increases from the stem-end to the stylar-end (Hendrickson et al., 1970; Sinclair, 1984). In the northern hemisphere, 'Valencia' orange peel discs had higher oil content on the southern side of fruit (Hendrickson et al., 1970). Fruit with thin rinds yield more oil than fruit with thick rinds, because thicker rinds have thicker albedo and the spongy albedo tends to absorb oil during the oil extraction process (Quaggio et al., 2002; Sinclair, 1984). Firmer fruit also yield more oil than flaccid (over-ripe) fruit, because oil glands under high turgor pressure release oil more readily (Sinclair, 1984).

2.5.1.3 *Stage of maturity*

Lemons at the silver-green stage (nearly mature) contain more oil than lemons of other stages of development (Sinclair, 1984). Rind oil content in young fruit are low and increase as the fruit mature until the silver-green stage (colour-break), when it reaches a maximum, and decrease as the fruit becomes mature and over mature (Kesterson and Hendrickson, 1962). Combariza et al. (1994) sampled lemon fruit at three stages of maturity, green, greenish-yellow and yellow-orange rind colouration, and found that maturity affects the concentration and composition of rind oil; limonene concentration, and other monoterpenes, reached a maximum in fruit of greenish-yellow colouration, and declined thereafter.

As a result of the above variation in rind oil content, it is imperative that fruit sampling methodology minimises this variation when controlled experiments are conducted on rind oil content.

2.5.2 Seasonal

In Italy, lemon fruit sampled in summer (May to Sept.) had the highest rind oil content, oil content decreased from Nov. to Feb. in winter samples, and then increased in spring samples (Feb. to May). Seasonal variation also has an influence on rind oil composition (Crescimanno et al., 1988). Sinclair (1984) reported that as fruit mature (from Aug. to Dec.), rind oil yield per ton of fruit decreases (mostly because fruit were larger), although oil yield per fruit increased with fruit development and maturity.

2.5.3 Genetic

Kesterson and Braddock (1977) compared 21 lemon selections on a single rootstock and Kesterson et al. (1974) compared eight lemon selections. In both experiments 'Bearss' lemon had the highest rind oil content, followed by 'Lisbon'. 'Bearss', also known as 'Sicilian', is known to have high rind oil content (Morton, 1987; Saunt, 2000).

Another lemon cultivar reported to have high rind oil content is 'Limoneira 8A', a 'Lisbon' selection (Saunt, 2000). When comparing 11 lemon cultivars and 10 selections, Foguet et al. (1996) showed 'Limoneira 8A' had the highest rind oil content, followed by 'Bearss', 'Genoa' and 'Feminello St Teresa'.

In Italy, 'Feminello' lemon had the highest rind oil content compared to 'Monachello' and 'Eureka' (Crescimanno et al., 1988). 'Feminello St Teresa' is also planted in Tucumán, Argentina, for its high rind oil yield (Saunt, 2000).

Rootstock influences vigour, fruit size and quality (Barry et al., 2004a; Barry et al., 2004b), and the choice of rootstock depends on soil type, climate and resistance to viruses, pathogens and nematodes (Castle, 1987). Rough lemon (*C. jambhiri* Lush.) is the rootstock most commonly used for lemons in South Africa. Citrus trees on rough lemon rootstock grow rapidly and are highly productive, yielding large fruit with low internal quality under sweet oranges and mandarins (Castle, 1987). Six rootstocks with 'Eureka' lemon and 12 with 'Lisbon' lemon were evaluated for fruit yield at Addo Research Station, South Africa (Koekemoer et al., 2004). With 'Eureka' as scion, X639 [*C. reshni* Hort. ex Tan. × *Poncirus trifoliata* (L.) Raf.] and MxT [(*C. reticulata* Blanco × *C. paradisi* Macf.) × *P. trifoliata* (L.)

Raf.] had the highest fruit yield, whereas rough lemon and citremon [*P. trifoliata* (L.) Raf. × *C. limon* (L.) Burm. f.] had the lowest. MxT also had the highest yield with 'Lisbon', but X639 had the lowest. Carizzo citrange [*P. trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck] and rough lemon also had low yield.

Numerous studies have shown the effects of rootstock on many fruit quality variables, but few studies report on rootstock effects on lemon rind oil content. Bitters and Scora (1970) tested 'Valencia' orange on 25 rootstocks for differences in rind oil content. 'Valencia' orange budded on Savage and Troyer citrange rootstocks had the highest rind oil content, whereas on rough lemon it had the second lowest rind oil content. When 'Bearss' lemon was budded on seven rootstocks, there was no difference in rind oil content among rootstocks (Kesterson and Braddock, 1977; Kesterson et al., 1974).

2.5.4 Environmental

Despite the various uncontrolled variables of, for example, microclimate and soil type, and their affects on rootstocks choice, and irrigation and fertilizer requirements, the distant differences in geographical origin of lemon fruit are considered to play a central role in differences in rind oil content among growing regions.

As stated previously, climate, which implicates growing region, has the largest influence on rind oil composition and content (Misitano, 2000; Staroscik and Wilson, 1982). Bartholomew and Sinclair (1946) compared rind oil content of 'Navel' and 'Valencia' oranges from various areas in southern California and found rind oil content to be highest in inland fruit and progressively lower in areas grown closer to the coast. In South Africa, rind oil content was higher for 'Eureka' lemon sampled from Messina (hot, dry inland climate) than for Addo (cool, coastal climate) (Barry 2000). Barry (2000) also sampled 'Eureka' lemon from various production areas throughout the country and found cooler regions generally produced fruit with lower rind oil content.

2.5.5 Nutritional

Nutrition can have direct and indirect effects on rind oil content. Direct effects of fertilisation on rind oil content have been shown, e.g., increased N application increased oil yield, whereas increased application of K decreased oil yield (Kesterson and Braddock, 1977; Kesterson et al., 1974). High P applications, which might lead to excessive accumulation in the soil, decreased rind oil content (Quaggio et al., 2002). Quaggio et al. (2002) also found that N application of higher than 100 $kg \cdot ha^{-1}$ caused a sharp decrease in rind oil content of lemon compared to rates of 20 $kg \cdot ha^{-1}$. Potassium had a less pronounced, but still decreasing effect on rind oil content. However, when rind oil yield was expressed per production area, total rind oil production (per hectare) increased with higher K applications due to positive effects of K on fruit size and yield.

Fertilisation may also influence rind oil content indirectly via the effects of mineral nutrients on fruit yield, fruit size and rind condition i.e. rind colour, thickness or creasing. For example, increased N application increased fruit production (Koo et al., 1974), fruit were greener (Embleton et al., 1973; Koo et al., 1974) and smaller (Du Plessis et al., 1975; Embleton et al., 1973; Quaggio et al., 2002). Increased K resulted in increased fruit size (Du Plessis et al., 1975; Embleton et al., 1973; Embleton et al., 1967; Quaggio et al., 2002). Phosphorus had no effect on fruit size (Du Plessis et al., 1975; Embleton et al., 1973; Quaggio et al., 2002), but decreased rind thickness (Embleton et al. 1973).

2.5.6 Other cultural practices

Fruit position within a tree canopy also affects fruit quality (Barry et al., 2000; Morales et al., 2000; Reitz and Sites, 1948; Syvertson and Albrigo, 1980), which can be managed by row orientation, tree architecture (pruning) and tree spacing. Spacing affects tree growth and fruit yield. When citrus trees are closely spaced, the lower parts of the trees are shaded which reduces fruit yield and leaf oil content (Weiss, 1997). Spacing might also influence rind oil content through the effects of tree spacing on light interception, but no reports were found to support this speculation. Kesterson and Braddock (1977) found increased frequency of irrigation also increased lemon oil yield ($kg \cdot ha^{-1}$), although this may be an indirect effect through increased fruit yield.

2.5.7 Hormonal

2.5.7.1 *Gibberellins and cytokinins*

Gibberellins (GA) applied during flower initiation decrease the number of reproductive buds (Harty and Van Staden, 1988; Lord and Eckard, 1987; Monselise, 1979; Moss, 1975). When GA was applied to reproductive buds before a full set of sepals were initiated, 50% to 80% of the reproductive buds reverted to the vegetative state (Lord and Eckard, 1987). GA is also applied on flowers and young fruit to improve set, especially in parthenocarpic citrus cultivars (Monselise, 1979; Moss, 1975). GA delays fruit senescence, rind aging and softening, as well as rind pigmentation (Coggins et al., 1964; El-Otmani and Ait-Oubahou, 1999; Monselise, 1979). García-Luís et al. (1992) showed that GA delayed rind pigmentation and early applications reduced peel thickness at maturation, but had no effect on fruit growth. Cytokinins act in a similar way to GA in preventing degreening of fruit (Eilati et al., 1969).

Wilson et al. (1990) found that GA treatment increased rind oil concentration of grapefruit. McDonald et al. (1997) found significantly higher rind oil content in grapefruit treated with GA in the second year of their experiment, but did not find any differences in the first year. Barry (2000) repeated the experiment on lemons and found that GA treatment increased lemon rind oil content by 5.7%.

Growth-retardants, such as the triazoles and prohexadione-Ca, are gibberellins biosynthesis inhibitors, and have been shown to enhance flowering in citrus (Harty and Van Staden, 1988; Monselise, 1979). However, this group of plant growth regulators is not used commercially in citrus production.

2.5.7.2 *Ethylene*

Ethephon, which stimulates ethylene synthesis, causes fruit thinning when applied during the post-bloom period thereby improving fruit size (El-Otmani and Ait-Oubahou, 1999; Moss, 1975). Ethephon can also be used to improve rind colour (El-Otmani and Ait-Oubahou, 1999). However, these effects might lead to lower rind oil yield through the relationships of lower rind oil yield with increasing fruit size (see 2.5.1.1.) and lower rind oil content with increasing fruit maturity, and indirectly rind colour, as discussed earlier (see 2.5.1.3.).

Aminoethoxy-vinylglycine (AVG) inhibits ethylene production by inhibiting ACC-synthase, which prevents ACC formation, one of the intermediates in ethylene biosynthesis. When studying the role of ethylene in mango (*Mangifera indica* L.) fruit aroma volatiles biosynthesis, Lalel et al. (2003) found that applied AVG decreased the production of aromatic volatile compounds. Ethephon-treated fruit had significantly higher total aroma volatile compounds than AVG-treated fruit. These volatile compounds include monoterpenes (limonene), sesquiterpenes and aldehydes. Although mango is a climacteric fruit, ethephon applied at specific stages of fruit development may affect lemon rind oil synthesis.

2.5.7.3 *Auxins*

Synthetic auxins, such as 2,4-dichlorophenoxyacetic acid (2,4-D), applied at colour-break prevent pre-harvest fruit drop (Hield et al., 1964; Monselise, 1979; Moss, 1975). 2,4-D, 2,4-Dichlorophenoxy propionic acid (2,4-DP) and 3,5,6-trichloro-2-pyridyloxy acetic acid (3,5,6-TPA) are used to increase fruit size (El-Otmani and Ait-Oubahou, 1999; Hield et al., 1964; Monselise, 1979; Moss, 1975;). On 'Satsuma' mandarin (*C. unshiu* Marc.), 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-TPA) both increased fruit size, and peak responses were obtained when applied 26 days after anthesis (Guardiola and Lázaro, 1987). There is no evidence in the literature to suggest that synthetic auxins directly affect rind oil content, although an indirect effect might be found through their affects on increasing fruit size.

2.6 Techniques to determine oil content

2.6.1 Clevenger method

The Clevenger method to determine recoverable oil makes use of an oil trap apparatus to distil the volatile oil fraction off the juice and quantify the oil distillate (A.O.A.C., 1980).

For example, to extract oil from juice, 1 L of juice is transferred to a 3 L Clevenger flask. The flask is connected to an oil trap and placed on a heating element. The stopcock is closed and the oil trap is filled with distilled water. The condenser is set in the trap so that it does not touch the side of the trap and cold water is run through it from the bottom to the top. The solution is brought to the boil, while stirring continuously with a magnetic stirrer. It should not be heated to such an extent that over 75% of the condenser is necessary for condensation of the vapours. It must be boiled for 1 hour at a rate that condensate approaches 50 drops per minute, but does not exceed that rate. After removing the condenser, the trap is left to cool to room temperature. Then the oil is lowered into the graduated portion of the separation trap. With the meniscus adjusted to zero, the amount of oil recovered is recorded. This value divided by 10 will give the recoverable oil in mL·100 mL juice (% by volume) (A.O.A.C., 1980). The disadvantages of the Clevenger method are the wide variation in results (Scott, 1968; Scott and Veldhuis, 1966) and it takes about 1.5 hours to complete one sample.

2.6.2 Scott (bromate titration) method

The Scott method is a quick and accurate method developed by Scott and Veldhuis in 1966 to estimate oil content by determining limonene content, the major component of citrus oils. This method is based on the quantitative combination of *d*-limonene with bromine in an acid solution (Scott and Veldhuis, 1966).

In the basic method, 25 mL juice is placed in a distillation flask with a few silicon-carbon chips and 25 mL isopropanol is added. The flask is connected to a condenser and boiled on an electric heater until completion of distillation, which is when 30 mL or more distillate is collected (≈5 to 10 minutes). The flask is removed from the heater. 10 mL HCl and methyl orange indicator are added. This is titrated with a standardised 0.025 N KBr-$KBrO_3$ solution, while stirring with a magnetic stirrer, until the endpoint is reached. The percent recoverable oil is calculated by multiplying the amount of titrant (after the blank value is subtracted) with 0.004 (A.O.A.C., 1980; Scott, 1968; Scott and Veldhuis, 1966). The isopropanol is added to the juice to ensure solubility and dispersion of oil in the flask and to facilitate rapid volatilisation and carry-over. In acid solution, bromate releases bromine, and therefore HCl is added to the titrant to acidify the solution. The bromine reacts quantitatively with *d*-limonene through saturation of the double bonds. At the endpoint, excess bromine completely destroys the colour of methyl orange (Scott and Veldhuis, 1966).

The advantages of the Scott method are that it is more precise than the Clevenger method (Scott, 1968) and it takes <0.5 hours to determine the oil content of a sample.

To determine the oil content in citrus rind, the Scott method was modified (Dickon Hall Products, 2001). Whole fruit are homogenised in a blender (Waring, Torrington, Conn., USA) with an equal weight of distilled water added. A 20 g sample is taken and the weight recorded. The sample is then distilled with 25 mL isopropanol and 50 mL distilled water, and the rest of the procedure is the same as described above for the determination of recoverable oil in a juice sample. The percentage available oil is calculated by the following equation:

$$\%\,\text{available oil} = \frac{(\text{Titre - blank})\times(0.01)\times(0.853)\times\text{CF}}{\text{Sample}\times 0.5}$$

Where blank = mL bromide-bromate solution used to cause the indicator to go clear when only 50 mL distilled water and 25 mL isopropanol are distilled and titrated;

0.01 = mL of *d*-limonene per mL of bromide-bromate solution;

0.853 = specific gravity of lemon oil;

CF = correction factor for the normality of the bromide-bromate solution;

Sample × 0.5 = sample weighed off into the boiling flask, divided by two, to compensate for the water in the sample.

Melendreras (1991) studied the influence of different fruit sampling methods on the yield of essential oil in lemon rind using the Scott method. Whole fruit, peeled shells and peel disks were compared, and it was found that the whole fruit and peel disks methods were "more sound" than the peeled shell method (possibly because some of the rind oil was lost during peeling) and a higher reproducibility index resulted when whole fruit were used, possibly due to variations found in rind oil content from the stem-end to the stylar-end.

2.7 Commercial extraction of citrus oils

2.7.1 Hand process or sponge-pressing method

The sponge-pressing method is the traditional method to extract citrus rind oil and is still used in Italy. This method yields the finest quality oil (Anon., 1984; Sinclair, 1984), but it is a very labour intensive and time-consuming process. It takes 4 to 5 hours to treat 100 kg of rind (Misitano, 2000). The reason for the high quality oil produced is because the oil never makes contact with the juice, which is acidic and enzymatic. Further, the oil is extracted in water, which reduces oxidation.

In the sponge-pressing method fruit is halved crosswise and the pulp is removed with a sharp spoon (rastello). The rind is then hand-squeezed, while turning the halved fruit into a cup-like sponge. The sponge is slowly saturated with the oil and pressed into a special jar (concolina). This jar has a depression just below the lip, which, when decanting the oil, holds the water and impurities back. This method yields 0.15% to 0.3% of the fruit weight (Braverman, 1949; Sinclair, 1984).

Since this method requires expertise and hard labour, efforts were made in the 1920s to mechanise rind oil extraction. Nevertheless, this method is still used in Italy to produce high quality oil for the perfume industry.

2.7.2 Mechanical extraction methods.

2.7.2.1 *Pipkin roll*

The Pipkin roll machine was first patented in 1931 and modified in 1935. It consists of two stainless steel drums, rotating in opposite directions. The rinds are passed between the rollers, which apply just enough pressure to puncture the oil glands, without rasping the rind. The drums are grooved, receiving the oil and keeping it out of reach of the rind and absorption of oil by the albedo. This extraction method is considered to be low-yielding (Braverman, 1949; Kesterson et al., 1971; Sinclair, 1984).

2.7.2.2 *Screw press*

A tapered screw presses the rind, after juice is extracted, against a perforated screen. The screen ruptures the oil cells to release oil from the flavedo. Usually water is sprayed on the screen to pick up oil-bearing liquors pressed from the rind. Oil yield is dependent on the surface area of rind coming in contact with the screen (Kesterson et al., 1971; Sinclair, 1984).

2.7.2.3 *Fraser-Brace excoriator*

Abrasive rollers remove the flavedo of whole fruit. Water is sprayed continuously onto the fruit and rollers to wash the abraded material away. The oil and water emulsion is passed over a screen to remove solid particles and then it is transferred to a settling tank to allow the oil emulsion to float to the surface of the water. The emulsion is then decanted and centrifuged. Oil yield from this machine is exceptionally good, but it has a limited capacity (Kesterson et al., 1971; Sinclair, 1984).

2.7.2.4 *AMC scarifier*

The AMC scarifier was developed by the American Machinery Corporation, USA, and it also operates by rasping the whole fruit. Fruit in a processing line, goes through rotating steel cylinders that are pierced so that sharp points puncture oil cells in the rind. A water mist in the cylinders washes the emulsion away and keeps the flavedo saturated to prevent re-absorption (Kesterson et al., 1971; Sinclair, 1984).

2.7.2.5 *Brown peel shaver*

The Brown peel shaver is manufactured by the Brown Citrus Machinery Corporation, USA. After juicing, the rind cups are fed into the machine. A knife slices the rind, separating the albedo and flavedo. The flavedo slice is flattened and passed through a knurled roll to release the oil in water (Kesterson et al., 1971; Sinclair, 1984).

2.7.2.6 *Brown oil extractor (BOE)*

The Brown oil extractor replaced the Brown peel shaver. This machine is installed at the start of the processing line. Fruit is passed over a series of rotating toothed rollers in a pool of water. The oil glands are punctured to release their oil into the water. The fruit move onto the juice extractors and the oil and water emulsion is separated and refined (Braddock, 1999; Sinclair, 1984). This process results in high oil yields.

2.7.2.7 *FMC Process*

The FMC process is manufactured by the Food Machinery Corporation, USA. Juice and oil is extracted simultaneously, thus minimising the space and energy for high rind oil yields. Fruit are placed in a cup, another cup descends onto the fruit and plugs are cut from the fruit through the bottom cups. As the upper cup descends further, the juice and inner fruit is forced out through the bottom tube for juice recovery. The rind is shredded, rupturing the oil glands. Water is sprayed over the rind, emulsifying the oil as it is released. The emulsion is then further processed (Braddock, 1999; Sinclair, 1984). This machine also has high oil yield and is used by many commercial citrus processing companies.

2.7.3 Refining and finishing cold-pressed oils

After rind oil is extracted from fruit and washed away with the waterbed, the emulsion is centrifuged to separate the oil from the water. The separated oils are blended and then "winterised" in tall stainless steel tanks at -10 °C for up to 3 weeks. This is done to let the natural waxes in the oil precipitate. The clean oil is then decanted and sealed in drums for distribution (Sinclair, 1984).

2.7.4 Other types of citrus oils

2.7.4.1 *Distilled oils*

Citrus oils can also be recovered by distillation. This is a by-product when concentrating juice, or oil can be distilled from pulp residue. Distilled oil varies in type and quality. Oil may be distilled by one of two methods. It can either be distilled under a vacuum or at atmospheric pressure. Essential oil that is steam-distilled at atmospheric pressure is of fairly good quality, but inferior to cold-pressed oil, or even to oil that has been steam-distilled under a vacuum (Kesterson et al., 1971; Sinclair, 1984). The reason for the differences in oil composition and quality is because distilled oil is exposed to air and is oxidised.

2.7.4.2 *Stripper oil*

Stripper oil is 90% *d*-limonene and is steam-distilled from press-liquor during manufacturing of citrus molasses (Kesterson et al., 1971; Sinclair, 1984).

2.7.4.3 *Essence oils*

Essence oils are obtained from juice during concentration. The essence oil is obtained by using a stripper column and condenser. The less-fragrant terpenes and sesquiterpenes are eliminated, leaving the flavour-bearing components (Kesterson et al., 1971; Sinclair, 1984).

2.7.4.4 *Petitgrain oil*

Petitgrain oil is obtained by steam distilling citrus leaves and twigs. Compared to other citrus oils with high *d*-limonene content, this type of oil has a citral content of 50% (Weiss, 1997).

CHAPTER 3

LIGHT EXPOSURE AFFECTS LEMON [*Citrus limon* (L.) Burm. f.] RIND OIL CONTENT

Abstract

Light distribution within a citrus tree's canopy is affected by row orientation, tree architecture, pruning, and tree spacing, as well as canopy position within the tree. The effect of light on rind oil content of lemon fruit [*Citrus limon* (L.) Burm. f.] is not known. Therefore, this study was conducted to quantify the effects of light exposure and canopy position on rind oil content, by quantifying within-tree variation in rind oil content of 'Eureka' lemon. Rind oil content was determined for fruit sampled through the canopy, from different heights in the tree and from different cardinal points around the tree. Fruit borne on the outside of trees, higher in the tree, north-facing or not within the hedgerow had the highest rind oil content. Photosynthetically active radiation (PAR) was also measured and correlated with rind oil content. Although the PAR measurements were not taken in the same season within which fruit were sampled, the PAR data supports the hypothesis that rind oil content appears to be correlated with light exposure. To optimise rind oil content of lemons, trees should not be too dense or too high as to overshadow the lower parts of adjacent trees.

Introduction

A citrus tree's canopy is very dense, which results in only a small percentage of its leaves being exposed to direct solar radiation. Only the outer 0.3 m of the tree canopy receives enough light to actively photosynthesise (Greene and Gerber, 1967; Jahn, 1979). Greene and Gerber (1967) and Jahn (1979) took light measurements throughout the tree canopy and found that at 1 m into the canopy photosynthesis was very low because most light was intercepted by the outer layers of leaves. At more than 1.3 m into the canopy there was less than 1% full sunlight. Citrus trees, however, can survive in these low light conditions, because the leaves are anatomically and physiologically adapted to low light conditions (Syvertsen and Smith, 1984).

Light distribution within a tree's canopy is affected by row orientation, tree architecture (pruning) and tree spacing as well as canopy position within the tree. Spacing affects tree growth and fruit yield. When citrus trees are closely spaced, the lower parts of the trees are shaded, which reduces fruit yield and leaf oil content (Weiss, 1997). Spacing might also influence rind oil content through the effects of tree spacing on light interception, but no reports were found to support this speculation.

Light effects on tree growth, fruit yield and fruit quality are well-known. Light exposure also affects juice quality in 'Valencia' sweet orange [*Citrus sinensis* [L.] Osbeck] (Barry et al., 2000; Morales et al., 2000; Reitz and Sites, 1948; Syvertsen and Albrigo, 1980). Differences in fruit quality for mandarin types (*C. reticulata* Blanco) borne on different canopy positions is also known (Verreynne et al., 2004). According to Reitz and Sites (1948) differences in juice quality among canopy positions can be attributed to differences in photosynthetically active radiation (PAR), however, this has not been quantified. Not all differences in juice quality may be due to differences in photosynthesis. These differences may also be caused by differences in sugar accumulation due to differences in water stress resulting in less dilution of sugars in canopy positions with higher radiation exposure (Syvertsen and Albrigo, 1980). In Florida, northeast lower canopy positions were compared with southwest upper canopy positions in 'Valencia' sweet orange trees. The mean maximum temperature was significantly higher in the southern canopy positions than in the northern positions (Barry et al., 2000). Annual mean temperature was also higher for the southern canopy positions.

No reports were found on the effects of light exposure on rind oil content of lemon [*C. limon* (L.) Burm. f.]. Therefore, this study was conducted to quantify the effects of light exposure and canopy position on lemon rind oil content by quantifying within-tree variation in rind oil content.

Materials and Methods

Site and plant material. 'Eureka' lemon on rough lemon [*C. jambhiri* Lush.] rootstock from River Farm, Simondium, Western Cape province, South Africa (33°52'S, 19°01'E; 164 m alt.) was used. To quantify the relationship between light exposure and rind oil content, three experiments were conducted over two growing seasons in three adjacent orchards of differing row orientation and tree size.

<u>Inside vs. outside and Upper vs. lower canopy positions.</u> Trees were planted in an east-west row orientation in 1981 with a 7 x 3 m spacing and are over 7 m high and almost 5 m in diameter.

<u>Cardinal points.</u> Trees were either planted in 1997 in an east-west row orientation with a 5 x 2 m spacing or in 1981 in a north-south row orientation with a 7 x 3 m spacing. The trees planted east-west were about 3.5 m high and 2.5 m in diameter, forming a hedge-row, whereas the trees planted north-south were 4 m high with a diameter of 4 m and were free-standing.

Treatments and data collection. Inside vs. outside canopy positions. To determine the variation in rind oil content within the tree canopy, from the outside to the inside, the trees were divided into five canopy positions. These canopy positions were: outer canopy (0.5 to 0.6 m from the outside), north and south; inner canopy (next 1 m to the inside), north and south; and the inner shell (1 m radius around the trunk). Fruit were sampled between 0.5 and 2 m height from each of the five canopy positions on 31 Mar. 2003 and 1 Apr. 2004.

Upper vs. lower canopy positions. The same trees as for the first experiment were used. The trees were divided into three canopy positions, viz. lower (0.5 to 2 m), middle (2.5 to 4.5 m) and upper (5 to 7 m). Samples were taken on 10 Apr. 2003 from both the northern and southern sides of the trees, resulting in six canopy positions.

Cardinal points. To determine the variation in rind oil content among the four cardinal points, samples were taken from the northern, southern, eastern and western sides of trees. Samples were taken in May 2003 and again in May 2004. In 2003, samples were only taken from the east and west on the north-south row-oriented trees.

For all three experiments, five fruit per canopy position from each of six single-tree replicates were sampled. All fruit sampled were of similar size and maturity (colour plate T6; CRI, 2004; Appendix 1). Fruit length and equatorial diameter were measured with a digital calliper, from which fruit volume and surface area-to-volume ratio (SA:V ratio) were calculated (Braddock, 1999). Rind oil content was determined using the modified Scott method (Dickon Hall Products, 2001; Scott and Veldhuis, 1966; Chapter 2) and calculated as percentage mass of the whole fruit.

PAR measurements. PAR was measured on 4 Aug. 2005 between 11:00 and 13:00 with a light meter (LI-250 light meter with a LI-190SA quantum meter, LI-COR, Lincoln, Neb., USA), which took point measurements integrated over 15 seconds. The measurements were taken at the same positions as fruit were sampled.

Statistical analysis. Rind oil content, fruit size and PAR were subjected to analysis of variance using the general linear model procedure of SAS (SAS Inc., Cary, N.C.), followed by mean separation using Fisher's LSD. Where fruit diameter or SA:V ratio was significantly different among treatments, analysis of covariance was conducted with fruit diameter and SA:V ratio as covariates.

Results

Inside vs. outside canopy positions. In 2003, rind oil content was highest in fruit from the northern outer canopy position, followed by the northern inner canopy (Table 3.1). Rind oil content was lowest in the inner shell and southern canopy positions (inner and outer), but did not differ significantly in these positions. In 2004, there was no significant difference in rind oil content among canopy positions. Although fruit size among canopy positions differed, after covariance analysis for fruit size, differences in rind oil content among canopy positions was still not significant ($P = 0.6330$).

Upper vs. lower canopy positions. Irrespective of canopy quadrant, rind oil content was affected by canopy height (Table 3.2). Fruit from upper and middle canopy positions had significantly higher rind oil content than fruit from lower canopy positions.

Cardinal points. Rind oil content of fruit borne on the sides facing the row (northern and southern quadrants) in an east-west row orientation, forming a hedge, was higher than the quadrants (eastern and western) within the hedgerow in both seasons (Table 3.3). Whereas, in a north-south row orientation, with more free-standing trees, rind oil content from fruit borne in the eastern and western quadrants were not significantly different in 2003. In 2004, when rind oil content was adjusted for fruit size (Table 3.3), fruit from the northern quadrant had the highest rind oil content, and fruit from the eastern and western quadrants the lowest rind oil content, but there was no difference in rind oil content in fruit from the eastern and western quadrants.

Relationship between PAR and rind oil content. Unfortunately PAR measurements were not taken in the same season within which fruit were sampled. Nevertheless, in an attempt to quantify the possible role of solar radiation on rind oil content of lemons, as evidenced by the association between canopy exposure and rind oil content, it was assumed that a point measurement of PAR on a typical sunny day would provide an indication of relative differences in PAR among canopy positions sampled.

There was a significant linear relationship between rind oil content and PAR in four of five examples (Table 3.4), with coefficients of determination ranging from 0.14 to 0.43, when the relationship was significant.

Discussion and Conclusions

Rind oil content of 'Eureka' lemon was higher in fruit from exposed canopy positions than in fruit from shaded or semi-shaded canopy positions. In the southern hemisphere, north-facing objects have the highest light exposure and, in this study, rind oil content was higher in fruit from the northern side of the tree than fruit from the southern side. Although the PAR measurements were not taken in the same season within which fruit were sampled, the PAR data supports the hypothesis that rind oil content appears to be correlated with light exposure.

The large, dense trees prevented light penetration and distribution within the canopy and the southern facing sides of the tree from receiving direct light. In the case of trees planted in an east-west row orientation in a hedgerow, the northern and southern sides of the tree were more exposed to light. The eastern and western sides were within the hedgerow and entwined with the next tree, resulting in poor light exposure.

It is not known how light exposure may affect rind oil content of lemon fruit, but it is tempting to speculate on some possible mechanisms. The relationship between higher light exposure and rind oil content may be associated with more carbohydrates being available for secondary metabolites entering the isoprenoid pathway. Alternatively, differences in rind oil gland size and density from fruit borne in exposed vs. shaded canopy positions may be comparable with anatomical differences found in sun vs. shade leaves (Syvertsen and Smith, 1984).

In conclusion, factors affecting light exposure of fruit, such as row orientation, tree architecture and tree spacing, appear to affect rind oil content. Fruit borne on the outside of trees, higher in the tree, north-facing or not within the hedgerow would have the highest rind oil content. Trees should not be too dense or too high as to overshadow the lower parts of adjacent trees. Further quantification of the relationship between PAR and rind oil content variation within a tree's canopy is required.

Table 3.1. Canopy position effects on rind oil content of 7 m tall and 5 m diameter 'Eureka' lemon trees, planted in 1981 in an east-west row orientation and spaced at 7 x 3 m (n=6, single-tree replicates).

	Rind oil content (%)	
Canopy position	31 Mar. 2003	1 Apr. 2004
Outer canopy, north	0.471 a[z]	0.542 NS
Inner canopy, north	0.445 b	0.496
Inner shell	0.413 c	0.491
Inner canopy, south	0.403 c	0.519
Outer canopy, south	0.405 c	0.500
P-value	0.0002	0.5759
LSD	0.025	0.060

[z]Means within a column followed by different letters are significantly different (P=0.10; LSD); NS = nonsignificant.

Table 3.2. Effect of canopy height and quadrant on rind oil content of 7 m high and 5 m diameter 'Eureka' lemon trees, planted in 1981 in an east-west row orientation at a 7 x 3 m spacing, sampled in Mar. 2003.

	Quadrant (Q)		
Height (H)[z]	North	South	Mean
Lower	0.404 b[y]	0.402 b	0.403 b
Middle	0.453 a	0.435 ab	0.444 a
Upper	0.463 a	0.452 a	0.457 a
Mean	0.440 NS[x]	0.430 NS	
P-values			
H	0.0031		
Q	0.4274		
HxQ	0.8574		

[z]Lower = 0.5 to 2 m; Middle = 2.5 to 4.5 m; Upper = 5 to 7 m.

[y]Means within a column followed by different letters are significantly different (n=6 single-tree replicates; P=0.10; LSD).

[x]NS = nonsignificant difference between north and south quadrants.

Table 3.3. Effect of cardinal point on rind oil content of 'Eureka' lemon (n=6 single-tree replicates) and effect of cardinal point on rind oil content adjusted for fruit surface area-to-volume ratio (covariate) sampled in May 2004.

	Rind oil content (%)	
Cardinal point	May 2003	May 2004
East-west row orientation		
North	0.515 a[z]	0.667 a[y]
South	0.504 a	0.637 ab
East	0.428 b	0.614 b
West	0.429 b	0.605 b
P-value	0.0372	0.0091
North-south row orientation		
North	-	0.672 a[x]
South	-	0.647 ab
East	0.458 NS	0.624 bc
West	0.444	0.610 c
P-value	0.4852	0.0206

[z]Means within a column followed by different letters are significantly different; NS = nonsignificant (P=0.10; LSD).

[y]Means within a column followed by different letters are significantly different (P=0.05; LSD).

[x]Adjusted means by analysis of covariance with fruit surface area-to-volume ratio and rind oil content as covariates.

Table 3.4. Coefficients of determination for linear relationships between lemon rind oil content and solar radiation (PAR) in various tree canopies.

Independent variable[z]	P-value	Coeff. of determination
Inside vs. outside 2003	0.1234	0.082
Upper vs. lower 2003	0.0269	0.140 *
East-west row orientation 2003	0.0096	0.268 *
East-west row orientation 2004	0.0006	0.425 *
North-south row orientation 2004	0.0168	0.233 *

[z]Rind oil content for experiments on these canopy positions.

*Significant at P=0.05.

CHAPTER 4

VARIATION IN LEMON [*Citrus limon* (L.) Burm. f.] RIND OIL CONTENT AMONG GROWING REGIONS IN SOUTH AFRICA

Abstract

Within a cultivar, climate has the largest influence on lemon [*Citrus limon* (L.) Burm. f.] rind oil composition and content. Previous studies showed that rind oil content was highest in inland-grown fruit and progressively lower in areas closer to the coast, and that cooler regions have lower rind oil content than warmer growing regions. South Africa has a diverse climate, and rind oil content from fruit produced in different growing regions was compared. Rind oil content was also regressed against cumulative heat units for the various growing regions to determine any relationship between these two variables. 'Eureka' lemon fruit from Upington, a hot, dry region, had the highest rind oil content in all seasons sampled. In Mar. and Apr. 2004, rind oil content in fruit from Malelane and Marble Hall ranked second to Upington. Rind oil content for fruit from Vaalharts was the lowest at each sampling time. In 2003, there was a positive linear relationship between rind oil content and cumulative heat units for the period Sept. to Apr., but in 2004 the relationship was weak. However, in general, rind oil content increased with increasing heat unit accumulation.

Introduction

Within a cultivar, climate has the largest influence on rind oil composition and content (Misitano, 2000; Staroscik and Wilson, 1982). When the rind oil content of 'Navel' and 'Valencia' sweet oranges [*Citrus sinensis* (L.) Osbeck] from various regions in southern California was compared, Bartholomew and Sinclair (1946) found rind oil content to be highest in inland fruit and progressively lower in areas grown closer to the coast. In South

Africa, rind oil content was higher for 'Eureka' lemon [*C. limon* (L.) Burm. f.] sampled from Messina (hot, dry inland climate) than for Addo (cool, coastal climate) (Barry, 2000). Barry (2000) also sampled 'Eureka' lemon from various production areas throughout South Africa and found cooler regions generally produced fruit with lower rind oil content than warmer regions.

South Africa has a diverse climate, from semi-tropical to Mediterranean and semi-desert and lemons are grown in many of these climatic regions. Barry (1996) divided South Africa into six climatic zones: 1) hot, humid, 2) hot, dry, 3) intermediate, 4) cool, inland, 5) cold, semi-coastal, and 5) semi-desert areas. According to Barry (1996) lemons can be commercially produced in all these climatic zones.

Temperature is the single biggest climatic factor affecting citrus growth. The threshold temperature for growth of citrus is 13 °C (Cassin et al., 1969; Davies and Albrigo, 1994). Cumulative effective heat units (EHU) have been used to compare growing regions with one another (Reuther, 1973) and to determine whether different cultivars can be commercially cultivated within a region (Barry et al., 1996). Lemons need between 1100 and 1500 EHU annually for commercial production in South Africa (Barry et al., 1996).

There are not many reports on the influence of environmental factors on various horticultural responses because the factors are complex and it is difficult to study these factors under field conditions without potential confounding effects (Stover and Greene, 2005). Also, there are few reports on the effect of growing region on rind oil content in lemons.

In light of the diversity in climate in South Africa and the large effect of climate on rind oil content, this study was conducted to determine the broad effects of growing region on rind oil content of lemon.

Materials and Methods

Plant material. 'Eureka' lemon on rough lemon rootstock (*C. jambhiri* Lush.) was used as the model scion-rootstock combination to quantify the variation in rind oil content among growing regions in South Africa.

Treatments. In this experiment "sites" served as "treatments", where site was considered as "overall growing environment" including, inter alia, weather conditions, soil type and cultural practices. Whereas, large differences in soil condition, for example, occur among sites, the overriding effect on rind oil content is thought to be local weather conditions during fruit development.

Lemon fruit samples were taken in South Africa from Malelane, Karino and Marble Hall (all in Mpumalanga province), Addo (Eastern Cape province), Simondium and Citrusdal (Western Cape province), and Vaalharts and Upington (Northern Cape province) representing hot, intermediate, cold and semi-desert citrus production areas (Table 4.1) (Barry, 1996).

Data collection. In 2003, fruit were sampled from Karino on 14 May, Simondium on 11 Jun., Addo on 7 Jul., Vaalharts on 15 Jul., and Citrusdal on 21 Jul. Except for Karino, all sites were sampled at similar dates to minimise possible seasonal variation on rind oil content.

In 2004, fruit were sampled at Karino on 17 March and 19 May, Malelane on 12 March, Marble Hall on 15 March, Addo on 13 April and 28 June, Simondium on 1 April and 22 June, Citrusdal on 7 July, Vaalharts on 16 July, and Upington on 16 April and 26 June. Data from similar sampling dates were analysed together.

All fruit were at a similar stage of maturity when sampled (colour chart T6; CRI, 2004; Appendix 1). Four single-tree replicates were used for each site, and four fruit were sampled per replicate. Where rows have a north-south orientation, samples were taken from the eastern side of the trees, whereas fruit were collected from the northern side of trees where rows were planted in an east-west orientation. Fruit were sampled at between 0.5 and 2 m height.

The length and diameter of each fruit were measured with a digital calliper, from which fruit volume and surface area-to-volume ratio (SA:V ratio) were calculated (Braddock, 1999). Oil content of the fruit was then determined, using the modified Scott method (Dickon Hall Products, 2001; Scott and Veldhuis, 1966; Chapter 2).

Temperature data and EHU calculation. Daily minimum and maximum temperatures were obtained for each of the growing regions used in this study from the Agricultural Research Council of South Africa, from Jan. 2002 until Aug. 2004. Average temperature for each month was calculated and, using 13 °C as a base temperature, monthly EHU were calculated from daily mean air temperature using the following equation:

$$\text{EHU} = (\text{mean montly air temperature} - 13) \times \text{days in the month.}$$

For each growing region the EHU for the growing season was then calculated for the period from September of the previous year until April of the year samples were taken.

Statistical analysis. Data were subjected to analysis of variance using the general linear model procedure of SAS Enterprise Guide (SAS Inc., Cary, N.C.) and means were separated using Fisher's LSD. Where fruit diameter or SA:V ratio were significantly different among sites, analysis of covariance was conducted with fruit diameter and SA:V ratio as covariates. Rind oil content was regressed against EHU for the various growing regions to determine any relationship between the two variables.

Results and Discussion

There were significant differences in rind oil content of lemon fruit sampled from different growing regions, but fruit sampled also differed in fruit size (data not shown). As a result of differences in fruit size among growing regions, rind oil content was adjusted for fruit size by covariance analysis. In 2003, lemon fruit sampled in Upington had the highest rind oil content and fruit from Karino and Addo ranked second, followed by Citrusdal, then Simondium (Table 4.2). Fruit from Vaalharts had the lowest rind oil content. In 2004, when fruit were sampled in Mar. and Apr., fruit from Upington had the highest rind oil content again (Table 4.2). Fruit from Marble Hall and Malelane had the second highest rind oil content, whereas, fruit from Karino and Addo had the lowest rind oil content. Vaalharts was not sampled at this time. When fruit were sampled in May and Jun. 2004, fruit from Addo and Upington had the highest rind oil content, fruit from Vaalharts had the lowest rind oil content, and rind oil content of fruit from Karino was intermediate (Table 4.2). The ranking of growing regions according to rind oil content from highest to lowest was similar at each sampling time.

In 2003, there was a positive linear relationship between rind oil content and cumulative EHU for the period Sept. to Apr. (Table 4.3). However, in 2004, the relationship was weak. Nevertheless, Simondium and Vaalharts both had relatively low rind oil content and both are cool growing regions, whereas Malelane and Marble Hall are warm growing regions, and so is Upington. These growing regions had high rind oil content. Even though Upington and Vaalharts are both semi-desert regions, Vaalharts has a cooler climate than Upington. Barry (2000) also concluded that cooler growing regions have lower rind oil content than warmer regions. Barry (2000) found that fruit grown in Messina, a hot, dry region, had the highest rind oil content, which was confirmed in this study when fruit from Upington, also with a hot, dry climate, had the highest rind oil content.

Fruit were sampled on different dates from the different growing regions in the 2004 season, and rind oil content varied between dates within a growing region. Although the purpose of the current study was not to quantify seasonal changes in rind oil content, future studies on rind oil content should include sampling at various times during the harvest period to determine the optimum harvest time in terms of rind oil content. Knowing the optimum harvest time will in turn optimise sampling time thereby allowing more accurate comparisons among growing regions.

Climatic factors, other than temperature, combine to form the environmental conditions which characterise a growing region. In general, rind oil content increased with increasing heat unit accumulation. However, due to the confounding effects of the various components of the climate of a growing region, it is difficult to examine the effect of these environmental factors under field conditions on rind oil content of lemon fruit.

Table 4.1. Summary of experimental sites, general planting conditions and effective heat units during two growing seasons, 2002 – 03 and 2003 – 04.

Climatic zone	Site	Lat., Long.	Altitude (m)	Planting date	Spacing (m)	Row orientation	Heat units[z]	
							2002 – 03	2003 – 04
Hot, humid	Malelane	25°33'S, 31°39'E	349	Dec. 2000	7 x 4	east-west	2962	2912
Intermediate	Karino	25°25'S, 31°06'E	674	Jan. 1999	5 x 2	north-south	2517	2519
	Marble Hall	25°01'S, 29°24'E	876	1998	6 x 2.5	north-south	2608	2676
Cold	Citrusdal	32°36'S, 19°02'E	326	1980	6 x 6	east-west	2262	2009
	Addo	33°33'S, 25°41'E	42	1992	6 x 3	east-west	1980	2030
	Simondium	33°52'S, 19°01'E	164	1997	5 x 2	east-west	1806	1648
Semi-desert	Upington	28°40'S, 20°25'E	780	Nov. 1999	6 x 2	east-west	2777	2473
	Vaalharts	27°57'S, 24°47'E	1137	Dec. 1995	5 x 2.5	east-west	1727	1928

[z]Effective heat units = (mean monthly air temperature - 13) x days in the month, for the period from Sept. to Apr.

Table 4.2. Effect of growing region on rind oil content of 'Eureka' lemon budded in rough lemon rootstock, adjusted for fruit size differences by covariance analysis.

	Rind oil content (%)		
Growing region	May/Jun. 2003[z]	Mar./Apr. 2004	May/Jun. 2004
Malelane	–	0.596 ab	–
Karino	0.654 b[z]	0.483 c	0.548 c
Marble Hall	–	0.602 ab	–
Citrusdal	0.599 bcd	–	0.677 ab
Addo	0.635 bc	0.493 c	0.739 a
Simondium	0.565 cd	0.547 bc	0.588 bc
Upington	0.788 a	0.657 a	0.735 a
Vaalharts	0.535 d	–	0.386 d
P-value	0.0267	0.0002	<0.0001

[z]Means within a column, followed by different letters, are significantly different (*P*=0.05; LSD).

Table 4.3. Coefficients of determination for linear relationships between lemon rind oil content and cumulative effective heat units, using 13 °C as base temperature, for the period from Sept. of the previous year to Apr. of the year sampled.

Independent variable	*P*-value	Coeff. of determination
Rind oil content, sampled May/Jun. 2003	0.0296	0.7326
Rind oil content, sampled Mar./Apr. 2004	0.3019	0.3402
Rind oil content, sampled May/Jun. 2004	0.5379	0.1382

CHAPTER 5

LEMON [*Citrus limon* (L.) Burm. f.] RIND OIL CONTENT VARIES WITH GENOTYPE

Abstract

A large variation exists among citrus cultivars and rootstocks and their effect on fruit growth and quality. Little is known about the effect of lemon [*Citrus limon* (L.) Burm. f.] cultivar and rootstock on rind oil content, and this study was conducted to determine this effect, as well as the relationship between seedless clones of cultivars and rind oil content. 'Limoneira 8A', followed by 'Cicily', 'Lisbon' and 'Genoa' had the highest rind oil content, whereas rind oil content of 'Eureka' was disappointingly low. 'Villafranca', 'Messina' and 'Yen Ben Lisbon' had the lowest rind oil content of cultivars budded on Carrizo citrange [*Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck] at Addo. Seedless cultivars, 'Eureka SL' and 'Lisbon SL' had ~18.0% higher rind oil content than the seeded cultivars from which they were derived. With regards to rootstock, fruit from lemon trees budded on non-invigorating rootstocks, e.g. X639 [*C. reshni* Hort. ex Tan. × *P. trifoliata* (L.) Raf.] and Carrizo citrange, had the highest rind oil content, whereas rind oil content was low on invigorating rootstocks, such as rough lemon (*C. jambhiri* Lush.). When considering the additive effects, 'Eureka SL' had the highest rind oil content with MxT rootstock [(*C. reticulata* Blanco × *C. paradisi* Macf.) × *P. trifoliata* (L.) Raf.], however, seedless cultivars have lower fruit yield than their seeded progenitors. Keeping this in mind, the best combination, therefore, would be 'Limoneira 8A' budded on X639 or Carrizo citrange, since 'Limoneira 8A' is also very productive.

Introduction

The choice of citrus cultivar and rootstock, and their combinations, play a critical role in fruit growth and development. However, relatively little is known about the effect of lemon [*Citrus limon* (L.) Burm. f.] cultivar and rootstock on rind oil content. Some lemon cultivars are known to have higher rind oil content than others. For example, 'Bearss' lemon, also known as 'Sicily', is known to have high rind oil content (Kesterson and Braddock, 1977; Kesterson et al., 1974; Morton, 1987; Saunt, 2000). In both experiments by Kesterson and co-workers, 'Bearss' lemon had the highest rind oil content and 'Lisbon' was ranked second. 'Limoneira 8A', a 'Lisbon' lemon selection, is also reported to have high rind oil content (Saunt, 2000). Foguet et al. (1996) compared 11 lemon cultivars and 10 selections and found 'Limoneira 8A' to have the highest rind oil content, followed by 'Bearss', 'Genoa' and 'Feminello St Teresa'. In Italy, 'Feminello' lemon had the highest rind oil content when compared to 'Monachello' and 'Eureka' (Crescimanno et al., 1988), and in Tucumán, Argentina, 'Feminello St Teresa' is also planted for its high rind oil yield (Saunt, 2000). Furthermore, 'Lisbon' and 'Limoneira 8A' both had high fruit yield, whereas 'Eureka' lemon performed poorly (Wright and Peña, 2000). Sinclair (1984) also reported that 'Lisbon' lemon selections have high productivity and vigour.

Rootstock influences tree vigour, and therefore fruit size and quality (Barry et al., 2004a; Barry et al., 2004b), and the choice of rootstock depends on soil type, climate and resistance to viruses, pathogens and nematodes (Castle, 1987). The rootstock most commonly used for lemons in South Africa is rough lemon (*C. jambhiri* Lush.), resulting in rapid growth with high productivity and large yields with low internal fruit quality (Castle, 1987). In Argentina, Foguet and Blanco (2000) evaluated new *Poncirus trifoliata* (L.) Raf. hybrid rootstocks for 'Eureka' and found the hybrid rootstock citrumelo 75-AB 12/14 [*C. paradisi* Macf.) × *P.*

trifoliata (L.) Raf.] had the highest fruit yield. These trifoliate hybrid rootstocks also restricted 'Eureka' tree growth compared to a standard rootstock like Volkamer lemon (*C. volkameriana* Ten. & Pasq.). Six rootstocks with 'Eureka' lemon and 12 with 'Lisbon' lemon were evaluated for fruit yield at Addo Research Station, South Africa (Koekemoer et al., 2004). With 'Eureka' as scion, X639 [*C. reshni* Hort. ex Tan. × *P. trifoliata* (L.) Raf.] and MxT [(*C. reticulata* Blanco × *C. paradisi* Macf.) × *P. trifoliata* (L.) Raf.] had the highest fruit yield, whereas rough lemon and citremon [*P. trifoliata* (L.) Raf. × *C. limon* (L.) Burm.f.] had the lowest. MxT also had the highest yield with 'Lisbon', but X639 had the lowest. Carrizo citrange [*P. trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck] and rough lemon also had low yield.

Numerous studies have shown the effects of rootstock on many fruit quality variables (Barry et al., 2004a; Barry et al., 2004b; Castle, 1987; Wutcher, 1979), but few studies report on rootstock effects on lemon rind oil content. Bitters and Scora (1970) tested 'Valencia' orange [*C . sinensis* (L.) Osbeck] on 25 rootstocks for differences in rind oil content. 'Valencia' orange budded on Savage and Troyer citrange rootstocks had the highest rind oil content, whereas on rough lemon it had the second lowest rind oil content. When 'Bearss' lemon was budded on seven rootstocks, there was no difference in rind oil content among rootstocks (Kesterson and Braddock, 1977; Kesterson et al., 1974).

Few studies report on the effect of lemon cultivar on rind oil content and almost nothing is known about the effect of rootstock. Therefore, this study was conducted to determine the effect of genotype, i.e. scion and rootstock, as well as the relationship between seedless clones of cultivars and lemon rind oil content.

Materials and Methods

Sites. Various lemon cultivars on various rootstocks were sampled in South Africa from Addo Research Station, Eastern Cape province, (33°33'S, 25°41'E; 42 m alt.) and in Mpumalanga province at Larten Lemon Project, Karino (25°25'S, 31°06'E; 674 m alt.) and Broham Farm, Nelspruit (25°26'S, 30°52'E; 748 m alt.).

Treatments and experimental design. Cultivar. At Addo, various seeded lemon cultivars budded on Carrizo citrange, were compared to determine their relative rind oil contents. The cultivars, planting dates and row orientations are summarised in Table 5.1. Also at Addo, 'Eureka', 'Lisbon' and 'Limoneira 8A' budded on rough lemon and X639 were compared. These trees were planted in 1992 at a 6 x 3 m spacing. 'Eureka' was planted with an east-west row orientation and 'Lisbon' and 'Limoneira 8A' with a north-south row orientation.

At Broham, 'Genoa', 'Lisbon' and 'Limoneira 8A' were budded on Swingle citrumelo (*C. paradisi* Macf. x *P. trifoliata*). The 'Genoa' and 'Lisbon' trees were planted in May 1999 at a 6 x 2.25 m spacing, while 'Limoneira 8A' was planted at a spacing of 7 x 2 m in Feb. 1999. All trees were planted with a north-south row orientation.

Seedless vs. seeded cultivars. At Addo and Larten, 'Eureka' was compared with 'Eureka SL', a seedless mutation of 'Eureka' (Miller et al., 2000), and at Addo, 'Lisbon' was compared with 'Lisbon SL', a seedless mutation of 'Lisbon' lemon. 'Eureka SL' is the world's first truly seedless lemon and is commercially produced in South Africa, whereas 'Lisbon SL' is not completely seedless, i.e. it is low-seeded, and is currently not yet released.

At Addo, 'Eureka' was budded on rough lemon, whereas 'Eureka SL' was budded on Volkamer lemon (*C. volkameriana* Ten. & Pasq.), a rootstock comparable with rough lemon in terms of vigour. The 'Eureka' trees were planted in 1992 in an east-west row orientation, while 'Eureka SL' was topworked in March 1999 and the trees were in a north-south row orientation. Both cultivars were planted at a 6 x 3 m spacing.

At Larten, both 'Eureka' and 'Eureka SL' were budded on three rootstocks, namely rough lemon, MxT and X639. Planting dates and spacing are summarised in Table 5.2. All trees were planted in a north-south row orientation.

At Addo, 'Lisbon' was budded on rough lemon in 1992, while 'Lisbon SL' was budded on rough lemon in Feb. 2000. Trees were planted at a 6 x 3 m spacing with an east-west row orientation.

<u>Rootstock</u>. At Addo, fruit were sampled from various scion-rootstock combinations, including 'Eureka', 'Lisbon' and 'Limoneira 8A' budded on rough lemon, Carrizo citrange and X639, except for the 'Eureka'-Carrizo citrange combination, due to the well-known rootstock/scion incompatibility of this combination (Bird, 1999) . All trees were planted at a 6 x 3 m spacing. These were the same trees as for the cultivar experiment at Addo.

At Larten, fruit were sampled from various scion-rootstock combinations, with either 'Eureka' or 'Eureka SL' as scion. These are the same trees as for the comparison of 'Eureka' with 'Eureka SL', except that 'Eureka' was also budded on Yuma citrumelo (syn. Sacaton citrumelo) (Table 5.2).

Data collection. Samples of lemon fruit were taken at Addo on 7 Jul. 2003, and 13 Apr. and 28 Jun. 2004. Fruit were sampled at Broham on 20 May 2003, and 20 Mar. and 17 May 2004, and from Larten on 19 May 2003, and 17 Mar. and 19 May 2004.

All the fruit were at similar stage of maturity when sampled (colour plate T6; CRI, 2004; Appendix 1). Where the row orientation was north-south, fruit were sampled from the eastern side of the trees, whereas with an east-west row orientation, fruit were sampled from the northern side of trees. Samples were taken between 0.5 and 2 m height. Four single-tree replicates were used with five fruit per sample representing one replicate.

Length and diameter of each fruit were measured with a digital caliper, from which fruit volume and surface area-to-volume ratio (SA:V ratio) were calculated (Braddock, 1999). Oil content of the fruit was then determined, using the modified Scott method (Dickon Hall Products, 2001; Scott and Veldhuis, 1966; Chapter 2).

Statistical analysis. Data were subjected to analysis of variance using the general linear model procedure of SAS Enterprise Guide (SAS Inc., Cary, N.C.) and means were separated using Fisher's LSD. Where fruit diameter or SA:V ratio were significantly different, analysis of covariance was conducted with fruit SA:V ratio and rind oil content as covariates.

Results and Discussion

Cultivar. In the first experiment at Addo, where different lemon cultivars budded on Carrizo citrange were compared, 'Limoneira 8A' had the highest rind oil content when sampled in Jun. 2003 and Jun. 2004 (Table 5.3). 'Cicily', 'Lisbon' and 'Genoa' were the other cultivars with consistently high rind oil content. When samples were taken in Apr. 2004, 'Fino' and

'Cicily' had the highest rind oil content, with 'Genoa', 'Limoneira 8A' and 'Lisbon' ranking second. 'Villafranca', 'Messina' and 'Yen Ben Lisbon' consistently had the lowest rind oil content (Table 5.3).

In a second experiment at Addo, where 'Lisbon', 'Limoneira 8A' and 'Eureka' lemons were budded on two rootstocks, 'Limoneira 8A' consistently had the highest rind oil content (Table 5.4). 'Eureka' lemon consistently had the lowest rind oil content, whereas rind oil content of 'Lisbon' tended to be intermediate.

When sampled in May (2003 and 2004) 'Limoneira 8A' at Broham had the highest rind oil content, with 'Lisbon' and 'Genoa' not significantly different from one another (Table 5.5). In Mar. 2004, there was no significant difference in rind oil content among cultivars when adjusted for fruit size (Table 5.5).

'Limoneira 8A' is known to have high rind oil content (Foguet et al., 1996; Saunt, 2000). On all rootstocks, both at Addo and at Broham, 'Limoneira 8A' had the highest rind oil content, followed by 'Cicily', 'Lisbon' and 'Genoa'. 'Villafranca', 'Messina' and 'Yen Ben Lisbon' had low rind oil content. Eureka, the standard lemon cultivar grown in South Africa for fresh fruit production (Veldman et al., 1996), had lower rind oil content than the 'Lisbon'-types. Foguet et al. (1996) and Crescimanno et al. (1988) also reported that rind oil content of 'Eureka' lemon was low. According to Sinclair (1984) and Wright and Peña (2000), 'Lisbon' lemon selections, which include 'Limoneira 8A', have high productivity and vigour, making these cultivars favourable choices for lemon oil production.

Seedless vs. seeded cultivars. At Addo, 'Eureka SL' had significantly higher rind oil content than 'Eureka' when sampled in Jun. 2003 and Apr. 2004 (Table 5.6). In Jun. 2004, there was no significant difference in rind oil content between the cultivars, however, when rind oil content was adjusted for differences in fruit size (Table 5.6), 'Eureka' had significantly higher rind oil content than 'Eureka SL'. On average, 'Eureka SL' had 13.1% higher rind oil content than 'Eureka'.

At Larten, with rough lemon as rootstock, 'Eureka SL' had significantly higher rind oil content than 'Eureka' when sampled in May (2003 and 2004) (Table 5.7). The most consistent effect of seed content on rind oil content was observed with MxT rootstock, where 'Eureka SL' had on average 34.1% higher rind oil content than 'Eureka'. With X639 rootstock, however, there was no significant difference in rind oil content between 'Eureka' and 'Eureka SL', except when sampled in May 2004 when rind oil content was significantly higher for 'Eureka SL' than 'Eureka'.

Rind oil content of 'Lisbon SL' was significantly higher than that of 'Lisbon' when sampled at Addo in Apr. and Jun. 2004 (Table 5.6). In Jun. 2003, rind oil content of 'Lisbon SL' was numerically higher than that of 'Lisbon', but there was no significant difference in rind oil content between cultivars. On average, rind oil content of 'Lisbon SL' was 23.7% higher than that of 'Lisbon'.

Seedless lemon cultivars tended to have higher rind oil content than seeded cultivars, both at Addo and Larten. Since seedless lemons have not previously been available, this is the first report of higher rind oil content in seedless lemon cultivars. However, 'Eureka SL' tends to have lower fruit yield than 'Eureka' (G.H. Barry, personal communication). Therefore, it must still be determined whether it would be economically viable to produce 'Eureka SL' solely for essential oil production. Fruit yield of 'Lisbon SL' is still unknown as it is still an experimental cultivar.

Rootstock. At Addo, rind oil content of 'Eureka' lemon fruit on different rootstocks was not significantly different when sampled in Jun. (2003 and 2004) (Table 5.8) However, when sampled in Apr. 2004 rind oil content of 'Eureka' fruit from trees budded on X639 had significantly higher rind oil content than fruit from trees on rough lemon rootstock. Rind oil content of 'Lisbon' and 'Limoneira 8A' lemon fruit trees budded on X639 and Carrizo citrange were significantly higher than on rough lemon, except for 'Lisbon' fruit sampled in Jun. 2003 and Apr. 2004 (Table 5.8). There was no difference in rind oil content of 'Lisbon' or 'Limoneira 8A' fruit from trees on Carrizo citrange or X639.

At Larten, rind oil content of 'Eureka' fruit from trees on X639 rootstock was the highest and on MxT the lowest (Table 5.9). In contrast to this, rind oil content of 'Eureka SL' fruit from trees on MxT rootstock was consistently the highest.

Fruit from trees on X639 rootstock consistently produced the highest rind oil content among the known cultivars tested. Furthermore, other non-invigorating rootstocks, e.g. Carrizo citrange, MxT and Yuma citrumelo (syn. Sacaton citrumelo), generally bore fruit with higher rind oil content than the invigorating rootstock, rough lemon. X639 and MxT are also known to give high fruit yield with 'Eureka' and 'Lisbon' lemons, respectively (Koekemoer et al., 2004). MxT rootstock gave the highest rind oil content with 'Eureka SL'.

The possible cause of these observed differences in rind oil content of lemon cultivars budded on different rootstocks imparting various levels of vegetative vigour to the scion may be attributed to both dilution and physiological effects (Albrigo, 1977; Barry et al., 2004a; Barry et al., 2004b).

While the objective of this research was to determine the role of scion and rootstock genotype on rind oil content of lemons, rind oil quality has not been determined. However, qualitative differences in rind oil content among scion and rootstock cultivars occur (Lota et al., 2001; Usai et al., 1996; Verzera et al., 2001), and the qualitative analysis of 'Eureka SL' rind oil has not yet been conducted.

Conclusion

Scion and rootstock cultivar choice affects rind oil content of lemon fruit. When considering the individual components, fruit of 'Limoneira 8A', followed by 'Cicily', 'Lisbon' and 'Genoa', had the highest rind oil content, whereas rind oil content of 'Eureka' was disappointingly low. Seedless cultivars had higher rind oil content than the seeded cultivars from which they were derived, by ~18.0%. With regards to rootstock, fruit from lemon trees budded on non-invigorating rootstocks, e.g. X639 and Carrizo citrange, had the highest rind oil content, whereas rind oil content was low in fruit from trees on invigorating rootstocks. It is appealing to make the conclusion that the best combination therefore would be 'Limoneira 8A' budded on X639 or Carrizo citrange, or 'Eureka SL' on X639 or MxT rootstocks. When considering the additive effects, 'Eureka SL' had the highest rind oil content with MxT rootstock. However, fruit yield, also affected by cultivar and rootstock, is an important factor when considering the best combination for total lemon rind oil yield per hectare, making 'Limoneira 8A' or 'Lisbon' lemons budded on MxT or X639 rootstocks the best combination.

Table 5.1. Summary of various lemon cultivars budded on Carrizo citrange rootstock at Addo Research Station, Addo.

Cultivar	Planted	Row orientation
Cicily	Nov. 1992	N-S
Fino	May 1992	N-S
Genoa	Oct. 1998	N-S
Lapithkiotiki	Jan. 1999[z]	E-W
Limoneira 8A	Oct. 1998	N-S
Lisbon	Oct. 1998	N-S
Messina	Jan. 1999[z]	E-W
Villafranca	Jan. 1999[z]	E-W
Yen Ben Lisbon	Jan. 1999[z]	E-W

[z]Topworked onto Carrizo citrange.

Table 5.2. 'Eureka' and 'Eureka SL' lemons on various rootstocks at Larten Lemon Project, Karino, showing the planting date and spacing.

Cultivar	Eureka		Eureka SL	
Rootstock	Planting date	Spacing	Planting date	Spacing
Rough lemon	Jan. 1999	5 x 2 m	Oct. 2000	5 x 1.5 m
MxT	Feb. 1999	5 x 2 m	Dec. 2000	5 x 1.5 m
X639	Dec. 2000	5 x 1.5 m	Dec. 2000	5 x 1.5 m
Yuma citrumelo	Feb. 1999	5 x 2 m	–	–

Table 5.3. Effect of various lemon cultivars at Addo Research Station, Addo, budded on Carrizo citrange rootstock, on rind oil content, adjusted for fruit surface area to volume ratio differences among the cultivars.

	Rind oil content (%)		
Cultivar	Jun. 2003	Apr. 2004	Jun. 2004
Limoneira 8A	0.730 a[z]	0.675 abcd	0.884 a
Cicily	0.633 b	0.732 ab	0.794 b
Lisbon	0.631 b	0.642 bcd	0.817 ab
Genoa	0.623 b	0.690 abc	0.767 bc
Fino	0.592 bc	0.751 a	0.698 cd
Lapithkiotiki	0.627 b	0.620 cd	0.678 cd
Villafranca	0.578 bcd	0.598 cd	0.684 d
Messina	0.518 cd	0.586 d	0.652 d
Yen Ben Lisbon	0.516 d	0.589 cd	0.564 e
P-value	<0.0001	0.0099	<0.0001
LSD (*P*=0.05)	0.074	0.097	0.075

[z]Means within a column followed by different letters are significantly different (*P*=0.05; LSD).

Table 5.4. Effect of 'Eureka', 'Lisbon' and 'Limoneira 8A' lemons budded on rough lemon and X639 rootstocks at Addo Research Station, Addo, on rind oil content.

	Rind oil content (%)		
Cultivar	Jun. 2003	Apr. 2004	Jun. 2004
Rough lemon rootstock			
Eureka	0.626 b[z]	0.486 b	0.755 NS
Lisbon	0.635 b	0.574 a	0.740
Limoneira 8A	0.688 a	0.578 a	0.760
P-value	0.1204	0.0006	0.4506
LSD (*P*=0.10)	0.052	0.031	0.096
X639 rootstock			
Eureka	0.650 b	0.658 b	0.760 b
Lisbon	0.676 b	0.651 b	0.859 a
Limoneira 8A	0.761 a	0.770 a	0.868 a
P-value	0.0221	0.0169	0.0407
LSD (*P*=0.10)	0.062	0.067	0.072

[z]Means within a column, followed by different letters are significantly different (*P*=0.10; LSD); NS=nonsignificant.

Table 5.5. Effect of 'Genoa', 'Lisbon' and 'Limoneira 8A' lemon budded on Swingle citrumelo rootstock at Broham, Nelspruit, on rind oil content.

	Rind oil content (%)			
Cultivar	May 2003	Mar. 2004	Adj. Mar. 2004[z]	May 2004
Limoneira 8A	0.777 a[y]	0.543 NS	0.568 NS	0.757 a
Lisbon	0.714 b	0.595	0.577	0.642 b
Genoa	0.716 b	0.618	0.610	0.614 b
P-value	0.0472	0.0861	0.4217	<0.0001
LSD (*P*=0.05)	0.056	0.086	–	0.041

[z]Rind oil content, adjusted for fruit size differences by covariance analysis.

[y]Means within a column followed by different letters are significantly different (*P*=0.05; LSD); NS=nonsignificant.

Table 5.6. Effect of seed content on rind oil content of lemon cultivars budded on rough lemon rootstock at Addo Research Station, Addo.

	Rind oil content (%)			
Cultivar	Jun. 2003	Apr. 2004	Jun. 2004	Adj. Jun 2004[z]
Eureka	0.626 b[y]	0.486 b	0.755 NS	0.806 a
Eureka SL	0.696 a	0.722 a	0.744	0.693 b
P-value	0.0815	0.0008	0.6935	0.0123
LSD (P=0.10)	0.065	0.066	0.052	–
Lisbon	0.635 NS	0.574 b	0.738 b	–
Lisbon SL	0.708	0.655 a	1.076 a	–
P-value	0.1674	0.0269	0.0030	–
LSD (P=0.05)	0.116	0.067	0.146	–

[z]Rind oil content, adjusted for fruit size differences by covariance analysis.

[y]Means within a column followed by different letters are significantly different (*P*=0.10; LSD); NS=nonsignificant.

Table 5.7. Effect of 'Eureka' and 'Eureka SL' lemons budded on rough lemon rootstock at Larten Lemon Project, Karino, on rind oil content.

	Rind oil content (%)		
Cultivar	May 2003	Mar. 2004	May 2004
Rough lemon rootstock			
Eureka	0.630 b[z]	0.505 NS	0.578 b
Eureka SL	0.739 a	0.553	0.621 a
P-value	0.0014	0.0697	0.0345
LSD (*P*=0.05)	0.055	0.054	0.075
MxT rootstock			
Eureka	0.687 b	0.413 b	0.523 b
Eureka SL	0.787 a	0.721 a	0.721 a
P-value	0.0014	<0.0001	0.0010
LSD (*P*=0.05)	0.050	0.038	0.082
X639 rootstock			
Eureka	0.777 NS	0.601 NS	0.589 b
Eureka SL	0.703	0.645	0.693 a
P-value	0.1015	0.0870	0.0176
LSD (*P*=0.05)	0.092	0.053	0.079

[z]Means within a column followed by different letters are significantly different (*P*=0.05; LSD); NS=nonsignificant.

Table 5.8. Effect of various rootstocks at Addo Research Station, Addo, on rind oil content of three lemon cultivars.

Rootstock	Rind oil content (%)		
	Jun. 2003	Apr. 2004	Jun. 2004
Eureka			
Rough lemon	0.626 NS[z]	0.486 b	0.755 NS
X639	0.650	0.658 a	0.760
P-value	0.4309	0.0009	0.9112
LSD (*P*=0.05)	0.068	0.070	0.097
Lisbon			
Rough lemon	0.635 NS	0.574 NS	0.739 b
Carrizo citrange	0.637	0.648	0.846 a
X639	0.676	0.651	0.860 a
P-value	0.5706	0.1394	0.0106
LSD (*P*=0.10)	0.077	0.072	0.061
Limoneira 8A			
Rough lemon	0.688 b[z]	0.578 b	0.688 b
Carrizo citrange	0.745 a	0.683 a	0.873 a
X639	0.761 a	0.770 a	0.868 a
P-value	0.0370	0.0054	0.0086
LSD (*P*=0.05)	0.056	0.098	0.116

[z]Means within a column, followed by different letters are significantly different (*P*=0.05 for 'Eureka' and 'Eureka SL' and *P*=0.10 for 'Lisbon'; LSD); NS=nonsignificant.

Table 5.9. Effect of various rootstocks at Larten Lemon Project, Karino, on rind oil content of 'Eureka' and 'Eureka SL'.

	Rind oil content (%)		
Rootstock	May 2003	Mar. 2004	May 2004
<u>Eureka</u>			
Rough lemon	0.630 b[z]	0.505 bc	0.538 bc
MxT	0.687 b	0.481 c	0.523 c
Yuma	0.686 b	0.533 b	0.600 a
X639	0.777 a	0.601 a	0.589 ab
P-value	0.0009	<0.0001	0.0438
LSD (*P*=0.05)	0.063	0.035	0.060
<u>Eureka SL</u>			
Rough lemon	0.739 ab[z]	0.553 c	0.621 b
MxT	0.787 a	0.721 a	0.721 a
X639	0.703 b	0.645 b	0.693 ab
P-value	0.0311	0.0002	0.0440
LSD (*P*=0.05)	0.061	0.048	0.077

[z]Means within a column, followed by different letters are significantly different (*P*=0.05; LSD).

CHAPTER 6

SCREENING OF PLANT GROWTH REGULATORS TO ENHANCE RIND OIL CONTENT OF LEMONS [*Citrus limon* (L.) Burm. f.]

Abstract

Oil gland initiation in citrus fruit occurs in the early stages of fruit development and oil glands are mature when fruit are still immature, but continue to enlarge as fruit grow. To manipulate rind oil content, oil glands must be manipulated either very early, to increase the number of glands, or later, to increase the size of the glands. Synthetic gibberellins, cytokinins, ethylene and auxins were applied to lemon trees [*Citrus limon* (L.) Burm. f.] at different times and concentrations to screen their ability to enhance rind oil content. Of all the gibberellin and cytokinins applied, Promalin®, a combination of gibberellic acid 4/7 and benzyl adenine-phosphate, a cytokinin, had a small, but nonsignificant effect on rind oil content. Ethephon, which induces ethylene synthesis, affected rind oil content in 2004, when applied 8 weeks before harvest. However, ethephon and aminoethoxyvinylglycine (AVG, an ethylene biosynthesis inhibitor) had an inconsistent effect on lemon rind oil content. Auxins did not affect rind oil content. As fruit of similar size and colour were sampled, the indirect effects of applied plant growth regulators, through their effect on fruit size or colour, were not investigated. Further experiments should be conducted, especially on the timing and concentration of applied gibberellins, e.g. Promalin®, and ethylene.

Introduction

Oil glands are initiated before flowering in 'Navel'orange [*C. sinensis* (L.) Osbeck] (Klieber et al., 2000), and are already observed in the ovary stage of lemon flower development (Ford, 1942). Knight and co-workers (2001) studied oil gland development in relation to fruit development in 'Washington Navel' orange. They also found oil glands to be present in pre-anthesis floral ovaries. As fruit develop, more glands are initiated until ≈20 mm fruit diameter, when oil gland initiation ceased. When fruit are 32 to 52 mm in diameter, all oil glands are fully developed. Between 52 and 88 mm fruit diameter, rapid oil gland enlargement was observed, which coincides with the cell growth period of fruit development. These observations confirm previous observations made by Bain (1958) working with 'Valencia' orange: new oil glands were formed and older glands enlarged during stage I of fruit development (cell division stage). During stage II of fruit development (cell enlargement), oil glands increased in size and this continued through stage III (maturation period). Rind oil production starts just as the glandular cavity starts to form.

From these observations we can conclude that oil gland initiation in citrus fruit occurs in the early stages of fruit development and all oil glands were mature when fruit were still immature, but continued to enlarge throughout fruit growth. Therefore, to manipulate rind oil content the oil glands must be either manipulated during oil gland initiation, to increase the number of oil glands, or during cell enlargement, to produce larger oil glands.

Synthetic plant growth regulators (PGRs) are used to manipulate fruit development in many horticultural crops. Different PGRs applied at various times have a variety of effects. Of interest to this study is whether any PGRs have possible direct or indirect effects on oil gland formation and/or rind oil production.

Gibberellins (GA) delay fruit senescence, rind aging and softening, as well as rind pigmentation (Coggins et al., 1964; El-Otmani and Ait-Oubahou, 1999; Monselise, 1979). GA also reduces rind thickness in mature fruit when applied early in the growing season (García-Luís et al., 1992). Cytokinins can also prevent degreening of citrus fruit.

Wilson et al. (1990) found GA treatment increased rind oil concentration of grapefruit (*C. paradisi* Macf.). In a two-year experiment, GA-treated grapefruit had significantly higher rind oil content in the second year, but there were no differences during the first year of the experiment (McDonald et al., 1997). Barry (2000) applied GA to lemons and found GA treatment increased lemon rind oil content by 5.7%.

Growth-retardants, such as the triazoles and prohexadione-Ca (Ph-Ca), are gibberellin biosynthesis inhibitors, and could be used to demonstrate the role of GA in rind oil content of lemons.

Ethephon, which stimulates ethylene synthesis, causes fruit thinning when applied during the post-bloom period thereby improving fruit size (El-Otmani and Ait-Oubahou, 1999; Moss, 1975). Ethephon can also be used to improve rind colour (El-Otmani and Ait-Oubahou, 1999). However, these effects might lead to lower rind oil yield through the relationships of lower rind oil yield with increasing fruit size (Chapter 2, 2.5.1.1.) and lower rind oil content with increasing rind colour, as discussed earlier (Chapter 2, 2.5.1.3.).

In mangoes (*Mangifera indica* L.), a climacteric fruit, aminoethoxyvinylglycine (AVG, an ethylene biosynthesis inhibitor) decreased the production of aromatic volatile compounds, whereas ethephon-treated fruit had significantly higher total aroma volatile compounds, including monoterpenes (limonene), sesquiterpenes and aldehydes (Lalel et al., 2003). However, this response has not been reported for lemons.

There is no evidence in the literature to support a direct effect of auxins on rind oil content, however, an indirect effect of auxins on rind oil content of lemons may occur via the well-known effect of auxins on fruit size (El-Otmani and Ait-Oubahou, 1999; Hield et al., 1964; Monselise, 1979; Moss, 1975).

While PGRs have a variety of commercial uses to manipulate fruit growth and development, only gibberellins are known to affect rind oil content in citrus and even this is not consistent. The objective of this study was to screen various PGRs applied at different times and concentrations on their ability to enhance rind oil content of lemons.

Materials and Methods

Treatments, experimental design and sampling.

Gibberellins and cytokinins. To determine the effect of gibberellins and cytokinins on lemon rind oil content an 'Eureka' lemon on rough lemon rootstock (*C. jambhiri* Lush.) orchard in Simondium, Western Cape Province, South Africa (33°52'S, 19°01'E; 164 m alt.) was used. Trees were planted in an east-west row orientation in 1997 and spaced at 5 x 2 m.

Seven different chemicals were applied on 19 Jun. 2003. This was a cool day (≈18 °C) with almost no wind. Table 6.1 summarises the chemicals applied, the commercial name and the concentration used. Samples for all treatments were taken 4 and 8 weeks after applying the chemicals and then again in May 2004 to assess possible return-bloom effects. Fruit from the control, ProGibb®, BA and Regalis® were also sampled at 2 and 6 weeks after application.

In another experiment, gibberellins were applied a month after full bloom, on 26 Nov. 2003, viz. GA_3 (ProGibb®) at 50 $mg{\cdot}L^{-1}$, $GA_{4/7}$ + BA (Promalin®) at 1 $mL{\cdot}L^{-1}$, and Ph-Ca (Regalis®) at 2000 $mg{\cdot}L^{-1}$. Fruit were sampled on 4 May 2004.

Based on the results from the first two experiments in 2003, the experiment was repeated in 2004. On 28 Apr. 2004 the following chemicals were applied: GA_3 (ProGibb®) at 50 mg·L^{-1}, $GA_{4/7}$ (ProVide®) at 2000 mg·L^{-1}, BA at 20 mL·L^{-1}, $GA_{4/7}$ + BA (Promalin®) at 2 mL·L^{-1}, and $GA_{4/7}$ (ProVide®) + BA at 20 mL·L^{-1} each. Fruit were sampled at 6, 8 and 12 weeks after application.

In all experiments, treatments were compared to an untreated control. Approximately 6 L spray material was applied per tree using a high-pressure hand-held spray gun. Untreated, buffer trees were left between treated trees. Six single-tree replicates were used in a randomised complete block experimental design.

Fruit of similar size and maturity (colour plate T6; CRI, 2004; Appendix 1) were sampled. All samples were taken between 0.5 and 2 m height. Five fruit per replicate were sampled from the northern side of trees.

Ethylene. To determine the effect of ethylene on rind oil content an 'Eureka' lemon on rough lemon rootstock orchard planted in 1997 in an east-west row orientation and at a 5 x 2 m. spacing at Simondium, Western Cape Province, South Africa (33°52'S, 19°01'E; 164 m alt.) was used.

In 2003, ethephon (Ethrel®) at 0, 200 and 400 mg·L^{-1} and AVG (ReTain®) at 0, 200 and 400 mg·L^{-1} were applied on 27 Jun., a mild, sunny day (≈19 °C). A randomized complete block design was used with six single-tree replicates. Fruit were sampled 2, 4, 6 and 8 weeks after treatment application and then again in May 2004 to assess possible return-bloom effects of the treatments on rind oil content.

Based on the results from 2003, the experiment was repeated in 2004 with the best two treatments, AVG and ethephon, both at 400 $mg \cdot L^{-1}$, and compared to an untreated control. The chemicals were applied on 28 Apr. on a warm, sunny day. A randomized complete block design with six single-tree replicates was used.

Approximately 6 L spray material was applied per tree using a high-pressure hand-held spray gun. Untreated, buffer trees were left between treated trees.

Fruit of similar size and maturity (colour plate T6; CRI, 2004; Appendix 1) were sampled 6, 8 and 12 weeks after treatment application. All samples were taken between 0.5 and 2 m height. Five fruit were sampled per replicate from the northern side of trees.

Auxins. To determine the effect of auxins on rind oil content of lemons an 'Eureka' lemon on rough lemon rootstock orchard, planted in 1994 with a north-south row orientation and at a 5 x 3 m spacing in the Wemmershoek area, Western Cape Province, South Africa (33°52'S, 19°02'E; 164 m alt.) was used. 2,4-Dichlorophenoxy propionic acid (2,4-DP; Citrimax®) at 100 $mg \cdot L^{-1}$ and 10 $mg \cdot L^{-1}$ 3,5,6-TPA (Maxim®) were applied as a medium-cover foliar spray in Nov. 2002 at the 12-mm or 18-mm stage of fruit development, respectively. Untreated trees were used as a control and no buffer trees were left between treated trees. A completely blocked design was used.

Fruit of similar size and maturity (colour plate T6; CRI, 2004; Appendix 1) were sampled from 0.5 to 2 m height. Fruit samples were taken on 14 Mar. 2003 from the eastern side of each tree. Ten single-tree replicates were used and each replicate consisted of five fruit.

Rind oil content determination. For all treatments, length and diameter of each fruit were measured with a digital caliper, from which fruit volume and surface area to volume ratio (SA:V ratio) were calculated (Braddock, 1999). Rind oil content was determined using the modified Scott method (Dickon Hall Products, 2001; Scott and Veldhuis, 1966; Chapter 2) and calculated as percentage mass of the whole fruit.

Statistical analysis. Analysis of variance (ANOVA) was performed using the general linear model procedure of SAS (SAS Inc., Cary, N.C.), followed by mean separation using Fisher's LSD. Where fruit diameter or SA:V ratio was significantly different among treatments, analysis of covariance was conducted with fruit diameter and SA:V ratio as covariates.

Results and Discussion

Gibberellins and cytokinins. In 2003, applied gibberellins and cytokinins did not significantly affect rind oil content (Table 6.2). At 4 and 8 weeks after treatment in 2003 and in May 2004, trees treated with Promalin® bore fruit with the highest numerical value of rind oil content, but this was not statistically significant. Even when rind oil content was adjusted for SA:V ratio (Table 6.2), there were still no significant effect of these PGRs on rind oil content. The Nov. 2003 application of Promalin® and ProGibb® did not significantly affect rind oil content the following year when samples were taken in May 2004 (Table 6.3). Even with rind oil content adjusted for differences in fruit size, there was no effect (P=0.4706; data not shown) on rind oil content. Ph-Ca, a gibberellin-biosynthesis inhibitor, did not affect rind oil content when applied in Jun. or Nov. 2003. In 2004, there were no differences in rind oil content (Table 6.4) among treatments. When rind oil content was adjusted for differences in fruit size there was still no significant difference in rind oil (Table 6.4).

Promalin®, which is composed of $GA_{4/7}$ and BA, had a small, but nonsignificant effect on rind oil content. However, this experiment did not give similar results to those of Wilson et al. (1990) and Barry (2000), where they reported an increase in rind oil content after gibberellin application.

Ethylene. In 2003, the treatments had no significant effect on rind oil content (Table 6.5), even when rind oil content was adjusted for differences in fruit size with covariance analysis (data not shown). In 2004, there was a significant difference among treatments for fruit sampled 8 weeks after application (Table 6.6). Ethephon-treated fruit had the highest rind oil content. Even after covariance analysis was performed with fruit SA:V ratio (covariate), at 12 weeks after application (Table 6.6), there were no significant differences in rind oil content among the treatments. Numerically, Ethephon-treated fruit had the highest rind oil content at each sampling time, however.

Ethephon affected rind oil content once, when applied 8 weeks before harvest. Ethephon treatment has been shown to increase aroma volatile compounds in mango (Lalel et al., 2003) However, these two years' data showed that ethephon and AVG did not have a consistent effect on lemon rind oil content. Because fruit of similar size and colour were sampled, the indirect effect of ethephon on rind oil content, via the effect of ethylene on advancing rind colour and possibly fruit maturity, was not investigated.

Auxins. Synthetic auxins applied during the physiological fruit drop period did not affect rind oil content at fruit maturity of similar sized fruit (P=0.5735; Table 6.7). At harvest it was observed that where auxins were applied, fruit were larger than fruit from the control.

However, fruit of similar size were sampled for rind oil analysis and the indirect effect of fruit size on rind oil yield per tree was not investigated.

Although auxins did not directly affect oil content, it seems to lead to larger fruit and might have an indirect effect on rind oil yield via fruit size. Bartholomew and Sinclair (1946) and Sinclair (1984) showed that larger lemon fruit had a lower oil yield per ton of fruit than smaller fruit, because larger fruit have a smaller SA:V ratio. Therefore, applied auxins, yielding larger fruit, might yield less oil.

Conclusion

In this screening experiment on the effects of PGRs on rind oil content of lemons, various PGRs were applied at full bloom, which is when oil gland formation occurs. However, the PGRs tested had no significant effect on rind oil content at fruit maturity. From these results we suggest that further experiments should be conducted, especially on the timing and concentration of applied PGRs. From the gibberellin experiment, Promalin® deserves further attention as earlier experiments (Barry, 2000; Wilson et al., 1990) showed that GA had a positive affect on rind oil content. It may also be worthwhile conducting further experiments with Ethrel® and ReTain® to elucidate the possible role of ethylene on rind oil content. The problem with timing of application with lemons is the fact that more than one crop per season is on the trees.

Table 6.1. Gibberellins and cytokinins applied in Jun. 2003 on 'Eureka' lemon budded on rough lemon rootstock.

Active ingredient	Chemical[z]	Concentration
GA_3, 400 $g \cdot kg^{-1}$	ProGibb®	50 $mg \cdot L^{-1}$
GA_3	Falgro®	0.64 $mL \cdot L^{-1}$
$GA_{4/7}$	ProVide®	2000 $mg \cdot L^{-1}$
$GA_{4/7}$ + BA	Promalin®	1 $mL \cdot L^{-1}$
$GA_{4/7}$ + BA	Perlan®	2.5 $mL \cdot L^{-1}$
Benzyl adenine-phosphate	BA	10 $mL \cdot L^{-1}$
Prohexadione-Ca, 10%	Regalis®	200 $mg \cdot L^{-1}$

[z]0.25 $mL \cdot L^{-1}$ ABG® was used as wetting agent for all chemicals except Regalis® where 0.3 $mL \cdot L^{-1}$ Dash® was used as the wetting agent.

Table 6.2. The role of gibberellins and cytokinins applied in Jun. 2003 on rind oil content of 'Eureka' lemon.

	Rind oil content (%)							
Treatment[z]	2 weeks[y]	4 weeks	Adj. 4 weeks[x]	6 weeks	8 weeks	Adj. 8 weeks	May 2004	Adj. May 2004
Control	0.650 NS[w]	0.589 NS	0.574 NS	0.577 NS	0.506 NS	0.503 NS	0.583 NS	0.575 NS
GA_3 (ProGibb®)	0.659	0.574	0.582	0.585	0.538	0.540	0.561	0.572
GA_3 (Falgro®)	0.639	0.600	0.612	0.574	0.505	0.504	0.549	0.566
$GA_{4/7}$ + BA (Promalin®)	-	0.632	0.630	-	0.574	0.574	0.635	0.628
$GA_{4/7}$ + BA (Perlan®)	-	0.597	0.611	-	0.558	0.559	0.587	0.588
$GA_{4/7}$ (ProVide®)	-	0.577	0.571	-	0.528	0.530	0 590	0.624
BA	-	0.608	0.607	-	0.553	0.554	0.600	0.584
Ph-Ca (Regalis®)	0.637	0.610	0.600	0.594	0.511	0.508	0.605	0.574
P-value	0.9045	0.4561	0.2425	0.8888	0.2915	0.3965	0.4895	0.3644

[z]Concentration of chemicals as per Table 6.1.

[y]Number of weeks after application.

[x]Rind oil content adjusted for fruit size by covariance analysis.

[w]NS = nonsignificant.

Table 6.3. The role of gibberellins applied in Nov. 2003 on rind oil content of 'Eureka' lemon sampled in May 2004.

Treatment	Rind oil content (%)
Control	0.6340 NS[Z]
GA_3 (ProGibb®)	0.6308
$GA_{4/7}$ + BA (Promalin®)	0.5870
Prohexadione-Ca (Regalis®)	0.6352
P-value	0.4319

[Z]NS = nonsignificant.

Table 6.4. Role of gibberellins and cytokinins applied Apr. 2004 on rind oil content of 'Eureka' lemon.

Treatment	Rind oil content (%)				
	6 weeks[z]	8 weeks	Adj. 8 weeks[y]	12 weeks	Adj. 12 weeks
Control	0.669 NS[x]	0.700 NS	0.694 NS[y]	0.618 NS	0.587 NS
GA_3 (ProGibb®)	0.740	0.751	0.746	0.627	0.627
$GA_{4/7}$ + BA (Promalin®)	0.740	0.729	0.736	0.650	0.651
$GA_{4/7}$ (ProVide®)	0.686	0.709	0.716	0.576	0.561
BA	0.710	0.710	0.714	0.612	0.641
$GA_{4/7}$ (ProVide®) + BA	0.707	0.742	0.735	0.589	0.605
P-value	0.3203	0.5918	0.6243	0.4633	0.2893

[z]Number of weeks after application.

[y]Rind oil content adjusted for fruit size by covariance analysis.

[x]NS = nonsignificant.

Table 6.5. Effect of ethephon (Ethrel®) and AVG (ReTain®) applied in Jun. 2003 on rind oil content of 'Eureka' lemon.

	Rind oil content (%)				
Treatment	2 weeks[z]	4 weeks	6 weeks	8 weeks	May 2004
Ethephon, 0 $mg \cdot L^{-1}$	0.571 NS[Y]	0.617 NS	0.560 NS	0.457 NS	0.645 NS
Ethephon, 200 $mg \cdot L^{-1}$	0.581	0.585	0.550	0.435	0.641
Ethephon, 400 $mg \cdot L^{-1}$	0.546	0.582	0.517	0.428	0.613
AVG, 0 $mg \cdot L^{-1}$	0.549	0.634	0.566	0.427	0.642
AVG, 200 $mg \cdot L^{-1}$	0.542	0.597	0.524	0.442	0.633
AVG, 400 $mg \cdot L^{-1}$	0.570	0.602	0.566	0.484	0.589
P-value	0.6146	0.4794	0.4494	0.1918	0.3501

[z]Number of weeks after application.

[y]NS = nonsignificant.

Table 6.6. Effect of ethephon (Ethrel®) and AVG (ReTain®) applied in Apr. 2004 on rind oil content of 'Eureka' lemon.

	Rind oil content (%)			
Treatment	6 weeks[z]	8 weeks	12 weeks	Adj. 12[y]
Control	0.673 NS[x]	0.694 b	0.583 NS	0.573 NS
Ethephon, 400 mg·L^{-1}	0.731	0.750 a	0.627	0.629
AVG, 400 mg·L^{-1}	0.701	0.666 b	0.583	0.592
P-value	0.1306	0.0104	0.3114	0.2591
LSD	0.057	0.051	–	0.069

[z]Number of weeks after application.

[y]Rind oil content at 12 weeks after application, adjusted for fruit size by covariance analysis.

[x]Means within a column, followed by different letters, are significantly different (*P*=0.05; LSD); NS = nonsignificant.

Table 6.7. Results from of applied auxins on rind oil content of 'Eureka' lemon.

Treatment	Rind oil content (%)
Control	0.585 NS[z]
2,4 DP (Citrimax ®) at 100 $mg \cdot L^{-1}$	0.605
3,5,6 TPA (Maxim ®) at 10 $mg \cdot L^{-1}$	0.585
P-value	0.5735

[z]NS=nonsignificant.

CHAPTER 7

OVERALL DISCUSSION AND CONCLUSIONS

The purpose of this study was to quantify possible factors affecting rind oil content of lemons [*Citrus limon* (L.) Burm. f.]. The factors studied were light exposure and canopy position, growing region in South Africa, genotype, i.e. scion and rootstock, as well as the relationship between seedless clones of cultivars and the cultivars from which the seedless clones were derived, and various plant growth regulators (PGRs) were screened to determine whether they influenced rind oil content.

Factors affecting light exposure of fruit, such as row orientation, tree architecture and tree spacing, appear to affect rind oil content. Rind oil content of 'Eureka' lemon was higher in fruit from exposed canopy positions than in fruit from shaded or semi-shaded canopy positions. Fruit borne on the outside of trees, higher in the tree, north-facing or not within the hedgerow had the highest rind oil content. To optimise rind oil content of lemons, trees should not be too dense or too high as to overshadow the lower parts of adjacent trees. Although the photosynthetically active radiation (PAR) measurements were not taken in the same season within which fruit were sampled, the PAR data supports the hypothesis that rind oil content appears to be correlated with light exposure. Further quantification of the relationship between PAR and rind oil content variation within a tree's canopy is required.

The mechanism by which light exposure affected rind oil content is unknown, but it is tempting to speculate on some possibilities. The relationship between higher light exposure and rind oil content may be associated with more carbohydrates being available for secondary metabolites entering the isoprenoid pathway. Alternatively, differences in rind oil gland size and density from fruit borne in exposed vs. shaded canopy positions may be comparable with anatomical differences found in sun vs. shade leaves (Syvertsen and Smith, 1984).

Climate has the largest influence on rind oil content. South Africa has a diverse climate, and rind oil content from fruit sampled from different growing regions differed significantly. Rind oil content was also regressed against cumulative heat units for the various growing regions to determine any relationship between these two variables. 'Eureka' lemon fruit from Upington, a hot, dry region, had the highest rind oil content in all seasons sampled. In Mar. and Apr. 2004, rind oil content in fruit from Malelane and Marble Hall ranked second to Upington. Rind oil content for fruit from Vaalharts was the lowest at each sampling time. In 2003, there was a positive linear relationship between rind oil content and cumulative heat units for the period Sept. to Apr., but in 2004 the relationship was weak. However, in general, rind oil content increased with increasing heat unit accumulation.

Climatic factors, other than temperature, combine to form the environmental conditions which characterise a growing region. In general, rind oil content increased with increasing heat unit accumulation. However, due to the confounding effects of the various components of climate of a growing region and other growing conditions, it is difficult to examine the effect of these environmental factors under field conditions on rind oil content of lemon fruit. Climate also affects fruit maturation, and rind oil content varies according to fruit maturity. Therefore, knowing the optimum harvest time for each growing region would allow more accurate comparisons among growing regions.

Scion and rootstock cultivar choice affects rind oil content of lemon fruit. When considering the individual components, fruit of 'Limoneira 8A', followed by 'Cicily', 'Lisbon' and

'Genoa', had the highest rind oil content, whereas rind oil content of 'Eureka' was disappointingly low. 'Villafranca', 'Messina' and 'Yen Ben Lisbon' also had low rind oil content. Seedless cultivars had higher rind oil content than the seeded cultivars from which they were derived, by ~18.0%. With regards to rootstock, fruit from 'Eureka' lemon trees budded on non-invigorating rootstocks, e.g. X639 [*C. reshni* Hort. ex Tan. × *Poncirus trifoliata* (L.) Raf.] and Carrizo citrange [*P. trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck], had the highest rind oil content, whereas rind oil content was low in fruit from trees on invigorating rootstocks such as rough lemon (*C. jambhiri* Lush.). It is appealing to make the conclusion that the best combination therefore would be 'Limoneira 8A' budded on X639 or Carrizo citrange, or 'Eureka SL' on X639 or MxT rootstocks. When considering the additive effects, 'Eureka SL' had the highest rind oil content with MxT rootstock. However, fruit yield, also affected by cultivar and rootstock, is an important factor when considering the best combination for total lemon rind oil yield per hectare, making 'Limoneira 8A' or 'Lisbon' lemons budded on MxT or X639 rootstocks the best combination.

PGRs, i.e. synthetic gibberellins, cytokinins, ethylene and auxins, applied on lemon trees at different times and concentrations did not affect rind oil content and fruit maturity. Promalin®, which is composed of $GA_{4/7}$ and BA, had a small, but nonsignificant effect on rind oil content. These results are in contrast with earlier experiments by Wilson et al. (1990) and Barry (2000), where they reported an increase in rind oil content after gibberellic acid application. Ethephon, which induces ethylene synthesis, affected rind oil content in 2004, when applied 8 weeks before harvest. Therefore, it may be worthwhile conducting further experiments with Ethrel® and ReTain® to elucidate the possible role of ethylene in rind oil content. Because fruit of similar size and colour were sampled, the indirect effect of ethephon on rind oil content, via the effect of ethylene on advancing rind colour and possibly fruit maturity, was not investigated. Auxins had no direct effect on rind oil content. Further experiments should be conducted, especially on the timing and concentration of applied gibberellins, e.g. Promalin®, and ethephon.

Earlier research (Crescimanno et al., 1988; Sinclair, 1984) showed differences in lemon rind oil content within a season. Nutrition also affected rind oil content (Kesterson and Braddock, 1977; Kesterson et al., 1974). Applied N increased lemon rind oil content, whereas applied K decreased rind oil content (Kesterson and Braddock, 1977; Kesterson et al., 1974). High P applications, which might lead to excessive accumulation in the soil, decreased rind oil content (Quaggio et al., 2002). Fertilisation may also influence rind oil content indirectly via the effects of mineral nutrients on fruit yield, fruit size and rind condition, i.e. rind colour, rind thickness or creasing. Also, Mg^{2+} and Mn^{2+} are divalent ions required in the terpenoid biosynthesis pathway, whereas K^{+} inhibited the enzymes involved in this pathway (Lüker et al., 2002). Therefore, future research on factors affecting rind oil content of lemons should include seasonal variation in rind oil content, including within-season variation, and nutritional and irrigation experiments.

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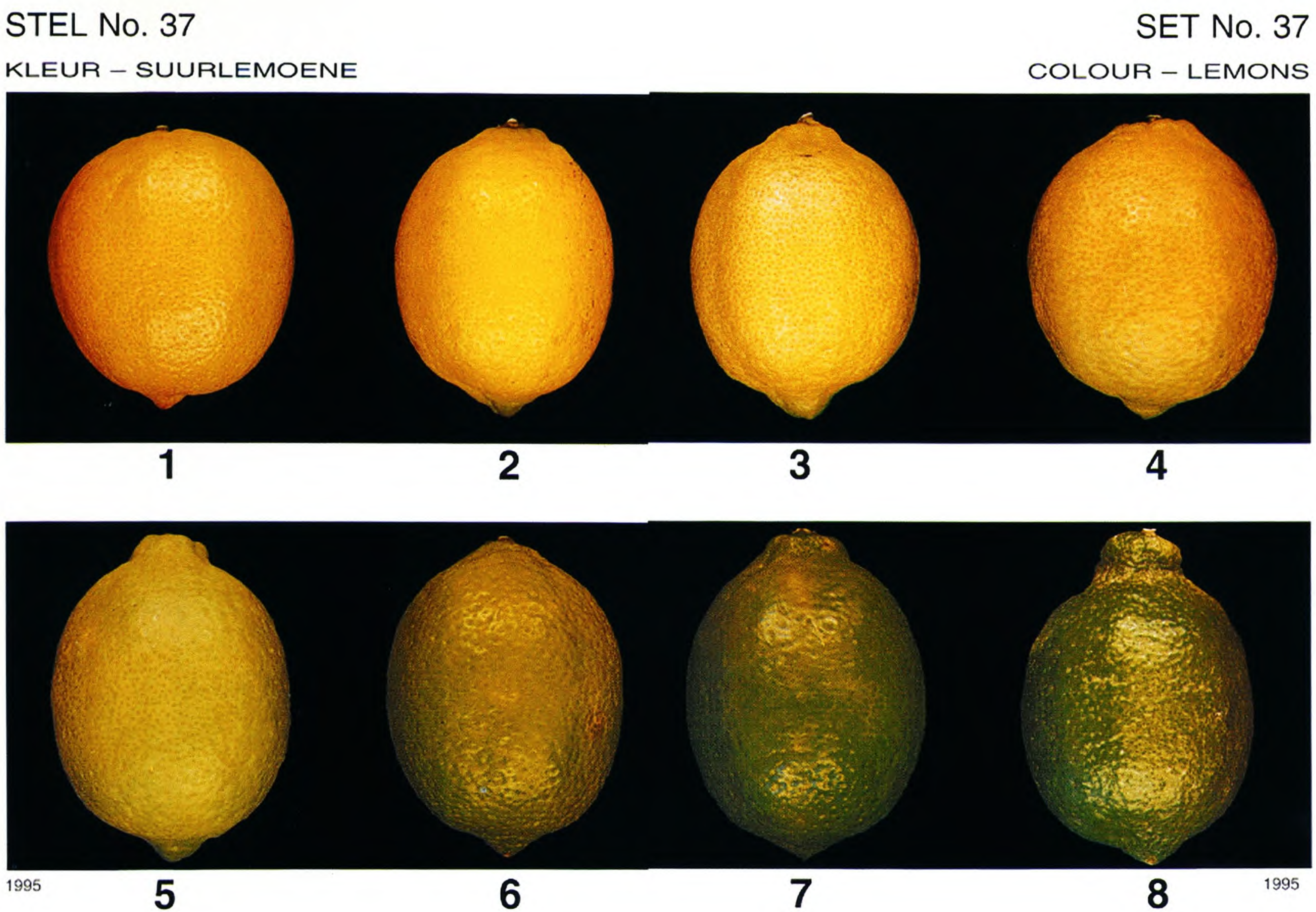

Appendix 1. Rind colour rating chart for lemons (CRI, 2004).