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Influence of soil water management on plant growth, essential oil yield and oil composition of rose-scented geranium (*Pelargonium* spp.)

by

Bahlebi Kibreab Eiasu

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Department of Plant Production and Soil Science
In the Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

Supervisor: Dr J.M. Steyn
Co-supervisor: Prof. P. Soundy

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LIST OF SYMBOLS AND ABBREVIATIONS

A	Plot area
ASW	Available soil water
cv.	Cultivar
D	Water deep percolation
DM	Dry mass
ET	Evapotranspiration
FAO	Food and Agricultural Organization of the United Nations (Rome, Italy)
FM	Fresh mass
GC	Gas chromatography
G:C ratio	Geraniol to citronellol ratio
G_s	Stomatal conductance
H_{LA}	Harvested land area
I	Irritation water applied
IW:CPE ratio	Irrigation water to cumulative pan evaporation ratio
ℓ	Litre
LAI	Leaf area index
LA (LA)	Leaf area
MAD	Maximum allowable depletion
n	Number of soil layers
P	Rainfall (precipitation)
PRD	Partial root zone drying
R	Water runoff
R_A	Neutron probe reading in air
R_S	Neutron probe reading in soil
R_t	Transpiration rate
RWC	Relative water content
R_z	Plant root zone
SANDA	South African National Department of Agriculture
SFE	Supper fluid extraction
S_1	Initial soil water content of a cropping season (regrowth period)
S_2	Final soil water content of a cropping season (regrowth period)

THRIP	Technology and Human Resources for Industry Programme (South Africa)
UIDEA	Uganda's Investment in Developing Export Agriculture (Uganda)
V_I	Volume of irrigation water
WUE	Water use efficiency
θ_d	Depleted water
θ_{FCi}	Volumetric soil water content at field capacity
θ_i	Measured volumetric soil water content
θ_{PWPi}	Volumetric soil water content at plant permanent wilting point
ψ_W	Water potential

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INFLUENCE OF SOIL WATER MANAGEMENT ON PLANT GROWTH, ESSENTIAL OIL YIELD AND OIL COMPOSITION OF ROSE-SCENTED GERANIUM (*PELARGONIUM* SPP.)

by

Bahlebi Kibreab Eiasu

SUPERVISOR: Dr J.M. Steyn

CO-SUPERVISOR: Prof. P. Soundy

DEGREE: PhD

ABSTRACT

Introducing effective irrigation management in arid and semi-arid regions, like most areas of South Africa, is an indispensable way of maximising crop yield and enhancing productivity of scarce freshwater resources. Holistic improvements in agricultural water management could be realised through integrating the knowledge of crop-specific water requirements. In order to develop effective irrigation schedules for rose-scented geranium (Pelargonium *capitatum* x *P. radens*), greenhouse and field experiments were conducted at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa, from 28 October 2004 to 2006.

Results from 20, 40, 60 and 80% maximum allowable depletion (MAD) levels of the plant available soil water (ASW) indicated that plant roots extracted most of the soil water from the top 40 cm soil layer, independent of the treatment. Both essential oil yield and fresh herbage mass responded positively to high soil water content. Increasing the MAD level to 60% and higher resulted in a significant reduction in herbage mass and essential oil yields. An increase in the degree of water stress apparently increased the essential oil concentration (percentage oil on fresh herbage mass basis), but its contribution to total essential oil yield (kg/ha oil) was limited. There was no significant relationship between MAD level and essential oil composition. For water saving without a significant reduction in essential oil yield of rose-scented geranium, a MAD of 40% of ASW is proposed.

Response of rose-scented geranium to a one-month irrigation withholding period in the second or third month of regrowth cycles showed that herbage mass and oil yield were positively related. Herbage yield was significantly reduced when the water stress period was imposed during the third or fourth month of regrowth. A remarkable essential oil yield loss was observed only when the plants were stressed during the fourth month of regrowth. Essential oil content (% oil on fresh herbage mass basis) was higher in stressed plants, especially when stressed late, but oil yield dropped due to lower herbage mass. The relationship between essential oil composition and irrigation treatments was not consistent. Water-use efficiency was not significantly affected by withholding irrigation in the second or in the third month of regrowth. With a marginal oil yield loss, about 330 to 460 m^3 of water per hectare per regrowth cycle could be saved by withholding irrigation during the third month of regrowth. The overall results highlighted that in water-scarce regions withholding irrigation during either the second or the third month of regrowth in rose-scented geranium could save water that could be used by other sectors of society.

In greenhouse pot experiments, rose-scented geranium was grown under different irrigation frequencies, in two growth media. Irrigation was withheld on 50% of the plants (in each plot) for the week prior to harvesting. Herbage and essential oil yields were better in the sandy clay soil than in silica sand. Essential oil content (% oil on fresh herbage mass basis) apparently increased with a decrease in irrigation frequency. Both herbage and total essential oil yields positively responded to frequent irrigation. A one-week stress period prior to harvesting significantly increased essential oil content and total essential oil yield. Hence, the highest essential oil yield was obtained from a combination of high irrigation frequency and a one-week irrigation-withholding period. In the irrigation frequency treatments, citronellol and citronellyl formate contents tended to increase with an increase in the stress level, but the reverse was true for geraniol and geranyl formate.

Leaf physiological data were recorded during the terminal one-week water stress in the glasshouse pot trial. Upon rewatering, stomatal conductance (G_s) and transpiration rate (R_t) were significantly lower in the less often irrigated than in the more often irrigated treatments, while leaf water potential (ψ_w) and relative water content (RWC) were the same for all plants, indicating that water stress had an after-effect on G_s and R_t. At the end of the stress period, G_s, R_t,

ψ_w and RWC were lower in the plants from the more often irrigated than from the less often irrigated treatments. Irrespective of irrigation treatment, one type of non-glandular and two types (different in shape and size) of glandular trichomes were observed. In water stressed-conditions, stomata and trichome densities increased, while the total number of stomata and trichomes per leaf appeared to remain more or less the same. Water stress conditions resulted in stomatal closure.

Keywords: Citronellol; citronellyl formate; essential oil content; geraniol; geranyl formate; herbage yield; irrigation-withholding period; maximum allowable depletion level; relative water content; stomatal conductance; transpiration rate; trichomes; water potential; water stress; water use; water-use efficiency

CHAPTER 1

GENERAL INTRODUCTION

Rose-scented geranium (*Pelargonium* species) is an aromatic plant that belongs to the family Geraniaceae. Southern Africa, particularly South Africa, is the centre of origin for most of the *Pelargonium* species (Lis-Balchin, 2002a). The plant is cultivated for its essential oil (commonly referred to as geranium oil), which is extracted from tender stems, leaves and flowers by water- and/or steam-distillation techniques (Rajeswara Rao, Kaul, Syamasundar & Ramesh, 2002). Geranium oil is characterised by its rose-like odour (Wüst, Beck & Mosandl, 1998; Ravindra, Kulkarni, Gayathri & Ramesh, 2004), and is widely used in the perfumery industry. In addition, the oil is extensively used in the production of flavouring and cosmetic products (Singh, 1999; Babu & Kaul, 2005).

Geranium oil is among the top 20 valuable natural plant essential oils (Williams & Harborne, 2002; Ravindra *et al.*, 2004). The current world geranium oil demand is estimated to be around 600 tons per annum (Bhan, Dhar Choudhary, Rekha, Balyan, Khan, Agarwal & Shawl, 2006). China is the world-leading geranium oil producer, followed by Egypt, Réunion Island and India (Demarne, 2002). According to Demarne (2002), an additional 20 to 25 tons of quality geranium oil should be produced to satisfy the world's essential oil demand.

Rose-scented geranium is also cultivated in South Africa. The annual production is about 3 tons of essential oil (WESTGRO, 2006). The contribution of the country to international market is small (Weiss, 1997; Bhan *et al.*, 2006). As a result of the growing number and preferences of consumers, and a growth in the use of essential oil constituents in the fields of cosmetics, food-processing, pharmaceutical and agrochemical industries, trade in essential oils is expected to increase in the future (Kumar, Bahl, Bansal & Naqvi, 2001; Sangwan, Farooqi, Shabih & Sangwan, 2001). Hence, the potential share for South Africa in the world geranium oil trade is expected to increase to about 50 tons per year (WESGRO, 2006).

Geranium oil is a mixture of more than 120 compounds belonging to different classes of organic compounds, including acids, alcohols, aldehydes, esters, and ketones (Demarne & Van der Walt, 1993; Williams & Harborne, 2002). Citronellol, geraniol and linalool with or without their respective esters comprise 60-70% of the essential oil (Williams & Harbone, 2002). The perfumery value of geranium oil is determined by the proportion of three major constituents, namely citronellol, geraniol and iso-menthone (Weiss, 1997). Yield and composition of geranium oil are affected by factors such as location, climate, cultivar and shoot age (Rajeswara Rao, Kaul, Mallavarapu & Ramesh, 1996; Weiss, 1997).

Water is a major environmental factor that directly or indirectly controls various physiological and metabolic processes, and determines crop yield (Sangwan *et al.*, 2001; Lawlor, 2002). The available reports on response of essential oils to soil water availability, to a certain degree, are contradictory. Rajeswara Rao *et al.* (1996) reported that moist seasons encouraged vegetative growth of rose-scented geranium and resulted in higher oil yield. Similarly, Singh (1999) demonstrated that maintaining soil water at 0.6 IW:CPE (irrigation water to cumulative pan evaporation) ratio improves both herbage and essential oil yield, without bringing a significant change in essential oil composition in *Pelargonium graveolens* grown on alfisols. A report by Ram, Ram and Singh (2006) also indicated that high soil water regimes (maintained at 1.2 IW:CPE) promoted vegetative growth, enhanced essential oil yield, changed essential oil composition and increased water-use efficiency in menthol mint (*Mentha arvensis* L.). Previous work of Ram, Ram and Roy (2003) also showed that conserving soil water by organic mulching resulted in improved herbage and essential oil yield of *Pelargonium graveolens* by about 32 and 47% respectively. The findings of Kumar *et al.* (2001) and Motsa, Soundy, Steyn, Learmonth, Mojela & Teubes (2006) that higher vegetative growth resulted in higher total essential oil yield are in agreement with the current results.

On the other hand, there is a general understanding that a certain degree of water stress induces production of secondary metabolites, such as essential oils (Sangwan *et al.*, 2001). Simon, Reiss-Bubenheim, Joly and Charles (1992) found that mild to moderate water stress improved oil content and resulted in higher total oil yield per plant in basil. In addition, the authors indicated that water stress changed essential oil composition. In water-stressed conditions,

levels of geraniol and citral increased, while total essential oil yield remained the same or increased in lemongrass (Singh-Sangwan, Farooqi & Sangwan, 1994). Geranium oil yield also showed a mild increase under water-stressed conditions (Weiss, 1997). Such evidences imply that both high and low soil water availability have positive contributions to essential oil yields and, possibly, change essential oil composition.

The available freshwater for irrigation purposes seems to be approaching its lowest limit as a result of over-irrigating tendencies of farmers, climatic changes, and an increase in population and industrial growths (Seckler, Barker & Amarasinghe, 1999; Plusquellec, 2002; FAO, 2007a). South Africa is among the most drought-prone countries. Most of the country's fresh water (about 60%) is utilised for irrigation purposes (Nieuwoudt, Backeberg & Du Plessis, 2003). In the near future, part of the freshwater that has been used for irrigation will be shifted to other public and economic sectors (Conley, 1997). Since water-use efficiency in agriculture is generally low, it is suggested that increasing productivity of the available irrigation water could be among the major means by which freshwater scarcity could be relieved (Hamdy, Ragab & Scarascia-Mugnozza, 2003).

Water productivity could be increased by improving crop water-use efficiency (Shi-Wei, Yi, Na & Qi-Rong, 2006). Several promising results have so far been achieved on water productivity by different irrigation- scheduling strategies. Deficit irrigation, for instance, improved water productivity in crops such as maize (Kang, Shi & Zhang, 2000), wheat (Zhang, Li, Huang, Cheng & Zhang, 2006) and soybean (Karam, Masaad, Sfeir, Mounzer & Rouphael, 2005). Optimum irrigation schedules based on maximum allowable depletion of the plant available soil water were recommended for okra (Home, Panda & Kar, 2001), maize (Panda, Behera & Kashyap, 2003) and potato (Kashyap & Panda, 2003; Eiasu, Soundy & Hammes, 2007). In addition, less water stress- sensitive crop growth stages have been identified for several crops, including sorghum (Mastrorilli, Katerji & Rana, 1995), pearl millet (Winkel, Renno & Payne, 1997), and wheat (Gupta, Gupta & Kumar, 2001a).

Results from the aforementioned irrigation techniques indicated that efficacy of irrigation scheduling depends on crop type, physiological stage of the plant and the soil type. Since

photosynthate partitioning to different plant parts varies according to soil water level (Boogaard, Alewijnse, Veneklaas & Lambers, 1997), the magnitude of water-use efficiency induced by different soil water management techniques will depend on the harvestable plant part(s). Thus, all crop species in combinations with different soil types and soil water monitoring techniques need to be investigated for development of best irrigation management strategies (Kirda, 2000; Jalota, Sood, Chahal & Choudhury, 2006).

In South Africa, rose-scented geranium is commonly produced under full or supplementary irrigation (Learmonth, 2008, personal communication). Probably due to more irrigation water availability and relatively higher rainfall in those areas (Davies & Day, 1998), cultivation of rose-scented geranium is mainly limited to the Mpumalanga Lowveld, KwaZulu-Natal and Limpopo provinces (SANDA, 2006). For introduction and a sustainable production of the crop in the arid and semi-arid regions of the country, locally developed irrigation schedules are needed. Therefore, the objectives of the present study were (1) to acquire a sound knowledge of the impact of different soil water management strategies on the physiology, morphology, essential oil yield and essential oil composition of a locally grown rose-scented geranium (*Pelargonium capitatum x P. radens* cv. Rose*)*, and (2) to develop irrigation schedules that will improve productivity of scarce freshwater resources.

The following studies were conducted to achieve the set objectives:

1. Investigation of herbage growth and essential oil yield and essential oil composition under different maximum allowable depletion (MAD) soil water schedules at field level (Chapter 3);
2. Identification of the most sensitive stages to soil water stress by withholding irrigation for one month at varying regrowth stages at field level (Chapter 4);
3. Study the response of essential oil yield and composition to long- and short-term stresses by means of irrigation frequency and a one-week terminal stress period in greenhouses pot trials (Chapter 5); and
4. Determination of morphological, anatomic, and physiological changes in leaves associated with different soil water levels in greenhouse pot trials (Chapter 6).

CHAPTER 2

LITERATURE REVIEW

This chapter covers three major topics, namely (1) essential oils: their nature and composition, (2) rose-scented geranium: uses and production of the crop, and (3) irrigation: importance of irrigated agriculture, and coping with freshwater scarcity. Topics on effects of soil water/irrigation techniques on rose-scented geranium plant growth, essential oil yield and oil composition have been introduced under pertinent chapters.

2.1 ESSENTIAL OILS

2.1.1 Nature and composition of essential oils

Essential oils are plant secondary metabolites that impart the aroma and flavour characteristic to the plant (Salisbury & Ross, 1992). Plant essential oils are classified under secondary metabolites because of lack of sufficient evidence that shows they are directly involved in normal plant metabolic processes such as growth and viability (Lambers, Chapin & Pons, 1998). Probably due to the low boiling characteristics of most of the compounds that constitute them, essential oils are commonly known as 'volatile oils' (Hay & Waterman, 1993).

Essential oils are a complex mixture of a large number of individual compounds with a variety of highly functionalised chemical entities (Kayser, Latté, Kolodziej & Hammerschmidt, 1998). Most of the essential constituents belong to the terpenoids, specifically monoterpenes (C_{10}) and sesquiterpenes (C_{15}), and, to a lesser degree, to different low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes and acyclic esters; also in rare cases, nitrogen and sulphur-containing compounds, coumarins and homologue of phenylpropanoids are present (Dorman & Deans, 2000; Iijima, Davidovich-Rikanati, Fridman, Gang, Bar, Lewinsohn & Pichersky, 2004).

Synthesis of essential oils is not a characteristic of a specific class of plants. Although variable in amount and composition, they are metabolised throughout the plant kingdom (Hay & Svoboda, 1993). Most of the cultivated *Pelargonium* species are, for instance, rich in terpenoids (Turner, Gershenzon & Croteau, 2000), while some sweet basil (*Ocimum basilicum*) lines are characterised by high phenylpropenoids (Gang, Wang, Dudareva, Nam & Simon, 2001). In Salvia *sclarea*, linalool and linalyl acetate are the major constituents of essential oils (Lattoo, Dhar, Dhar, Sharma & Agarwal, 2006).

Essential oil composition may show dramatic variation among cultivars/lines. Based on the levels of essential oil constituents, Viljoen, Subramoney, Vuurena, Baser and Demirci (2005) could identify five chemotypes, namely (1) a myrcenone-rich type (36–62%), (2) a carvone-rich type (61–73%), (3) a piperitenone-rich type (32–48%), (4) an ipsenone-rich type (42–61%) and (5) a linalool-rich type(>65%) of *Lippia javanica* (Verbenaceae) in South Africa. Composition and amount of volatile oils also vary among parts/organs of the same plant (Kuiate, Bessière, Vilarem, & Zollo 2006), and plant physiological stage (Góra, Lis, Kula, Staniszewska & Wołoszyn, 2002; Kothari, Kumar, Bhattacharya & Ramesh, 2004; Lattoo *et al.*, 2006).

In several plant species, the non-woody plant materials are the major source of essential oils (Dorman & Deans, 2000). In most of the aromatic herbs of commercial interest, the essential oils are synthesised and/or stored within glandular trichomes that develop on the surface of leaves and other organs of the plants (Gershenzon, Maffei & Croteau, 1989; Hay & Waterman, 1993; Gaspar, Leeke, Al-Duri & Santos, 2003; Iijima *et al.*, 2004). Essential oils are extracted from the plant materials by water- and/or steam distillation techniques (Dorman & Deans, 2000; Babu & Kaul, 2005).

2.1.2 Importance of essential oils to the host plant

So far, there is no concrete evidence that has implicated essential oils in growth and developmental processes of the plants in which they are metabolised. Essential oils are usually

produced and stored in glandular trichomes, typical structures on the surface of the aerial parts of the plants containing glandular cell(s) that synthesise the oils, and a cuticular sac in which the oils are stored (Iijima, *et al.*, 2004). Such storage in isolated anatomical structures indicates that essential oils are not directly involved in the normal plant metabolic process (Deans & Waterman, 1993).

Certain essential oils can be produced as a response to certain environmental factors such as temperature and soil water. Simon *et al.* (1992) and Singh-Sangwan *et al.* (1994) reported that production of essential oils in plants tends to increase in water-stressed conditions. Such evidence supports the idea of Yaniv and Palvich (1982), who suggested an increase in secondary metabolism to be an adaptative mechanism of plants to dry environments. According to the authors, some secondary metabolites are involved in the process of osmotic adjustments. Some plant species also survive a rapid temperature change (which would result in destabilising of the photosynthetic process) by emitting certain volatile oils that have a lower heat capacity than that of water (Sharkey & Yeh, 2001; Raven, Evert & Eichhorn, 2005).

Mahmoud and Croteau (2002) mentioned that certain essential oils play major ecological roles in plants. In lavenders (*Lavendula angustifolia* M.), essential oils repel potentially harmful insects (Mauchline, Osborne, Martin, Poppy & Powell, 2005). To the contrary, other plants release essential oils to attract potentially beneficial organisms for mutual benefit. Such phenomenon is common in flowering plants to ensure pollination (Deans & Waterman, 1993; Wink, 2003). Hence, it seems safe to refer to essential oils as useful compounds in interactions between the host plants and their environment (Mahmoud & Croteau, 2002; Sudha & Ravishankar, 2002).

2.1.3 Role of essential oils in history and at present

Knowledge of essential oils started with the struggle of human beings against nature for survival (Aburjai & Natsheh, 2003). Even before invention and use of modern extracting techniques to isolate plant extracts selectively, humankind used crude forms of these compounds in the fields of folk medicines, cosmetics and perfumery (El-Sakhawy, El-Tantawy,

Ross & El-Sohly, 1998; Aburjai & Natsheh, 2003). History mentions that plant essential oils were among the traded commodities during the ancient Egyptian era, although their production was enhanced with the development of improved extracting techniques by the Arabs, and later by the French (Verlet, 1993).

With the current advance in different analytical techniques in the field of biochemistry, it has been possible to test the individual and/or synergetic mode of action of essential oil components (Cakir, Kordali, Zengin, Izumi & Hirata, 2004). Terpenes, for instance, have been identified to be among the chemicals that qualify essential oils for culinary, medicinal and fragrance uses (Deans, 2002). Furthermore, Niagre, Kalck, Roques, Roux and Michel (1996) demonstrated that the presence of oxygen in the functional group in derivatives of terpenoids such as ketones enhance the antibacterial properties of essential oil constituents. Similarly, Dorman and Deans (2000) could investigate individual and synergic inhibition effects of volatile oils of black pepper (*Piper nigrum* L.), clove (*Syzygium aromaticum* L.), geranium (*Pelargonium graveolens* L.), nutmeg (*Myristica fragrans* H.) and oregano (*Origanum vulgare* sp. *hirtum* L.) against 25 genera of bacteria. The results revealed that the inhibition effects of the oils from different plant species vary with the variation in composition of the oils, structural configuration of individual constituents, functional groups they contain and interactions between constituents.

Research on antibacterial activity and potential uses of essential oils is increasing at a faster rate than ever before (Dorman & Deans, 2000), because natural plant extracts such as essential oils are believed to have low side effects on mammals. In addition, in recent years, antibiotic resistant infections have shown alarming increases (Santos, Cunha, Viana, Rao, Manoel & Silveira, 1997). Therefore, a major objective of the intensified research on these plant extracts is to get substitutes for the commonly used synthetic antimicrobials and cosmetics that have been of great concern for unprecedented side effects to the health of humans as well as that of other mammals (Aburjai & Natsheh, 2003; Matthys, Eisebitt, Seith & Heger, 2003; SANDA, 2006), and to find new prototype drugs to combat infections (Santos *et al.*, 1997). Because of the high growth rate in number and preference of consumers, accompanied by discoveries of more uses of essential oil constituents, trade in essential oils is expected to gain great momentum in the future (Sangwan *et al.*, 2001).

2.2. ROSE-SCENTED GERANIUM (*PELARGONIUM* SPECIES)

2.2.1 Botany and origin

The genus *Pelargonium* (L.), to which the rose-scented geranium belongs, is one of the five genera that are classified in the Geraniaceae family (Weiss, 1997; Miller, 2002). South Africa is the centre of origin of the genus *Pelargonium* (Lis-Balchin, 2002b). Eighty percent of the 270 distinct and so far discovered *Pelargonium* species are found in the Western Cape Province of South Africa (Miller, 2002). According to Goldblatt and Manning (2000), the *Pelargonium* species are still among the three largest genera in the Cape flora. Plant collection history states that the first *Pelargonium* species was collected from Table Mountain (Weiss, 1997). According to the author, interest in the *Pelargonium* species started to increase during the control of the Cape of Good Hope by the British colonisers. Similarly, Miller (2002) suggested that the discovery of *Pelargonium* species was connected with exploration and discovery of the trade route around the southern tip of Africa to the East.

Members of the genus *Pelargonium* include annuals and perennials of various anatomic and morphological features such as bulbs and tuberous roots, which could have contributed to the survival of the plants in harsh environmental conditions, and the long journey to Europe and other parts of the world (Miller, 2002; Lewu, Adebola & Afolayan, 2007). In addition some *Pelargonium* species are characterised by succulent stems that possibly enable them to undergo crassulacean acid metabolism (CAM) in water-stressed conditions (Jones, Cardon & Czaja, 2003), thereby improving water-use efficiency (Lambers *et al.*, 1998).

P*elargonium* species were among the thousands of herbs known for their medicinal value in folk medicines as anti-diarrhoeic, and as remedies for colds and infection of the lungs, by the Zulus and early settlers in South Africa (Kayser *et al.*, 1998; Lis-Balchin, 2002a; Lewu *et al.*, 2007). In Europe and possibly in some other parts of the world, which were under colonisation or trade centres, however, *Pelargonium* species were abundantly grown as ornamental plants (Weiss, 1997; Lis-Balchin, 2002b; James, 2002).

Some *Pelargonium* species are known for their rosy essential oil odour (Lis-Balchin, Buchbuer, Hirtenlehner & Resch, 1998), which is commonly known as geranium oil (Williams & Harborne, 2002). Geranium oil is produced and stored in glandular trichomes (an extension of the epidermal tissue) in flowers, leaves and tender shoots, and are usually extracted by steam- and/or water-distillation techniques (Kothari *et al.*, 2004).

2.2.2 Uses of geranium oil

Geranium oil is among the top 20 valuable plant volatile oils (Williams & Harborne, 2002; Ravindra *et al.*, 2004). Because of its agreeable rose-like odour, geranium oil is widely used in the soap, cosmetics and perfumery industries (Kayser *et al.*, 1998; Gauvin, Lecomte, Smadja, 2004). Rhodinol, an aromatic chemical used in high-grade perfumes, is also extracted from geranium oil (Weiss, 1997; Bhan *et al*., 2006). To a lesser extent, geranium oil is used as flavour and preservative in food-processing industries (Wells & Lis-Balchin, 2002).

Although its mode of action has not yet scientifically been proved, geranium oil is widely used in the field of aromatherapy (Lis-Balchin, 1997). Geranium oil is also believed to have a sedative effect for relieve from stress, and its aroma could help fade negative memories (SANDA, 2006). A survey conducted in Australia showed that using essential oils of *Pelargonium graveolens* and other aromatic plants in the field of aromatherapy, resulted in a moderate reduction in the use of pharmaceutical products, mainly sedatives and analgesics (Bowles, Cheras, Stevens & Myers, 2005).

Extracts from the *Pelargonium* species have been used to treat diabetes, diarrhoea, gastric ulcers, jaundice, sterility and urinary stones in traditional pharmacology (Peterson, Machmudah, Roy, Goto, Sasaki & Hirose, 2006). A recent study indicated that essential oil of *Pelargonium graveolens* combined with Norfloxacin (an antibiotic) was effective in reducing bacterial infections, and at the same time, it reduced the side effects that would have resulted from a high dosage of the antibiotic Norfloxacin (Rosato, Vitali, De Laurentis, Armenise & Milillo, 2007). In addition, the essential oil was found to be an effective food preservative (Lis-Balchin *et al*., 1998; Lis-Balchin & Roth, 2000).

2.2.3 History of *Pelargonium* species cultivation for oil production

Cultivation of P*elargonium* species for oil was started in the early nineteenth century in the Grasse region of France (Weiss, 1997). It was started in the search for a substitute for the real 'Rose of the Lavant', an essential oil obtained from *Rosa damasscena*, with a similar odour as the geranium oil (Demarne, 2002). Because of the high cost of cultivation and pronounced frosty winter seasons, production of rose-scented geranium in Europe was not economical. Hence, perfumers introduced rose-scented geranium cultivation to some regions of Africa and Asia, where mild to high temperatures and cheap labour were available (Weiss, 1997; Demarne, 2002).

Annually, about 600 tons of geranium oil, estimated at 12.5 million US dollars, is delivered to the international markets (Williams & Harborne, 2002; Bhan *et al.*, 2006). China is the world-leading geranium oil producer (80-110 tons per year) followed by Egypt (50-55 tons per year), Réunion Island (6 tons per year) and India (around 2 tons per year) (Demarne, 2002). Rose-scented geranium is also cultivated in South Africa, mainly in the Mpumalanga Lowveld, KwaZulu-Natal, Western Cape and Limpopo provinces (SANDA, 2006), but most of the essential oil produced is absorbed by the domestic markets (Weiss, 1997).

To satisfy the present world essential oil demand, an additional 20 to 25 tonnes of high quality geranium oil should be produced (Demarne, 2002). In addition, as a result of the growing number and preferences of consumers, and the ever increasing number of uses of the individual essential oil constituents, an increase in essential oil demand is expected in the future (Sangwan *et al.*, 2001).

2.2.4 Geranium oil yield and composition

Geranium oil is a complex mixture of compounds such as terpene hydrocarbons, alcohols, aldehydes, acids esters and oxides (Deans, 2002). Kayser *et al.* (1998), for instance, detected about 230 distinct molecules in essential oils extracted from *Pelargonium sidoides* (D.) and

Pelargonium reniforme (C.). Profitability of geranium oil depends on the yield per hectare and composition, mainly the relative proportions of citronellol, geraniol, linalool and isomenthone (Weiss, 1997). The geranium oil industries suffer from variability in essential oil composition and inconsistency in yield because these parameters are affected by several factors such as cultivar, climate, shoot stage, soil fertility (Lis-Balchin, 2002c) and distillation/extraction techniques (Machale, Niranjan & Pangarkar, 1997; Babu & Kaul, 2005).

Chemotypes (Cultivars)

Geranium oil is obtained from various cultivars, mainly derived from crosses among *Pelargonium graveolens*, *P. capitatum* and *P. radens*, which are commonly known as the rose-scented geraniums (Lis-Balchin, 2002c). The commercially available rose-scented geranium cultivars or chemotypes are distinguished by the country of origin, and the Bourbon, Egyptian, Moroccan, Algerian and Chinese cultivars are the major ones (Weiss, 1997; Williams & Harborne, 2002).

Essential oil composition varies with cultivar/chemotype (Demarne, 2002). The Bourbon-type oil is, for instance, characterised by a 1:1 citronellol to geraniol ratio, lower citronellol and citronellyl ester levels, and high contents of geranyl esters, linalool, guaia-6,9-diene and isomenthone (Gupta, Mallavarapu, Banerjee & Kumar, 2001b; Williams & Harborne, 2002). Oil from the Egyptian type has citronellol to geraniol ratio (C:G ratio) similar to that of Bourbon type, but with lower guaia-6,9-diene contents. In addition, the Bourbon-type oil is devoid of 10 *epi*-γ-eudesmol (Gupta *et al.*, 2001b). Oils from the Chinese and the Algerian types are known for high C:G ratios, ranging between 3 and 4 (Kulkarni, Mallavarapu, Baskaran, Ramesh & Kumar, 1998). Geranium oil produced in South Africa is said to have a composition similar to that of the Bourbon type (SANDA, 2006).

From a marketing point of view, the Bourbon-type oil is regarded as the best in quality, and is priced higher than the other oils (Weiss, 1997; UIDEA, 1998). Qualitatively, oils from the Moroccan, the Algerian and the Egyptian types rank next to the Bourbon type, and presumably,

earn a premium over the oil from the Chinese type, which has a highly variable odour and is the cheapest in price (Weiss, 1997).

Apart from the commercially renowned rose-scented geranium cultivars, several essential oil-rich members of the genus *Pelargonium* and their hybrids have been reported (Viljoen, Van der Walt, Swart & Demarne, 1995; Kulkarni *et al.*, 1998). Geranium oil obtained from a *Pelargonium* sp. cultivar grown on the Réunion Island, for instance, is characterised by high *p*-cymene content (35.8%), and a pleasant 'citrusy-peppery-spicy' and herbaceous scent (Gauvin *et al.*, 2004). Essential oil of *Pelargonium graveolens* cv. Kunti (grown in India) is rich in geraniol (consisting of 40-50% of the total essential oil), whereas essential oil of a somaclonal mutant of the same cultivar was found to contain isomenthone (71%) as its major constituent (Gupta *et al.*, 2001b). High isomenthone contents (64.4% and 67.7%) have also been recorded in essential oils extracted from two other clones of *Pelargonium* species (Kulkarni *et al.*, 1998).

Climate

Climatic parameters such as temperature, rainfall and photoperiod are among the major role players in growth and biosynthetic processes in plants. Rose-scented geranium is a warm- to hot-climate plant in origin (Lis-Balchin, 2002b). Although the *Pelargonium* species are able to survive even short night chills below 0°C without permanent physiological damage (Stolarski, 1979), maximum temperature in the range between 20 and 25°C resulted in maximum leaf growth and high essential oil content (Weiss, 1997). Motsa *et al.* (2006) reported that a warm climate increased herbage growth and total essential oil yield. Results reported by Kumar *et al.* (2001) also indicated that *P. graveolens* gave a higher yield in sub-tropical (hot) areas than in temperate regions.

Reports on variability of geranium oil composition with change in seasonal as well as diurnal temperatures seem to be contradictory. Doimo, Mackay, Rintoul, D'arcy and Feltcher (1999), who monitored rose-scented geranium essential oil on a monthly basis for a duration of four years, reported that geraniol concentration declined in winter, and spring favoured citronellol. In addition, the authors indicated that the C:G ratio increased in midwinter. In data presented by

Motsa *et al.* (2006), geraniol content also tended to decline with a decrease in night temperatures in winter seasons. In contrast, Rajeswara Rao *et al.* (1996) and Rajeswara Rao *et al.* (2002) stated that concentration of geraniol increased during the cool winter, and decreased during the hot summer season, while the opposite was true for citronellol concentration and C:G ratios.

Little information on response of plant growth, essential oil yield, and essential oil composition of rose-scented geranium to light intensity and day length is available in literature, if any. Studies on other essential oil crops indicate that essential oil yield and/or essential oil compositions are affected by photoperiod (Yamaura, Tanaka & Tabata, 1989; Fahlèn, Welander & Wennersten, 1996). According to Yamaura *et al.* (1989), thymol content in essential oil of thyme increased with the number of light hours. Similarly, menthol content in *Mentha species* was enhanced by a long photoperiod (Fahlèn *et al.*, 1996).

It is a common phenomenon that in water-stressed environments plant growth is negatively affected (Turtola, Manninen, Rikala, & Kainulainen1, 2003). The reverse may be true in the case of biosynthesis of secondary metabolites such as essential oils, depending on species, degree of water stress and shoot age (Yaniv & Palevitch, 1982; Simon *et al.*, 1992; Singh-Sangwan *et al.*, 1994; Turtola *et al.*, 2003). *Pelargonium* species are characterised as drought tolerant, but in prolonged drought conditions, they show poor vegetative growth (Weiss, 1997). A rainy season favoured herbage growth and essential oil yield (Rajeswara Rao *et al.*, 1996). Weiss (1997), however, observed that a three-month dry season induced a mild increase in essential oil yield, despite a noticeable decrease in total fresh herbage mass.

Soil fertility

Studies have confirmed that total essential oil yield positively responds to fertility, particularly to nitrogen level. Singh (1999) treated rose-scented geranium with 0, 100, and 200 kg/ha nitrogen (N). The author did not observe any change in oil composition, but the highest plant growth and total essential oil yield were obtained from the plots that received 200 kg/ha N. Ram *et al.* (2003) also studied the response of rose-scented geranium to 0, 80, 160 and 240

kg/ha N with and without organic mulching. The results indicated that both fresh herbage mass and essential oil yield were improved by the 160 kg/ha N application with organic mulching. The authors also stated that the major essential oil constituents, citronellol and geraniol, were not affected by N levels. Similarly, Araya, Soundy, Steyn, Teubes, Learmonth and Mojela (2006) studied the response of rose-scented geranium to rates and sources of nitrogen (conventional and organic). Their report highlighted that the response of essential oil composition was not consistent, but herbage growth and essential oil yield were higher when organic rather than conventional nitrogen source was used. Observing a similar response of Melaleuca *alternifolia* to nitrogen and phosphorus, List, Brow and Walsh (1995) suggested that nutrient availability indirectly affects essential oil yield by controlling vegetative growth.

Ideal soil for rose-scented geranium should be rich in organic matter with soil pH between 5.5 and 6.5 (UIDEA, 1998). To avoid iron and manganese toxicity, SANDA (2006) advised to keep soil pH in the range between 5.8 and 6.2. A report by Ram, Prasad, Gupta and Kumar (1997) also indicated that both herbage growth and essential oil yield were slightly higher at a soil pH of 8.4 than at a soil pH of between 4.5 and 5.1 in a calcareous sandy loam soil. Work by Prasad, Chattopadhyay, Chand, Naqvi and Yada (2006) suggested that *Pelargonium* species are slight to moderately tolerant of soil sodicity stress.

Shoot age

Essential oil yield and composition vary with developmental stages of the whole plant, plant organs, tissues and cells (Sangwan *et al.*, 2001; Góra *et al.*, 2002). In *Erigeron canadensis,* the content of limonene in leaves declined with advance in leaf age, while the opposite was true in flower oil (Góra *et al.*, 2002). Southwell and Stiff (1989) investigated essential oil composition obtained from *Melaleuca alternifolia* leaves at different growth stages. The results revealed that with progress in leaf maturity, the content of cis-sabinene hydrate in the oil decreased from 40 to 1%, but the content of terpinene-4-ol increased from 10 to 30%. Based on these results, the authors suggested cis-sabinene hydrate to be a precursor to terpinene-4-ol. Similarly, a report by Luthra, Singh & Sharma (1991) indicated that citronellol and geraniol content in essential oil of *Cymbopogon winterianus* tended to increase with leaf age, while the reverse was true for

geranyl acetate and citronellyl acetate contents. In addition, the authors observed a positive relationship between total essential oil yield and dry matter accumulation. Góra *et al.* (2002) also agreed that essential oil yield and composition vary with overall plant growth stage as well as individual leaf age.

Information on geranium oil yield and composition at different shoot ages is limited in literature. Motsa *et al. (*2006) investigated the impact of shoot age on essential oil yield and composition of rose-scented geranium (*Pelargonium capitatum* x *P. radens*). The authors did not see a clear relationship between shoot age and essential oil concentration. Total oil yield per harvest showed an increasing tendency, and reached a maximum some time around the 19th week of each regrowth cycle. For high essential oil yield with high economic returns per annum, Kothari *et al.* (2004) recommended that rose-scented geranium should be harvested at the stage when four leaves are fully expanded. According to Góra *et al.* (2002), maximum essential oil accumulation in geranium (*Pelargonium graveolens*) is reached just before blooming. Based on their own work and reports in literature, Góra *et al.* (2002) suggested that generalisations about the biosynthesis of essential oil at different plant growth stages might possibly be based on extensive data sets from methodical studies within a particular species and subspecies.

Distillation methods

At a commercial level, geranium oil is extracted from the plant shoots by steam- and/or water-distillation techniques (Babu & Kaul, 2005). Plant essential oils are a mixture of several compounds with a wide range of chemical and physical properties (Deans, 2002; Williams & Harbone, 2002). Hence, different distillation methods and distillation phases are expected to have different effects on the chemical as well as physical state of the compounds (Amin, Pangarkar & Beenackers, 2001; Peterson *et al.*, 2006; Babu, Shanmugam, Ravindranath & Joshi, 2007).

Babu and Kaul (2005) investigated the impact of different hydrodistillation techniques (water distillation, water-steam distillation and steam distillation) with or without recycling the

hydrosol on the amount and composition of essential oil of a rose-scented geranium. Their results showed that hydrolysis of some constituents resulted in changes in essential oil composition. The authors also realised that the amount and composition of the recovered oil depend on the solubility of the essential oil constituents. Thus, the authors suggested that using steam distillation in combination with water distillation (in the later distillation phase) would give the desired essential oil yield and quality. The above discovery and suggestion supports a previous report (Rajeswara Rao *et al.*, 2002), which indicates that an average 7% of the total essential oil yield could be recovered from hydrosol by hexane extraction. In addition, Rajeswara Rao *et al.* (2002) revealed that the blend of the recovered and primary oil (oil obtained directly by distilling) had better perfumery note than either the recovered or the primary oil.

Peterson *et al.* (2006) also investigated the effect of the supercritical fluid extraction (SFE) technique on the amount and composition of rose-scented geranium essential oil. The authors used supercritical carbon dioxide as a solvent in combination with different pressure, temperature and carbon-dioxide flowing rates and extraction durations. The results showed that pressure and extraction time significantly affected essential oil composition. The authors highlighted that, at optimum temperature, extracting time and carbon-dioxide flow rate, the SFE technique improved essential oil recovery to nearly 17 times (2.53%) that of the essential oil extracted by the steam-distillation technique (0.15%).

The above findings are in agreement with results reported by Machale *et al.* (1997), who demonstrated that the commercial essential oils of basil and *Mentha arvensis* obtained by steam distillation lacked the natural aroma because some of the essential oil components originally present in the plant remained dissolved in the hydrosol (water condensate). The authors separated the essential oil constituent from the water condensate using Amberlite XAD-4 and ethanol as adsorbent and as eluent, respectively. The results showed that a blend of the oil recovered from the hydrosol and the oils obtained by steam distillation had a more natural and richer aroma.

The overall results confirm that the composition of essential oils obtained by the different extracting techniques could vary in amount and composition, which may result in products that would misrepresent the essential oil yield, oil composition and the natural aromatic characteristics of the oil in the source plant (Amin *et al.*, 2001). In addition, these reports indicate that it might be possible to maximise the present yield and quality of plant essential oils by improving existing extracting techniques.

2.3 IRRIGATION

2.3.1 History of irrigation

Irrigation could be explained as artificially supplying water to an agricultural/cropped land to avert crop failure due to shortage of natural precipitation (as supplementary) in semi-arid regions or to permit farming in arid regions as substitute for rainfall (Hillel, 1990; Bazza, 2007). Irrigated agriculture was defined by FAO (1999) as the practice of increasing the supply of water by using water-controlling technologies, including a drainage system to dispose of excess water.

Despite the variation in water conveyance (ranging from carrying water with buckets to a complex canal system), history has recorded that irrigated agriculture started thousands of years back (Brady & Weil, 1999). It is believed that irrigated agriculture started in the Near East, particularly in the Egypt (along the banks of the Nile River) and Mesopotamia (between the Tigris and Euphrates rivers) areas, which suffered form severe aridity (Bazza, 2007). Hoffman, Howell and Solomon (1990) mentioned that irrigated agriculture was observed in Egypt and Mesopotamia some time around 6000 BC and 4000 BC, respectively. Irrigated farming was introduced to the rest of the North African and Mediterranean regions some time around 800 BC (Bazza, 2007). Drawing groundwater for crop production was first developed by the Persians 2 500 years ago, first in Middle East, and later, with expansion of their rule (550 BC-331 BC), it was introduced to some regions of Asia and Africa (Bazza, 2007).

Out of the 1 560 million hectares cultivated lands in the world in 1961, only 136 million hectares were under irrigated agriculture. During the last four to five decades, the area of irrigated agriculture showed a high expansion rate; hence, in 2000 it doubled to about 273 million hectares (FAO, 1999).

2.3.2 Contribution of irrigated agriculture

Irrigation has played an indispensable role in coping with agricultural productivity and the ever-increasing demands from the continuously growing world population. The proportion of irrigated land is as small as 17% of the total cropped area, but its contribution is as high as 40% of humankind's food demands (Hamdy *et al.*, 2003; Smith, 2004). A report from the Food and Agriculture Organisation (FAO) of the United Nations (UN) indicated that under irrigated conditions, the yield of most crops could increase by 100 to 400% (FAO, 1996).

In irrigated agriculture, farmers are able to predict the timing of irrigation and supply the required amount of irrigation water for each crop. In regions where there is no shortage of freshwater, a year-round cropping would be possible through irrigation (Hussain & Hanjra, 2004), if other climatic conditions are favourable. In addition, in irrigated agriculture, farmers have a better chance to be flexible in planning what crop to plant in response to season and market demands than in dryland agriculture (FAO, 1999).

As the farm productivity increases, the income of the landowners rises, wages of farm employees improve, and employment opportunity increases (Smith, 2004). Such an increase in agricultural productivity would lower food prices in the rural communities, who spend about 50 to 80% of their income on purchasing staples (FAO, 1999; Hasnip, Mandal, Morrison, Pradhan & Smith, 2001). Irrigated agriculture could be explained as a pillar of economic growth because an increase in agricultural output and population concentration attract other services and infrastructures (Bazza, 2007). Hussain & Hanjra (2004) highlighted that an increase of 1% in agricultural productivity could result in an equivalent reduction in the number of people who live below the poverty line. Poverty is estimated to be 20 to 30% lower in communities where

irrigated farming is practised, compared to those communities fully dependent on rainfed agriculture (Hussain & Hanjra, 2004).

2.3.3 Freshwater scarcity

Water scarcity is an arbitrary term that could be defined as the failure of the available water in a region to fully meet the demand of all users (including the environment) in quantitative and/or qualitative terms, which could be characterised by severe environmental degradation, declining groundwater level, and problems of allocation (FAO, 2007a). Freshwater scarcity is becoming a common phenomenon worldwide (Shi-Wei *et al.*, 2006). Seckler *et al.* (1999) described water scarcity in arid and semi-arid regions as an unparalleled threat to food security, human health and environmental sustainability. Shi-Wei *et al*. (2006) also considered freshwater scarcity as the most limiting resource in agriculture.

The major contributors to freshwater scarcity are climatic change (FAO, 2007a) and overexploitation of water resources for agricultural, domestic, and industrial uses (Hussain & Hanjra, 2004). According to a meteorological analysis in the United Kingdom, the incidence of extreme drought that now occurs once every 50 years is predicted to increase to every other year by the end of the 21st century because of climatic changes (FAO, 2007a).

On average, about 70% of the total freshwater withdrawn worldwide is allocated to the agricultural sector, including livestock (FAO, 2007b), although this figure may vary at regional or continental level, depending on economic growth and climatic conditions. FAO (2007b), for instance, pointed out that nearly 50% of the freshwater withdrawn in the European and North American countries goes to industries, whereas in Africa only about 15% of the total freshwater is taken for industrial and domestic purposes.

Water scarcity is expected to be even worse in the near future because population numbers will continue to grow with a parallel increase in food demand, and rapid urbanisation and expansion of economic activities that would demand more water for domestic and industrial purposes

(FAO, 2007b; Fereres & Soriano, 2007). Countries that have already suffered from a shortage of freshwater for agricultural, industrial, domestic and environmental sectors (Plusquellec, 2002), will face more challenges in the future, because their population is expected to increase by more than 8 000 million, accompanied by a 100% increase in demand for agricultural products by the year 2025 (FAO, 1999). In addition, the present world population (6 000 million) is expected to increase by 35% (to about 8 100 million) by the year 2030 (Playán & Mateos, 2006). In line with this, the World Summit 1996 estimated that about 60% of the extra agricultural products to satisfy the needs of the ever-growing population should be contributed by irrigated agriculture (FAO, 1999; Plusquellec, 2002). To provide the world with enough food, Serageldin (2001) also suggested that irrigated agriculture should be expanded by 20% in 2025. Hence, the available information confirms that conflicts about water will increase among the agriculture, domestic and economic sectors and the environment (Hussain & Hanjra, 2004).

2.3.4 Coping with freshwater scarcity in agriculture

Demand for agricultural products in the 1960s to 1980s ('green revolution' era) was met by increasing cultivated yield per area by expanding the irrigated land (by developing more new water supplies), intensifying fertiliser application and introducing high-yielding cultivars (Rockström & Falkenmark, 2000; FAO, 2003; Smith, 2004). These days, there may still be a possibility of developing new water supplies in some regions (Brooks, 2006). In most arid and semi-arid regions, however, the available freshwater resources for agriculture and other social and economic sectors are being fully exploited (Seckler *et al.*, 1999; Plusquellec, 2002).

In arid and semi-arid regions, water recycling and improving water-use efficiency are among the possible strategies for solving freshwater scarcity (Pimentel, Berger, Filiberto, Newton, Wolfe, Karbinakis, Clark, Poon, Abbet & Nandagopal, 2004). Pereira, Oweis and Zairi (2002) and Hamdy *et al.* (2003) suggested that increasing the productivity of the limited available water, in all the water-using sectors, particularly in agriculture (where water-use efficiency is at most 45%), will be a major player in boosting agricultural production, easing competition for water and ensuring environmental sustainability.

In searching for innovative irrigation management methods that would boost the productivity of scarce water in dry regions, more focus has been given to different irrigation managements, including irrigation scheduling techniques such as deficit irrigation (Nautiyal, Joshi & Dayal, 2000), applying maximum allowable depletion levels (Panda *et al.*, 2003; Panda, Behera & Kashyap, 2004; Eiasu *et al.*, 2007) and irrigation withholding at less sensitive crop growth stages (Mastrorilli *et al.*, 1995).

Deficit irrigation

In deficit irrigation, also termed regulated deficit irrigation (Panda *et al.*, 2003), a certain level of water stress is imposed on the crop during a particular period or throughout the whole growing season, depending on the yield response of specific crops and plant growth stage (Singh & Singh, 1995; Kirda, 2000; Moutonnet, 2000; Pereira *et al.*, 2002). Deficit irrigation is usually achieved by irrigating the crop with an amount of water less than full evapotranspiration (Singh & Singh, 1995; Girona, Gelly, Mata, Arbonès, Rufat & Marsa, 2005) or field capacity (Kang *et al.*, 2000).

As reviewed by Pereira *et al.* (2002), increasing water productivity by adopting deficit irrigation may compromise total crop yield per unit land area. This technique is practised under circumstances of limited water supply to obtain the maximum crop yield per unit of water (Hamdy *et al.*, 2003), and to save water that would be available to irrigate more land (Pereira *et al.*, 2002). Pereira *et al.* (2002) highlighted that the adoption of deficit irrigation needs sound knowledge on crop water demand, yield response to water stress, and comparative economic advantage of the technique. Similarly, Shock and Feibert (2000) reported deficit irrigation to be a successful means of improving water productivity and coping with prevailing water scarcity, but all crop species in combination with different soil water monitoring techniques need to be investigated.

Partial root zone drying

Partial root zone drying (PRD) is a new irrigation management technique, where at each irrigation event, soil on one side of the plant (in a row) is supplied with water (commonly to field capacity) while the complement side is left to dry to a predefined depletion level. The wetted and dry root zones are interchanged in the subsequent irrigation events (Zegbe-Domínguez, Behboudian, Lang & Clothier, 2003; Kirda, Cetin, Dasgan, Topcu, Kaman, Ekici, Derici, & Ozguven, 2004). The PRD irrigation technique was based on the knowledge that when part of the plant root system experiences water stress, chemical signals produced by the root system induce reduction in stomatal conductance, while the leaf water potential is still high (Stoll, Loveys & Dry, 2000; Kirda *et al.*, 2004). In addition, the biochemical response of plants to PRD is believed to regulate the balance between vegetative and reproductive development (McCarthy, Loveys, Dry & Stoll, 2000).

It was confirmed that PRD irrigation is a viable irrigation management technique for grapevine. McCarthy *et al.* (2000) reported that PRD improved the water-use efficiency of grapevine without any significant crop yield reduction. Similarly, Dos Santos, Lopes, Rodigues, De Souza, Maroco, Pereira, Silva & Chaves (2003a, 2003b) reported that the PRD irrigation technique increased water-use efficiency by up to 80% without a significant reduction in leaf water potential in grapevine. A recent report by De la Hera, Romero, Gómez-Plaza & Martinez (2007) also indicated that PRD irrigation improved fruit yield and water-use efficiency of grapevine grown under semi-arid conditions.

Maximum allowable depletion level

With maximum allowable depletion irrigation scheduling, crops are irrigated to field capacity when a certain amount (or fraction) of the plant available soil water in the active root zone has been depleted (Panda *et al.*, 2003; Panda *et al.*, 2004; Eiasu *et al.*, 2007). This irrigation technique has been reviewed in depth in Chapter 3 of this thesis.

Withholding irrigation at different growth stages

An irrigation-withholding schedule is an irrigation management technique that is practised by stopping irrigation during certain periods of the growing season, when the crop is at a less water-stress-sensitive physiological stage (Mastrorilli *et al.*, 1995). This irrigation management technique has been reviewed and discussed in Chapter 4 of this thesis.

Certain authors classify both the irrigation-withholding and maximum allowable depletion irrigation schedules under deficit irrigation techniques (Gorantiwar & Smout, 2003; Panda *et al.*, 2003; Karam *et al.*, 2005). Regardless of the terminologies used to explain them, the overall objective of adopting the different irrigation scheduling techniques remains the same: to improve water productivity, thereby saving sufficient amounts of water that could be used to irrigate more crop land area and/or alleviate water shortage in other economic and social sectors without sacrificing agricultural production.

CHAPTER 3

GROWTH, ESSENTIAL OIL YIELD AND OIL COMPOSITION OF ROSE-SCENTED GERANIUM GROWN AT DIFFERENT MAXIMUM ALLOWABLE SOIL WATER DEPLETION LEVELS

3.1 ABSTRACT

Effective irrigation management in arid and semi-arid regions, like South Africa, could increase crop yield and thereby improve productivity of scarce freshwater resources. Experiments were conducted at the Hatfield Experimental Farm of the University of Pretoria, South Africa, from 2004 to 2006, to investigate the effects of different maximum allowable depletion (MAD) levels of plant available soil water (ASW) on rose-scented geranium (*Pelargonium capitatum x P. radens* cv. Rose) essential oil yield, essential oil composition and water-use efficiency in an open field and a rain shelter. Four predefined MAD levels, namely 20, 40, 60 and 80% of ASW in the top 0.8 m root zone, were applied as treatments. Plant roots extracted most soil water from the top 0.4 m soil layer. Increasing the MAD level of ASW to 60% and higher resulted in a significant reduction in herbage mass and essential oil yield. Water stress apparently increased the essential oil concentration (percentage oil on fresh herbage mass basis), but its contribution to total essential oil yield (kg/ha oil) was limited. Irrigation treatments did not affect essential oil composition. A reduction in leaf area and an increase in leaf to stem fresh mass ratio were common responses to an increase in MAD level. Up to 28% of irrigation water could be saved by increasing maximum allowable depletion level of ASW from 20 to 40%, without a significant reduction in essential oil yield.

Keywords: Citronellol; citronellyl formate; essential oil composition; fresh herbage mass; geraniol; maximum allowable depletion level; plant available soil water; water stress

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3.2 INTRODUCTION

South Africa is a drought-prone (mostly semi-arid) country with variable climate (Shand & Basson, 2003). Most of the freshwater resources of the country (about 60%) are used for irrigation (Conley, 1997; Enright, 2003). Because of population growth, it is expected that the annual water demand will have increased by 24.4% by the year 2025 (Shand & Basson, 2003). Since irrigation productivity is relatively low in the country, it is suggested that part of the water used in the agricultural sector be transferred to other non-agricultural economic sectors to maximise water productivity (Nieuwoudt *et al.*, 2003). Such a shift in the allocation of water resources could impose further restrictions on agricultural businesses, unless innovative irrigation management is introduced for all crops.

In searching for innovative irrigation-scheduling techniques, more focus has been given to different irrigation managements. One of the several irrigation-scheduling strategies investigated is the maximum allowable depletion (MAD) level of available soil water, an irrigation interval based on the soil water deficit (Panda *et al.*, 2003; Panda *et al.*, 2004; Eiasu *et al.*, 2007). With this irrigation-scheduling technique, crops are irrigated to field capacity when a certain amount (or fraction) of the plant available soil water in the active root zone has been depleted (Panda *et al.*, 2004).

Since the temporal soil water depletion rate varies with weather conditions (such as temperature and relative humidity) and plant growth stage (Gorantiwar & Smout, 2003), practising the fixed-day-based irrigation interval, could result in either overirrigation, wastage of water and fertiliser, or underirrigation (yield loss due to water stress) (Igbadun, Mahoo, Tarimo & Salim, 2006). Hence, the MAD level irrigation technique is probably preferred to an irrigation interval based on fixed date, especially for perennial crop, which have a well established/defined root zone.

Rose-scented geranium (*Pelargonium* species) is a perennial herb indigenous to South Africa (Lis-Balchin, 2002a). The plant is cultivated for its high-value essential oil, which is used for the production of high-grade perfumery, cosmetic products and aromatherapy (Rajeswara Rao *et al.*, 1996). Essential oil demand is expected to increase in the future as a result of the growing

number and preferences of consumers, and the continuously widening uses of essential oil constituents (Sangwan *et al.*, 2001).

Studies on the response of rose-scented geranium essential oils to soil water have come up with different results. Rajeswara Rao *et al*. (1996) reported that moist seasons resulted in higher essential oil yield. A report by Singh (1999) indicated that a soil water regime of 0.6 IW to CPE (irrigation water to cumulative pan evaporation) ratio gave a higher essential oil yield without a significant change in oil composition. Weiss (1997), on the other hand, reported that water-stressed conditions resulted in a mild increase in oil yield. Similarly, Simon *et al.* (1992) reported that a mild to moderate water stress imposed on sweet basil resulted in a higher oil content and higher total oil yield per plant.

In South Africa, rose-scented geranium production is limited to some areas in the Mpumalanga Lowveld, KwaZulu-Natal, Western Cape and Limpopo provinces (SANDA, 2006), all of which are relatively high rainfall regions in the country (Davies & Day, 1998). Weiss (1997) and Bhan *et al.* (2006) indicated that to date the South African geranium oil production business has not made a significant contribution to world essential oil markets. Low and erratic rainfall, and a lack of knowledge on the amount and time of application of irrigation could be among the major contributors to the low geranium oil production in the country. Hence, it was hypothesised that an irrigation schedule based on the MAD level of ASW would improve essential oil quality, boost essential oil yield per area and enable expansion of the cropping areas to the dry regions.

The main objective of the current study was, therefore, to acquire a sound knowledge of the response of rose-scented geranium herbage growth, essential oil yield and oil composition to different MAD levels of ASW, thereby to recommend effective irrigation management strategies for South Africa and other areas with similar agroclimatic conditions, where relevant experimental data are not available.

3.3 MATERIALS AND METHODS

3.3.1 Study area and duration

The experiments were conducted at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa (latitude 25° 45'S and longitude 28° 16'E, and an altitude of 1 372 m), from October 2004 to October 2006. The experimental site is situated in a region with an average annual rainfall of 670 mm, mainly in the summer season (during the months of October to March), and monthly average maximum and minimum temperatures of about 30°C (in January) and 1.5°C (in July), respectively (Annandale, Benadé, Jovanovic, Steyn & du Sautoy, 1999). The experiments were conducted in an open field and in a rain shelter (with movable roof, to screen out rainfall). Detailed data on soil chemical and physical properties, and weather conditions have been presented in Appendix A.

In the open field, two parallel trials were established on the same strip of land. Trial 1 was conducted only for one growth cycle (12 March to 11 July 2005), which in the results and discussion section is referred to as Harvest 1. Trial 2 continued for three regrowth cycles (14 May to 13 September, 14 September 2005 to 13 January 2006, and 26 June to 25 October 2006, for the first, second and third regrowth cycles, respectively). In the results and discussion section, the data from the first, second and third regrowth cycles of Trial 2 are referred to as Harvests 2, 3 and 4, respectively. The experiment in the rain shelter was conducted for two regrowth cycles, from 27 February to 26 June and from 27 June to 26 October 2006 (for Harvests 1 and 2, respectively).

3.3.2 Plant culture

Rose-scented geranium is commonly raised from stem cutting. At commercial level, planting to the first harvest takes five to six months. Duration for the subsequent regrowth cycles (from cut back to harvesting of regenerated shoot) is three to four months. Once the crop is established, it could be harvested for up to 10 years depending on standards of management (Weiss, 1998).

In the current experiments, rose-scented geranium (*Pelargonium capitatum* x *P. radens* cv. Rose) was used as plant material. For the open-field trials, about 45-day-old plantlets propagated from stem cuttings by a commercial nursery, were transplanted on 28 October 2004. For the rain shelter trial, healthy stem cuttings (taken from the open field trials) were planted in seedling trays filled with peat on 25 August 2005, and raised at high relative humidity (in a mist bed) in a greenhouse at the Hatfield Experimental Farm. Plantlets were transplanted on 1 October 2005. For both sites (the open field and the rain shelter), the plants were allowed to grow for about seven months until uniform establishment. Thereafter, the plants were cut back to start irrigation treatments. A regrowth duration of four months was decided on, according to local commercial farmer practices.

3.3.3 Field layout and treatments

Experimental layout

In the open-field trials, each experimental area was divided into four blocks. Each block consisted of four plots of 7.5 m long and 5 m wide. There was a buffer strip of 1.5 m between two adjacent blocks. Spacing between adjacent rows was 1 m, and plants in a row were planted 0.60 m apart. Each experimental plot consisted of five rows, and data recording was done on the three middle rows.

In the rain shelter, a higher plant density (0.75 m inter-row and 0.45 m intra-row spacing) was applied to ensure sufficient plant material from the smaller plots. Plastic sheets were installed vertically to a depth of 80 cm to avoid lateral water movement and root growth between adjacent plots. Each experimental plot consisted of four rows of 6 m long. In all trials, treatments were arranged in a randomised complete block design (RCBD).

Treatments

In all the trials, irrigation treatments were scheduled based on maximum allowable depletion (MAD) percentage of the plant available soil water (ASW). The following predefined MAD levels of ASW were applied as treatments:

1. Plots replenished when 20% of ASW had been depleted (20% MAD or control);
2. Plots replenished when 40% of ASW had been depleted (40% MAD);
3. Plots replenished when 60% of ASW had been depleted (60% MAD);
4. Plots replenished when 80% of ASW had been depleted (80% MAD).

During each irrigation event, plots were refilled to field capacity. No water stress was applied in the first month of each regrowth cycle (immediately after cutting back) to limit plant mortalities. Irrigation treatments were imposed during the remaining three months of regrowth cycles. During establishment (or when recovering from cutting), all plots were irrigated when 20% of the ASW had been depleted.

3.3.4 Irrigation monitoring

Neutron probe calibration

A neutron probe (Model 503 DR, CPN Corporation, CA, and USA) was used to measure soil water content. The neutron probe was first calibrated to establish a regression equation that would give the corresponding volumetric soil water content to standard reading ratio (Brady & Weil, 1999). To do the neutron probe calibration process, two wet spots were prepared by repeatedly ponding 2 m x 2 m areas, with an aluminium access tube inserted at the centre to a depth of 1.2 m. The ponding continued until the soil around the access tube was fully saturated. Immediately after the ponding process had been accomplished, the wet spots were covered with plastic sheets to avoid evaporative water loss. After 48 hours, during which the soil water status was supposed to be at field capacity, neutron probe readings and soil samples, from each 0.2 m soil depth increment, were taken. Similar data were also taken from dry spots. From before and

after oven-drying (for 24 hours at 105°C), mass of the soil samples, gravimetric and volumetric water contents for each soil layer were determined (detailed data are presented in Appendix B). From neutron readings in soil (R_S) and in air (R_A) of respective spots and soil layers, standard probe reading ratios (X) were calculated using Equation 3.1.

$$X = \frac{R_S}{R_A} \qquad (3.1)$$

Based on the regression relationship between volumetric water content and standard reading neutron probe ratios (run by Microsoft Excel), equations for each layer and trial site were derived (Table 3.1).

Table 3.1: Regression equations used to determine soil water content

Soil layer	Experimental site	
	Open field	Rain shelter
0.0 - 0.2 m	$Y = 0.1026X + 0.0459$ †	$Y = 0.1075X + 0.0961$
0.2 - 0.4 m	$Y = 0.2025X - 0.0560$	$Y = 0.5128X - 0.5081$
0.4 - 0.6 m	$Y = 0.1802X - 0.0684$	$Y = 0.0687X + 0.1304$
0.6 - 0.8 m	$Y = 0.134X + 0.0496$	$Y = 0.4819X + 0.5321$
0.8 - 1.0 m	$Y = 0.5759X + 0.1136$	$Y = 0.4563X - 0.4882$
1.0 - 1.2 m	$Y = 0.1211X + 0.0656$	$Y = 0.0936X + 0.1168$

† **Y and X are volumetric soil water content (%) and standard reading ratio, respectively**

Irrigation scheduling

For soil water monitoring neutron, neutron probe readings (at intervals of 0.2 m, to a soil depth of 1.2 m) were taken on every alternative day. A computer-controlled pressure-compensated drip irrigation system with water discharge rate of 2 ℓ/hr at pressure range of 120-200 kPa

(NETAFIM, Cape Town, South Africa) was used in both experiments. The drip lines were placed 0.5 m apart, and the in-line spacing between dripper emitters was 0.3 m. The percentage depletion of ASW (θ_d) and the volume of irrigation water (V_I) needed to refill the soil profile to field capacity were calculated using Equations 3.2 and 3.3, respectively (Kashyap & Panda, 2003; Panda *et al.*, 2004).

$$\theta_d = 100 \times \frac{1}{n} \sum_{i}^{n} \frac{\theta_{FCi} - \theta_i}{\theta_{FCi} - \theta_{PWPi}} \tag{3.2}$$

$$V_I = \theta_d R_Z A \left(\frac{1}{100}\right) \tag{3.3}$$

where θ_{FCi} represents volumetric soil water content (m^3/m^3) at field capacity for the ith layer, θ_i (measured) volumetric soil water content (m^3/m^3) for the ith layer, θ_{PWPi} volumetric soil water content at permanent wilting point (m^3/m^3) for the ith layer, n total number of layers under consideration, A area of plots (m^2), and R_z effective root zone depth (0.8 m in this case).

Based on preliminary observations of soil water depletion from the root zone during the six months of plant establishment period, effective plant root zone was considered to be the top 0.8 m soil layer. For observation of soil water dynamics, however, probe readings were taken to 1.2 m soil depth. In the rain shelter, rainfall was successfully excluded. In the open field, precipitation depth that exceeded the ASW deficit in the 0.8 m root zone was considered as deep percolation or runoff, and was excluded from effective evapotranspiration calculations. Evapotranspiration (ET) per regrowth cycle was calculated using Equation 3.4 (Wright & Smith, 1983; Çakir, 2004).

$$ET = P + I - (D + R) + (S_1 - S_2) \tag{3.4}$$

where P, I, D, R, S_1 and S_2 represent rainfall (mm), irrigation water applied (mm), water lost by deep percolation (mm), water surface runoff (mm), and initial and final soil water contents (mm), respectively.

3.3.5 Agronomic practices

During establishment, plants received 60 kg/ha nitrogen (N), 90 kg/ha phosphorus (P) and 60 kg/ha potassium (K). In the second week of each regrowth cycle, N, P and K were applied at rates of 30, 15 and 30 kg/ha, respectively. Hoeing was accomplished during the first month of each regrowth cycle. Hand-weeding, and standard pest and disease control measures were taken when necessary.

3.3.5 Data recorded

Plant growth data

Starting from the seventh week of each regrowth cycle, five plants per treatment were sampled on a biweekly basis to determine leaf area index (LAI) and dry herbage mass accumulation patterns. At the end of each regrowth cycle, all the plants left from biweekly sampling were harvested. During harvesting (sampling), plant shoots were cut to a height of about 0.15 to 0.2 m above ground. Herbage fresh mass was measured immediately after cutting. Leaves and stems of samples were separated. Leaf area (LA) was measured using an LI 3100 belt-driven leaf area meter (LiCor, Lincoln, Nebraska, USA), and leaf area index (LAI) was calculated from the measured LA and the harvested land area (H_{LA}), as expressed in Equation 3.5. The samples were oven-dried at about 70°C to a constant mass to determine the dry matter contents.

$$LAI = \frac{LA\ (m^2)}{H_{LA}\ (m^2)} \tag{3.5}$$

Essential oil yield components

At the final harvests of each regrowth cycle, freshly harvested herbage samples (of about 3-7 kg each) were taken for essential oil content determination. Essential oil was extracted by steam-

distillation technique (for one hour) using a 90 kg capacity custom-built distillation device Model KSST (Riebeek Kasteel 7306, Grahamstown, South Africa). From the essential oil content, the oil yield per treatment was determined.

Since cost for gas chromatography (GC) oil analysis is high, and the available budget was limited, oil composition was determined from oil samples that were pooled per treatment. In the pooling process, the same amount of oil from each replication of the same treatment was mixed and the GC analyses were conducted. For GC oil analysis, an Agilent GC (FID) model 6890N (Agilent Technologies, Inc., Santa Clara, CA), fitted with a 30 m x 0.25 mm fused silica capillary column and a film thickness of 0.25 µm, was used. Helium gas was used as a carrier. The temperature programme was from 50°C to 200°C with ramp amount of 5°C min^{-1}, and a detector and an injector temperature of 220°C. Constituents were identified based on their retention time as previously determined with pure chemicals as standards (Adams, 2004).

Water use and water-use efficiency

The sum of irrigation water applied and effective rainfall during the regrowth periods was considered as the total water used per regrowth cycle. Water-use efficiency was determined by dividing the dry herbage harvested (t/ha) or essential oil produced (kg/ha) by evapo-transpiration.

Where applicable, the recorded data were subjected to analysis of variance (ANOVA) using MSTAT-C, a data-analysing microcomputer program (MSTAT-C, 1991). Treatment means were compared using the least significant difference (LSD) test at 0.05 probability level.

3.4 RESULTS AND DISCUSSION

3.4.1 Soil water depletion patterns

Irrigation days and amounts (depths) of water applied to refill the root zone to field capacity for each treatment are presented in Tables 3.2 and 3.3. Only the data recorded during the winter (no-rain) season in the open-field trial and rain shelter trials are included to clearly illustrate the effect of the treatments on soil water depletion, without the interference of rain. The 20% MAD treatment was irrigated 15 times during the treatment application period (from about Day 30 until the end of each regrowth cycle), while the 80% MAD treatment was irrigated only once. The irrigation depth per irrigation event, on the other hand, increased from about 18 to 22 mm (for the 20% MAD treatment) to about 75 to 85 mm (for the 80% MAD treatment).

Examples of depth-wise temporal soil water content in the root zone of rose-scented geranium under the influence of irrigation treatments are illustrated in Figures 3.1 and 3.2. For more clarity, the figures include only the soil water content recorded during the last three months of regrowth cycles (when irrigation treatments were applied). Soil water depletion rate was higher in the top 0.2 m soil layer, where the density of fine roots is commonly higher (Goldhamer, Fereres, Mata, Girona & Cohen, 1999; Benjamin & Nielsen, 2006) than in the subsoil.

As the irrigation intervals became longer (higher MAD level), the top 0.2 m soil layer dried out, and the proportion of water taken up from the deeper soil layers increased. The water content of the top 0.2 m soil layer dropped below permanent wilting point by the time that the depletion threshold level for the 80% MAD treatment was reached. The reason could most probably is the direct evaporation of water from the topsoil, since it was unlikely that the root system could extract water at such a very low water potential (Laio, Porporato, Ridolfi & Rodriguez-Iturbe, 2001).

Table 3.2: Days of regrowth cycle and amount of irrigation applied to the different maximum allowable soil water depletion treatments in the open field

Experimental duration	Irrigation treatments							
	20% MAD†		40% MAD		60% MAD		80% MAD	
	Day	Amount (mm)	Day	Amount (mm)	Day	Amount (mm)	Day	Amount (mm)
12 March-11 July 2005 (Harvest 1)	1	20.5	5	21.5	1	20.2	3	19.3
	9	20.3	13	20.7	7	20.2	11	20.5
	15	20.7	19	18.0	13	19.7	17	19.2
	21	19.7	25	19.0	19	18.1	23	18.8
	27	18.6	31	17.0	25	18.3	29	19.7
	33	16.1	45	37.1	31	18.0	85	75.2
	39	16.8	61	38.4	57	56.1		
	45	18.1	77	37.1	81	56.6		
	51	18.9	91	37.4	113	56.7		
	57	19.6	105	37.2				
	63	19.2	117	37.0				
	69	18.1						
	75	18.2						
	81	19.1						
	87	19.1						
	93	18.9						
	99	18.6						
	105	17.4						
	111	18.8						
	117	18.8						
14 May-13 September 2005 (Harvest 2)	5	19.1	5	19.7	5	19.1	5	21.6
	11	20.8	11	20.7	11	20.5	11	20.7
	17	20.0	17	19.2	17	18.3	17	21.5
	23	19.8	23	20.0	23	18.2	23	20.3
	29	22.5	29	18.6	29	20.7	29	17.9
	37	21.0	49	39.8	61	55.9	101	77.0
	43	20.0	63	36.4	81	56.2		
	49	20.5	81	38.8				
	61	21.5	95	35.9				
	67	20.7	107	39.8				
	75	19.7						
	81	19..5						
	87	20.6						
	93	18.9						
	99	20.3						
	105	19.3						
	113	18.8						

†**MAD represents maximum allowable depletion of plant available soil water**

Table 3.3: Days of regrowth cycle and amount of irrigation applied to the different maximum allowable soil water depletion treatments in the rain shelter

Experimental duration	Irrigation treatments							
	20% MAD[†]		40%MAD		60% MAD		80% MAD	
	Day	Amount (mm)	Day	Amount (mm)	Day	Amount (mm)	Day	Amount (mm)
27 February-26 June 2006 (Harvest 1)	1	21.7	3	20.9	3	21.4	5	22.0
	9	17.3	11	22.2	13	26.0	13	23.4
	11	21.1	19	26.9	21	22.0	21	22.0
	19	21.0	27	21.2	29	24.5	29	22.0
	29	20.9	47	42.2	63	64.3	91	85.0
	39	19.3	65	43.7	91	64.0		
	51	20.6	81	42.4	119	64.3		
	59	20.7	97	43.6				
	67	21.0	111	43.2				
	77	22.3						
	83	20.9						
	89	22.0						
	95	21.8						
	101	21.1						
	107	21.6						
	113	19.6						
	119	21.9						
27June-26 October 2006 (Harvest 2)	1	22.2	3	21.4	1	21.5	5	19.0
	9	21.7	13	23.4	9	20.6	13	21.9
	17	21.4	21	22.5	11	23.0	21	25.9
	23	21.2	29	21.8	21	22.2	27	24.2
	29	20.5	45	42.3	29	24.7	79	85.3
	37	22.3	59	42.5	63	63.5		
	43	20.6	77	43.1	95	64.3		
	49	19.2	93	42.4				
	55	21.1	107	42.2				
	61	21.0						
	67	20.3						
	73	21.4						
	79	21.5						
	85	20.7						
	91	20.0						
	97	20.6						
	103	20.8						
	109	21.0						
	117	22.6						

[*]**MAD represents maximum allowable depletion of plant available soil water**

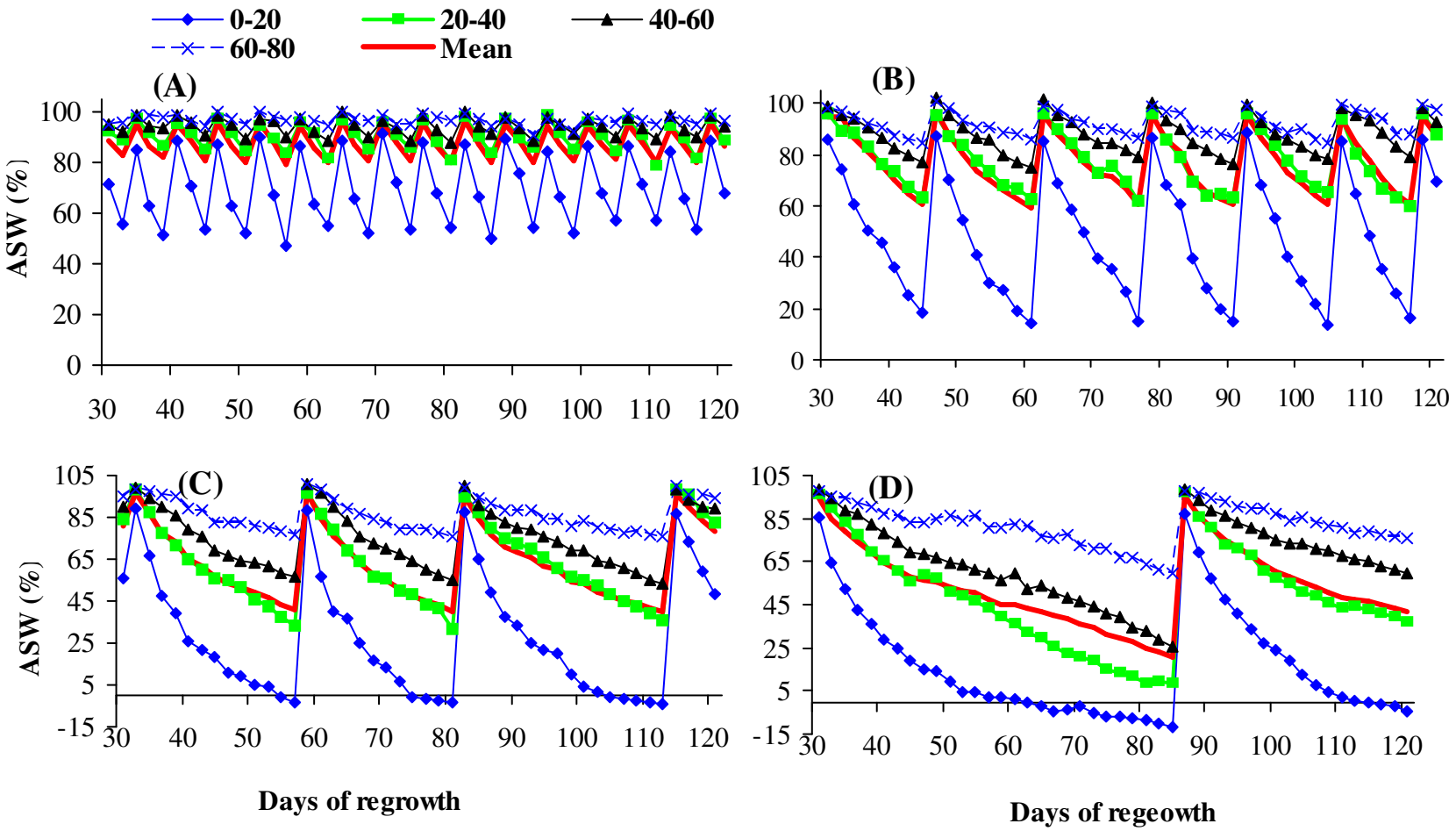

Figure 3.1: Temporal variations in plant available soil water (ASW) content in the layers of the root zone of rose-scented geranium in the open field trial: data for the 20 (A), 40 (B), 60 (C), and 80% (D) maximum allowable depletion (MAD) treatments for Harvest 1 (regrowth cycle during 12 May-11 September 2005)

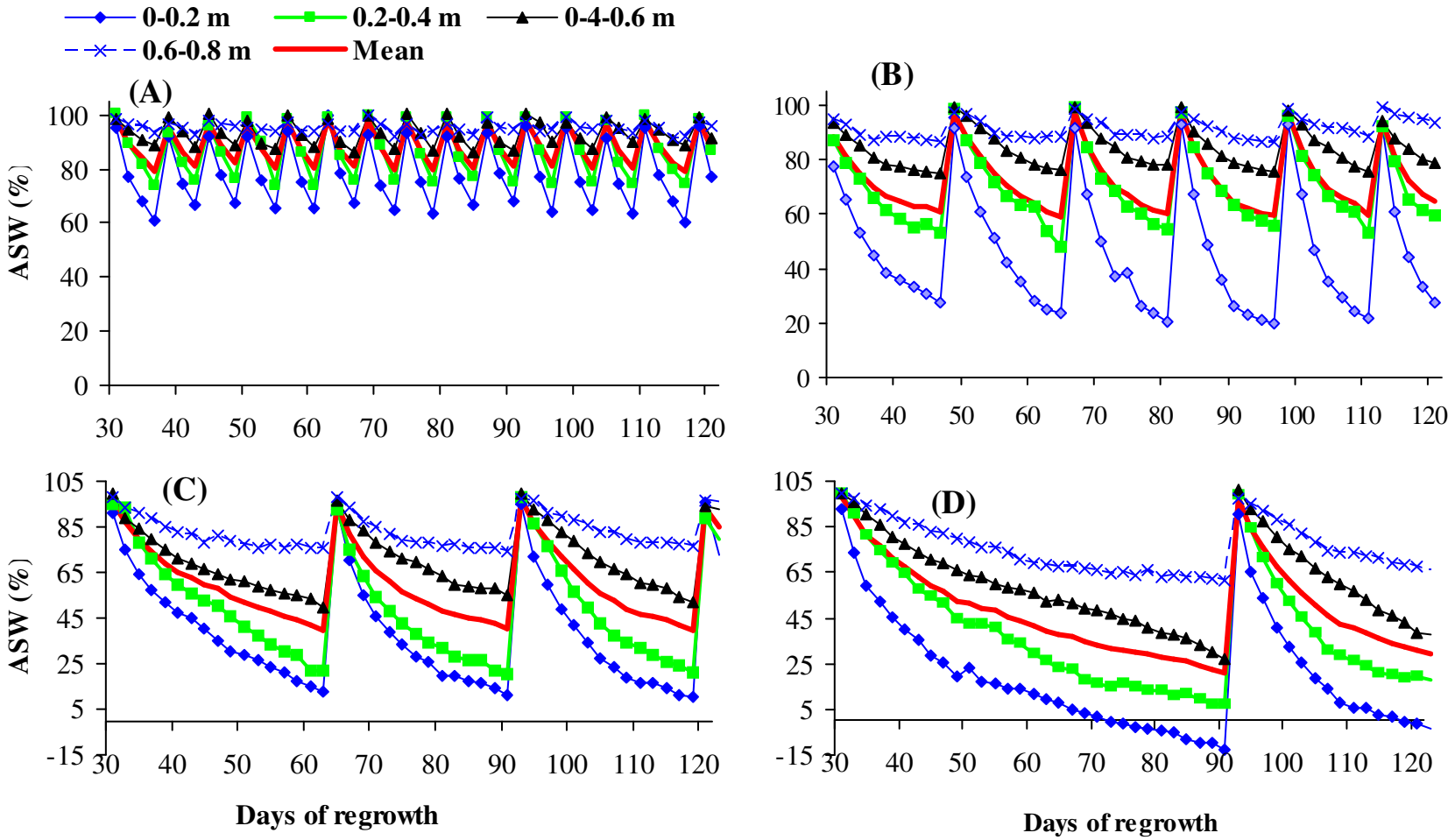

Figure 3.2: Temporal variations in plant available soil water (ASW) content in the layers of the root zone of rose-scented geranium in the rain-shelter trial: data for the 20 (A), 40 (B), 60 (C), and 80% (D) maximum allowable depletion (MAD) treatments in the rain shelter for Harvest 1 (regrowth cycle during 27 February-26 June 2006)

The percentage and absolute values of water depleted from the soil layers for each treatment are presented in Table 3.4. In the 20% MAD level irrigation schedule, about 77 to 80% of the total water depleted was from the top 0.4 m root zone. When the set depletion level for the 80% MAD treatment approached, the amount of water up taken from the soil layer between 0.4 and 0.8 m increased. As a result, the proportion of the water usage from the top 0.4 m soil layer was as low as 60 to 66%. The higher water loss from the top 0.4 soil layer at lower MAD treatments was, at least partly, associated with higher soil evaporation due to frequent wetting (Wallace, 2000) accompanied by a denser root system closer to the soil surface (Goldhamer *et al.*, 1999; Panda *et al.*, 2003; Benjamin & Nielsen, 2006).

Table 3.4: Percentage available soil water depleted and depth of irrigation applied (mm) per soil layer for the different maximum allowable soil water depletion treatments

Soil depth (m)	20% MAD†		40% MAD		60% MAD		80% MAD	
	%	mm	%	mm	%	mm	%	mm
……Open field (average for Harvest 1 and 2) …….								
0.0-0.2	49.8	10.2	86.3	17.6	100.6	20.5	109.8	22.4
0.2-0.4	17.9	5.0	37.3	10.5	72.2	20.4	89.9	25.4
0.4-0.6	9.4	2.3	22.0	5.3	42.1	10.2	73.6	17.8
0.6-0.8	5.3	1.5	13.0	3.6	23.3	6.4	43.8	12.0
……Rain shelter (average for Harvest 1 and 2) …….								
0.0-0.2	35.4	9.3	76.4	20.0	92.9	24.3	110.9	29.1
0.2-0.4	25.0	7.4	46.1	13.6	77.7	23.0	93.8	27.8
0.4-0.6	11.7	3.0	25.7	6.7	46.8	12.2	72.3	18.8
0.6-0.8	6.2	1.6	11.6	2.9	22.0	5.5	41.3	10.4

†**MAD represents maximum allowable depletion of the available soil water**

In general, the water depletion rate progressively declined as the water depletion level approached the set threshold value for the 80% MAD treatment, even though the ASW status in the 0.6 to 0.8 m root zone was still above 55%. This illustrates that the distal roots of rose-scented geranium were less effective in taking up water than the proximal roots. Goldhamer *et*

al. (1999) also reported that when peach trees were exposed to prolonged water stress, the lower profile contributed more water, but the magnitude of taking up water gradually declined with an increase in soil depth. To the contrary, Lai and Katul (2000) reported that, regardless of soil water status in the topsoil, the water depletion rate from the lower soil profile remained constant in a grass-covered forest.

3.4.2 Plant growth parameters

Leaf area index (LAI) accumulation pattern

LAI as affected by the different MAD levels during the regrowth time course is illustrated in Figure 3.3. In Weeks 7 and 9, LAI increased slowly. This could be explained by low metabolite sources for growth of the new leaves, as suggested by Fricke (2002) for similar observations for barley. A similar finding was also reported by Çakir (2004) in corn.

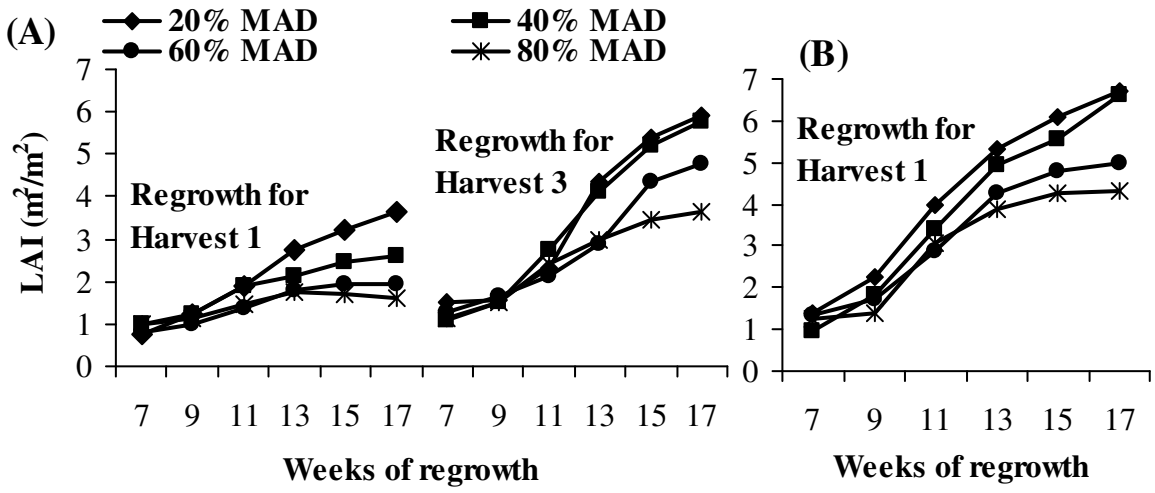

Figure 3.3: Leaf area index of rose-scented geranium as affected by different maximum allowable depletion (MAD) levels of plant available soil water. (A) Harvests 1 and 3 in the open, and (B) Harvest 1 in the rain shelter were conducted in July 2005, February 2006 and June 2006, respectively

In all irrigation treatments, LAI increased faster between Weeks 9 and 13, probably associated with an increase in assimilate supply for the new leaf growth. The declining tendency in LAI growth rate after the 15th week of regrowth in the 20% MAD level (control) could be attributed to the age of the whole plant and senescence of older leaves (Çakir, 2004).

Appearance of noticeable variations in LAI among the irrigation treatments coincided with the time of maximum leaf area expansion (Weeks 9 to 13). As a result, significant differences in LAI were recorded at the final harvesting (Table 3.5). Compared to the control (20% MAD schedule), LAI showed a significant reduction in the 60 and 80% MAD treatments in all harvests. These results support previous reports (Çakir, 2004; Karam *et al.*, 2005), which revealed that in water stress conditions leaf area declines in plants.

Table 3.5: Leaf area index of rose-scented geranium grown under different maximum allowable depletion (MAD) levels of plant available soil water for the final harvests

MAD	Open field				Rain shelter	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 1	Harvest 2
20%	3.62 a †	1.93 a	5.89 a	2.79 a	6.70 a	6.05 a
40%	2.62 b	1.73 a	5.78 a	2.54 b	6.62 a	5.65 a
60%	1.94 bc	1.39 b	4.79 b	1.62 c	4.99 b	4.14 c
80%	1.60 c	1.25 b	3.66 c	1.38 d	4.31 b	3.31 d
Grand mean	2.44	1.58	5.03	2.08	5.65	4.79
CV (%)	24.80	10.83	8.74	6.69	7.79	9.50
LSD (P < 0.05)	0.969	0.273	0.703	0.223	0.705	0.727

†**Values followed by the same letter in a column are not significantly different at $\propto = 0.05$; Harvests 1, 2, 3, and 4 in the open field were conducted in July and September 2005, and January and October 2006, respectively; Harvests 1 and 2 in the rain shelter were conducted in June and October 2006, respectively**

Fresh leaf mass to total fresh herbage mass ratio was inversely related to total fresh herbage yields and soil water levels (Table 3.6). The ratio was highest in the treatment that performed worst in total herbage yield and LAI (the 80% MAD). Such observations could be explained by

lower water content in the stem under water-stressed conditions, as opposed to the succulent nature of pelargonium stems observed under well-watered conditions (Jones *et al.*, 2003). Such results, at least partly indicate that rose-scented geranium plants could survive a short period of stress by using the extra water stored in the stems.

Table 3.6: Fresh leaf mass ratio (as a percentage of total fresh herbage mass) for rose-scented geranium grown at different maximum allowable depletion (MAD) levels of plant available soil water recorded at final harvests

MAD	Open field				Rain shelter	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 1	Harvest 2
20%	62.37 c†	69.82 c	61.11c	63.01 b	62.32 c	60.41 c
40%	63.88 c	71.57 b	62.76 c	63.48 b	62.77 c	62.83 b
60%	66.54 b	72.56 b	65.18 b	67.01 a	63.94 b	63.80 b
80%	69.07 a	75.84 a	70.92 a	69.24 a	66.05 a	65.59 a
Grand mean	65.46	72.44	64.99	65.68	63.77	63.15
CV (%)	2.02	1.36	1.82	2.24	1.15	1.18
LSD (P < 0.05)	2.11	1.575	1.89	2.547	1.17	1.191

†**Values followed by the same letter in a column are not significantly different at ∝ = 0.05; Harvests 1, 2, 3, and 4 in the open field were conducted in July and September 2005, and January and October 2006, respectively; Harvests 1 and 2, in the rain shelter, were conducted in June and October 2006, respectively**

Temporal herbage dry matter accumulation trends

Dry matter accumulation rate was low until the ninth week of the regrowth cycles (Figure 3.4). In most cases, growth rate was highest between the 11th and 13th week of regrowth cycles, indicating that dry matter accumulation is positively related with growth in LAI (Çakir, 2004). These results slightly differ from the fresh herbage yield accumulation pattern reported by

Motsa *et al.* (2006) for the same cultivar. According to the author, maximum herbage growth rate was noticed on either the fourth or fifth month of regrowth depending on season.

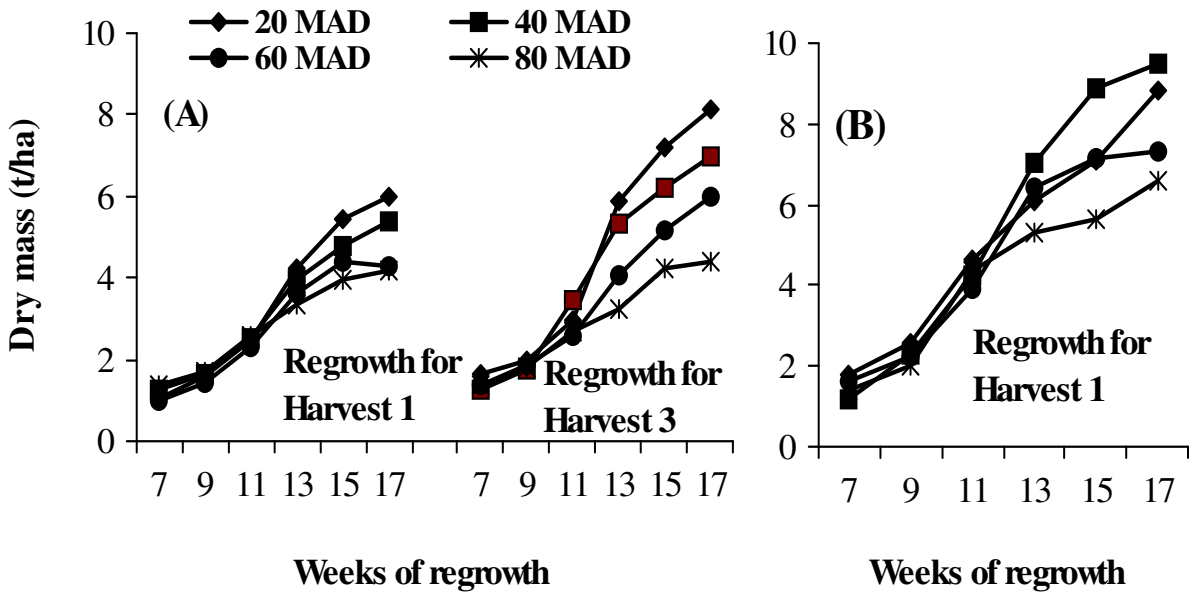

Figure 3.4: Herbage dry mass of rose-scented geranium grown at different maximum allowable depletion (MAD) levels of plant available soil water. (A) Harvests 1 and 3 in the open field and (B) Harvest 1 in the rain shelter were conducted in July 2005, February 2006 and June 2006, respectively

Noticeable differences in total dry matter accumulated amongst the irrigation treatments started in the second half of the third month (between Week 11 and 13) of the regrowth cycles, and the gap continuously widened with progress in shoot age (towards harvesting). The data show that plants in the 20 and 40% MAD treatments did not attain their maximum dry matter accumulation during the final harvesting periods. To a certain extent, these results are comparable to the results reported by Motsa *et al*. (2006), which indicated that maximum dry matter accumulation could be attained in the fifth month of regrowth cycles.

Leaf and stem dry matter contents at final harvesting

Data presented in Table 3.7 show that stem and leaf dry matter contents (%) tended to increase with an increase in MAD level. Within the same treatments and the same harvests, leaf dry matter content was consistently higher than stem dry matter content, confirming the succulent characteristics of stems in *Pelargonium* species (Jones *et al.*, 2003).

Table 3.7: Leaf and stem dry matter content (%) for rose-scented geranium grown at different maximum allowable depletion levels (MAD) of plant available soil water recorded at final harvests

Irrigation levels	Open field				Rain shelter			
	Harvest 1		Harvest 2		Harvest 1		Harvest 2	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
20% MAD	18.40 c[†]	14.23 c	19.81 c	14.23 a	15.14 c	12.92 c	15.64 b	12.42 b
40% MAD	19.33 b	16.44 b	20.79 b	16.44 b	17.30 b	13.87 b	16.71ab	13.27 a
60% AMD	20.34 a	16.68 b	21.67 a	16.67 b	17.71ab	14.14 ab	17.02 a	13.45 a
80% MAD	20.68 a	17.99 a	21.61 ab	17.99 a	18.54 a	14.74 a	17.64 a	13.84 a
Grand mean	19.64	16.33	20.97	16.33	17.18	13.92	16.75	13.25
CV (%)	2.12	1.74	2.51	1.74	4.05	3.78	4.15	3.98
LSD ($\alpha = 0.05$)	0.669	0.455	0.843	0.45	1.11	0.842	1.11	0.842

[†]**Values followed by the same letter in a column are not significantly different (at $\propto = 0.05$); Harvests 1 and 2 in the open field were conducted in July and September 2005, respectively; Harvests 1 and 2 in the rain shelter were conducted in June and October 2006, respectively**

Fresh herbage yield per regrowth cycle

The results in Figure 3.5 highlight that herbage yield decreased with an increase in MAD level. In most cases, the effects of water stress on herbage yield became evident when more than 40% of the ASW was depleted. These results agree with a previous report (Rajeswara Rao *et al.*,

1996), which indicated that a rainy season (wet conditions) encouraged herbage growth of rose-scented geranium. Hence, for higher herbage yield of rose-scented geranium, it seems advisable to maintain ASW in the effective root zone above 60% (when a maximum of 40% of the ASW has been depleted), which is slightly higher than the soil water level (55 to 65% of soil water at field capacity) previously recommended for this crop (Weiss, 1997).

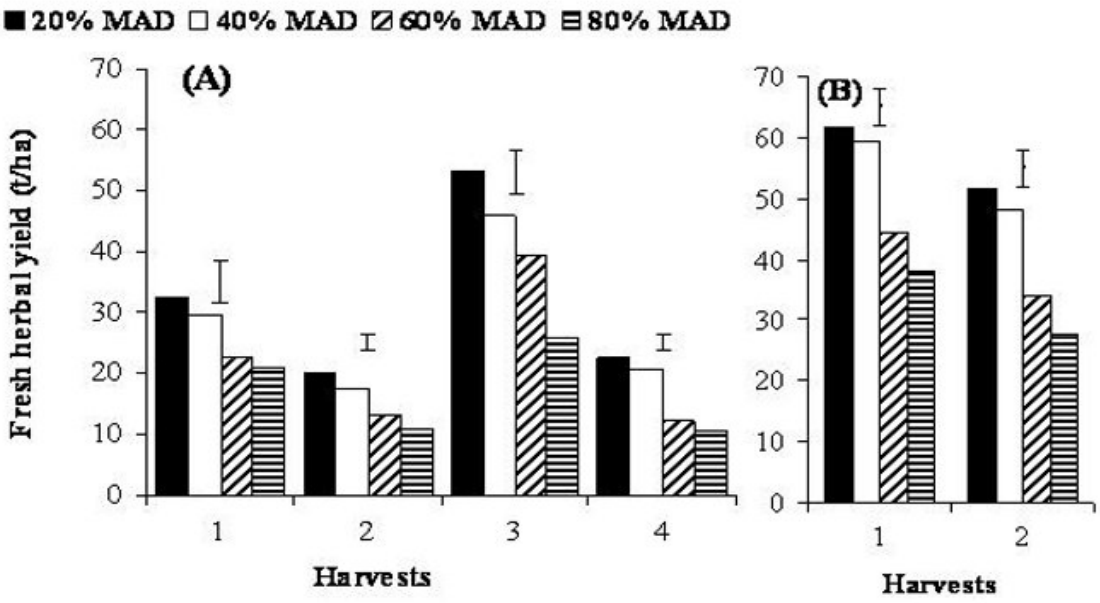

Figure 3.5: Fresh herbage yield of rose-scented geranium grown at different maximum allowable depletion (MAD) levels of plant available soil water. The vertical bars are LSD at α = 0.05; (A) Harvests 1, 2, 3 and 4 in the open field were conducted in July and September 2005, and January and October 2006, respectively; (B) Harvests 1 and 2 in the rain shelter were done in June and October 2006, respectively

The markedly high herbage yield differences between harvests could probably be attributed to seasonal variations. Herbage yields of regrowth cycles of Harvest 3 in the open field and Harvest 1 in the rain shelter, during higher average night temperatures (14 and 11°C, respectively), were highest. On the other hand, the herbage yields obtained from the other harvests, which experienced cool seasons and night temperatures in the range between 7 and 9 °C (see Appendix B), were lower. Similarly, Motsa *et al.* (2006) reported that higher

temperatures (in summer/spring season) increased herbage yield of rose-scented geranium. These results are also consistent with a previous report that characterised rose-scented geranium as a warm- to hot-season crop (Weiss, 1997; Lis-Balchin, 2002b).

3.4.3 Essential oil yield and quality parameters

Essential oil content

In the open-field trial, the effect of MAD treatments on essential oil content (percentage oil on fresh herbage mass basis) was not consistent (Figure 3.6a). In the rain shelter, this parameter showed more or less a consistent positive relationship with MAD level (Figure 3.6b).

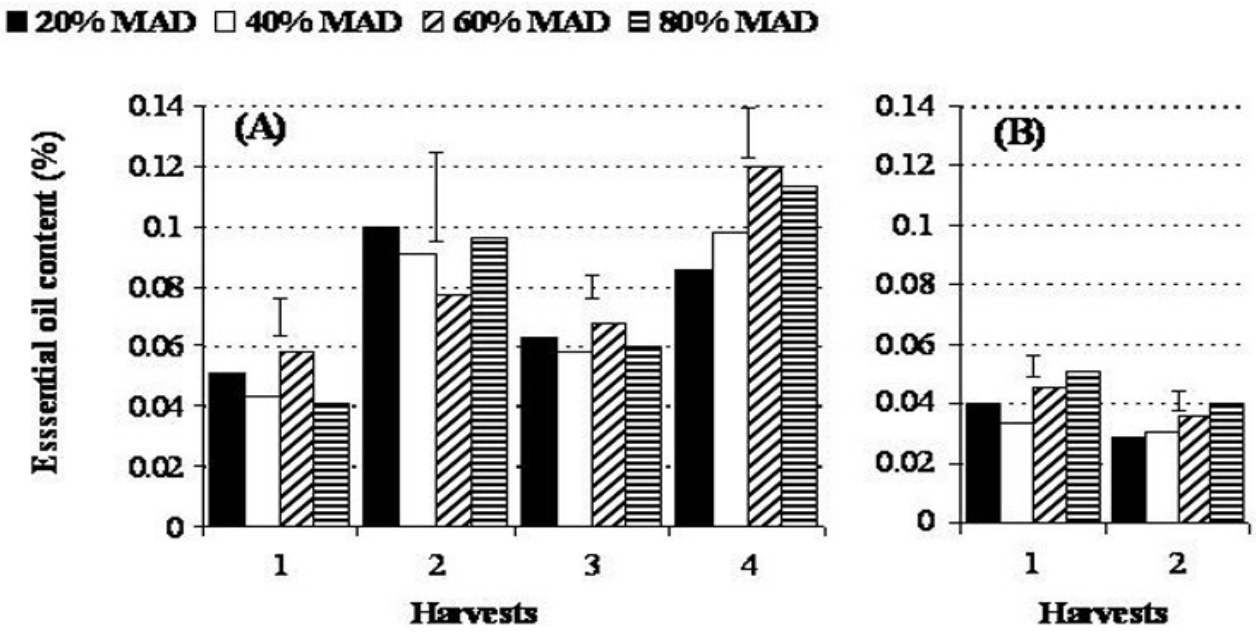

Figure 3.6: Essential oil content of rose-scented geranium grown at different maximum allowable depletion (MAD) levels of plant available soil water. The vertical bars are LSD at α = 0.05; (A) Harvests 1, 2, 3 and 4, in the open field, were conducted in July and September 2005, and January and October 2006, respectively; (B) Harvests 1 and 2, in the rain shelter, were done in June and October 2006, respectively

These results (from the rain shelter) are in agreement with commonly observed findings, namely an increase in essential oil concentration (percentage oil) in water-stressed conditions (Simon *et al.*, 1992; Singh-Sangwan *et al*, 1994; Weiss, 1997; Yaniv & Palevitch, 1982). Such a phenomenon could be explained by higher glandular trichome density due to smaller leaves under water-stressed conditions (Kothari *et al.*, 2004; Motsa *et al.*, 2006).

Essential oil yield

Essential oil yield data are presented on Figure 3.7. The positive relationship between herbage and essential oil yield in current results supports previous reports (Kumar *et al.*, 2001; Motsa *et al.*, 2006), which indicated that higher herbage yield resulted in higher total essential oil yield. Hence, it is safe to conclude that essential oil yield is a function of herbage yield (Murtagh & Smith, 1996).

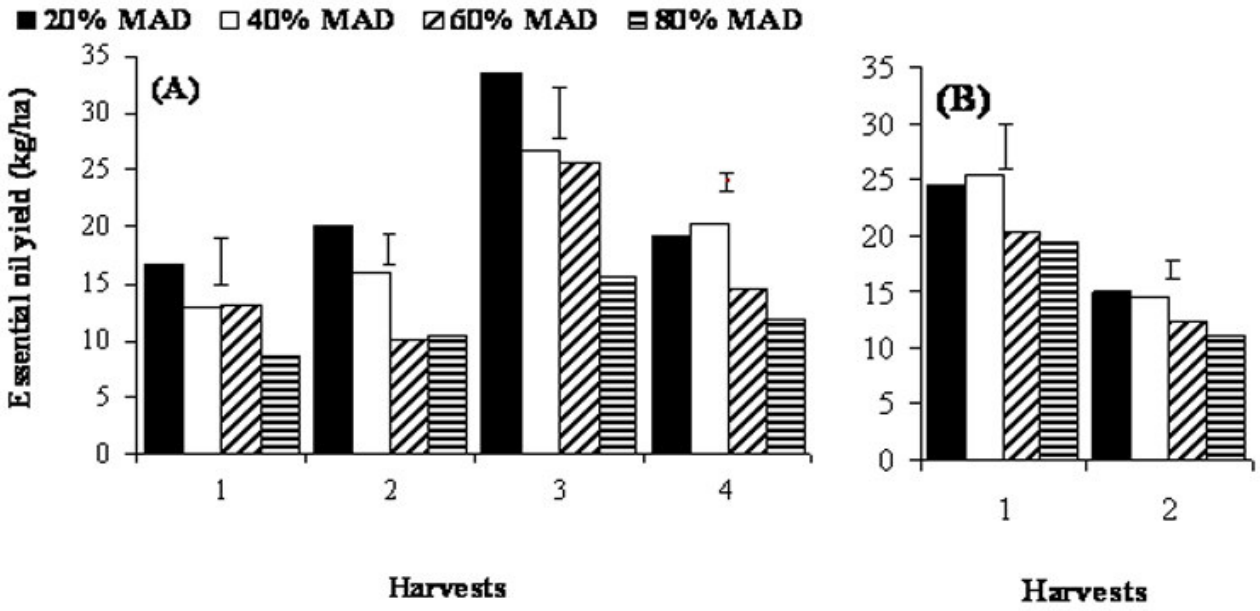

Figure 3.7: Essential oil yield of rose-scented geranium grown at different maximum allowable depletion (MAD) levels of plant available soil water. the vertical bars are LSD at α = 0.05; (A) Harvests 1, 2, 3 and 4, in the open field, were conducted in July and September 2005, and January and October 2006, respectively; (B) Harvests 1 and 2, in the rain shelter, were done in June and October 2006, respectively

Depending on cultivar and number of possible harvests, geranium oil yield commonly ranges between 5 and 20 kg/ha per year (Weiss, 1997). Assuming that Harvests 1, 3 and 4 in the open field were for growth cycles in the same year, the annual yield is estimated at about 69 and 60 kg/ha for the 20 and 40% MAD treatments, respectively. Hence, the essential oil yields obtained in the current study can be considered above average.

Essential oil composition

Rose-scented geranium essential oil is a mixture of more than 120 organic compounds (Williams & Harborne, 2002) from different classes such as acids, alcohols, aldehydes, esters and ketones (Demarne & Van der Walt, 1993). Since the contents of most of the essential oil constituents were extremely low, in the current chromatographic oil analysis only the composition of the first seven principal compounds was considered (Figure 3.8). Because of technical problems, GC analysis was performed only for Harvests 1, 2, and 3 of the open-field trials. The results indicate that the composition of the seven major components was not significantly affected by soil water level. Similar to the current results, Singh *et al.* (1996) reported that essential oil composition did not respond to irrigation levels.

Prominent essential oil composition variations were observed among harvests. Irrespective of irrigation treatments, geraniol content tended to increase with a decrease in citronellol and citronellyl formate contents for Harvest 1 to Harvest 3. Rajeswara Rao *et al.* (1996) also observed a negative relationship between geraniol and citronellol, and they stated that geraniol was converted into citronellol over time in the rose-scented geranium. To the contrary, Luthra *et al.* (1991) reported a positive relationship between geraniol and citronellol in *Cymbopogon winterianus*.

The present results show that the seven essential oil components considered in the GC analyses comprised 77.2 ± 2.9 % of the total essential oil recovered by the steam-distillation technique. Citronellol was the highest component (32.6± 4%), and linalool content (0.59 ± 0.2%) was the lowest. Citronellol to geraniol ratio (C:G ratio) varied among harvests (15.2, 3.7 and 2.2 for

Harvests 1, 2 and 3, respectively). The extremely high C:G ratio for Harvest 1 is not preferred by the perfumery industry. Although a C:G ratio in the range of one to three is acceptable, the most desirable in the perfumery and fragrance industries is a 1:1 ratio (Motsa *et al.*, 2006).

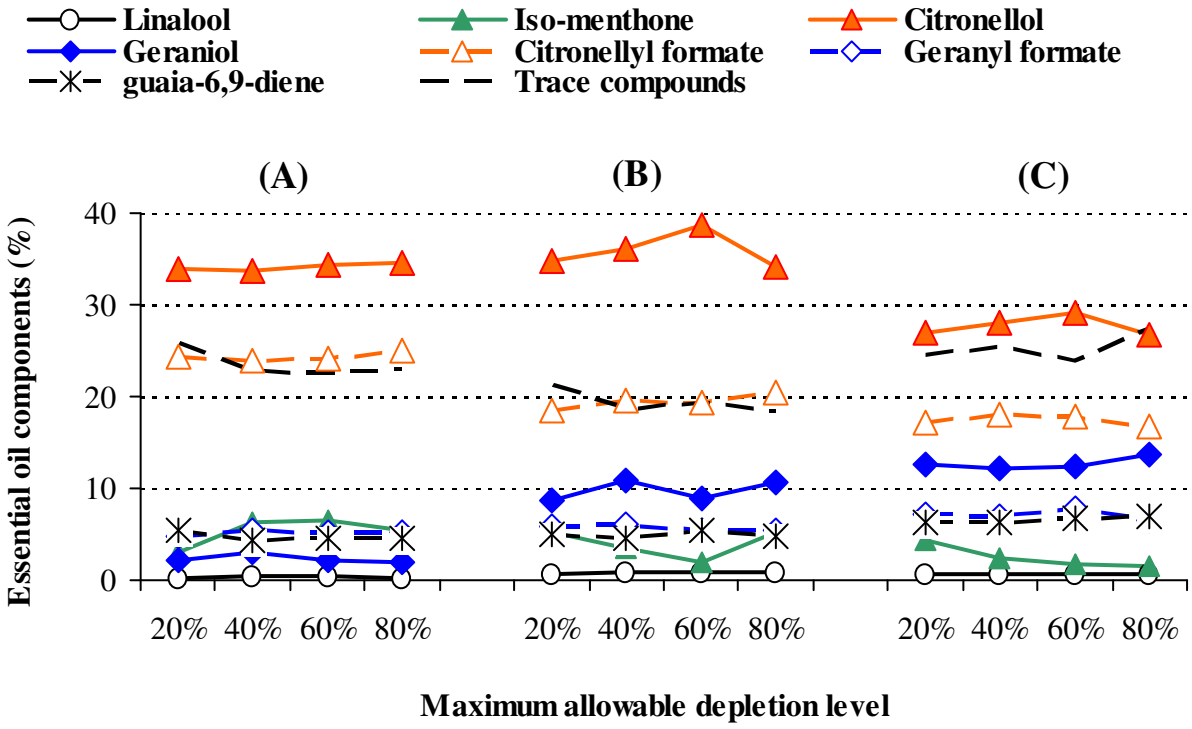

Figure 3.8: Major essential oil components (percentage of essential oil yield) of rose-scented geranium grown under different maximum allowable depletion levels of plant available soil water. (A) Harvest 1, (B) Harvest 2 and (C) Harvest 3, in the open field, were conducted in July and September 2005, and January 2006

3.4.4 Water use and water-use efficiency

Total water used and water-use efficiency (WUE) per regrowth cycles are presented in Table 3.8. Increased water use per regrowth cycle was observed for plants irrigated most frequently. Water-use efficiency, in terms of essential oil produced, did not show a consistent trend in the open-field trial. In the rain shelter, the highest WUE in terms of oil yield was recorded for the

80% MAD irrigation schedule. The highest water use observed in the 20% MAD irrigation schedule revealed that rose-scented geranium uses more water when it is irrigated more frequently. In such situations, more water is lost through evapotranspiration, but dry matter production might not increase proportionally (Salisbury & Ross, 1992; Lambers *et al.*, 1998).

Table 3.8: Average water use and water-use efficiency (expressed on essential oil yield and herbage dry mass basis) of rose-scented geranium grown at different maximum allowable depletion levels of plant available soil water

MAD†	Applied water		Evapo-	Water-use efficiency	
	Irrigation (mm)	Rainfall (mm)	transpiration (mm)	Oil (g ha^{-1} mm^{-1})	Dry herbage mass (kg ha^{-1} mm^{-1})
---------- Open field (Harvest 1, July 2005) ----------					
20%	485.3	0.0	476.6	34.9	11.5
40%	345.8	0.0	372.1	35.0	14.5
60%	310.6	0.0	310.0	42.2	13.8
80%	208.8	0.0	252.9	34.0	16.4
---------- Open-field (Harvest 3, January 2006) ----------					
20%	339.1	194.9	533.0	62.9	17.4
40%	281.0	203.2	488.3	52.6	15.8
60%	261.9	225.9	490.2	54.5	13.6
	171.0	214.9	383.0	40.8	12.6
----------Rain shelter (Harvest 1, June 2006) ----------					
20%	354.9	0.0	345.2	71.1	25.5
40%	306.2	0.0	332.0	76.7	28.6
60%	286.4	0.0	285.6	71.6	25.6
80%	174.3	0.0	238.1	81.6	27.6
----------Rain shelter (Harvest 2, October 2006) ----------					
20%	400.4	0.0	391.2	38.3	19.0
40%	310.5	0.0	335.8	43.1	22.2
60%	239.9	0.0	276.5	44.4	19.4
80%	176.3	0.0	226.3	48.9	20.0

†**MAD: maximum allowable depletion of plant available soil water; rainfall: effective rainfall**

Some reports (Zhang *et al.*, 2004; Zhang *et al.*, 2006) indicated that a certain degree of water stress improved WUE. In the current results, there was not consistent proof that water stress improved WUE. The contrasting results in the open field, Harvest 3 (where water-use efficiency was highest for the 20% MAD treatment), could probably be explained by the high rainfall (95 mm) during the last three weeks of this regrowth cycle (Appendix B). This increased the amount of water considered in the WUE calculations of all treatments, but there was probably only a marginal increase in vegetative growth, especially for the treatments scheduled at higher MAD levels. In most of the harvests presented in Table 3.7, the 40% MAD treatment ranked the highest or second highest in terms of dry herbage mass productivity per unit of water (water-use efficiency).

Inconsistency in the relationship between WUE and soil water status indicates that the interaction of these factors is influenced by certain plant and soil factors. Bessembinder, Leffelaar, Dhindwal and Ponsioen (2005) suggested that the declining tendency of WUE with an increase in soil water level reported in certain research works could have resulted from factors such as shortage of nutrients (to cope with fast growth rate) in non-stressed crops. According to Kadayifci, Tuylu, Ucar and Cakmak (2005), WUE depends on effective root depth of the crop species. The authors stated that plants with a deep effective root system could avoid water stress thereby improve WUE. WUE could also be crop species dependent.

Compared to the 20% MAD treatment, up to 28% of irrigation water could be saved in the open-field trial (Harvest 1) by applying the 40% MAD treatment, without any significant reduction in essential oil yield. In the rain shelter, between 13 (Harvest 1) and 22% (Harvest 2) water was saved by applying the 40% MAD treatment. The differences between harvests (in the rain shelter trials) could be explained by seasonal effects, as most of the regrowth period for Harvest 1 experienced cool temperatures (spring/winter), whereas the regrowth cycle for Harvest 2 was during a season with warm temperatures (autumn).

3.5 CONCLUSIONS AND RECOMMENDATIONS

The present study indicates that rose-scented geranium roots are most active in the top 0.4 m soil layer. Herbage yield significantly declined when more than 40% of ASW (40% MAD) was depleted from the root zone. An increase in leaf to stem ratio was a common response of plants to water-stressed conditions. In most cases, essential oil yield increased with higher herbage yield and soil water status (lower MAD level). Water stress apparently increased essential oil content (percentage oil on fresh herbage mass basis), but it was not sufficient to compensate for the yield loss due to reduced herbage yield. Composition of the seven principal essential oil components was not affected by the irrigation-scheduling regime. Essential oil composition variations among harvests indicated that geraniol and geranyl formate contents were inversely related to citronellol and citronellyl formate levels. For water saving, without a significant reduction in essential oil yield of rose-scented geranium, the author recommends a maximum depletion level of 40% of ASW in the 0.8 m root zone.

CHAPTER 4

RESPONSE OF ROSE-SCENTED GERANIUM GROWTH, ESSENTIAL OIL YIELD AND OIL COMPOSITION TO A ONE-MONTH IRRIGATION-WITHHOLDING PERIOD

4.1 ABSTRACT

Responses of plant growth, essential oil yield and oil composition of rose-scented geranium to a one-month irrigation-withholding period at different times of regrowth cycles were investigated at the Hatfield Experimental Farm of the University of Pretoria, South Africa, during 2004 to 2007, in an open field and a rain shelter. No-stress (control) and a one-month irrigation withholding period in the second, the third and the fourth month of regrowth were applied as treatments. Herbage yield showed a significant reduction when the water stress period was imposed during the third or fourth month of regrowth. Essential oil yield was reduced when the plants were stressed during the fourth month of regrowth cycles. Essential oil content (percentage oil on fresh herbage mass basis) apparently increased in the stressed treatments, but total oil yield dropped due to lower herbage mass. Essential oil composition changes in response to irrigation-withholding treatments were not consistent. Water-use efficiency was not significantly affected by withholding irrigation in the second and in the third month of regrowth. With a marginal oil yield loss, about 330 to 460 m^3/ha of water could be saved by withholding irrigation during the third month of regrowth cycles. Hence, in water-scarce situations, withholding irrigation during either the second or the third month of regrowth in rose-scented geranium could improve water productivity.

Keywords Herbage mass; essential oil content; essential oil composition; *Pelargonium* species; water use; water-use efficiency, water stress period

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4.2 INTRODUCTION

Rose-scented geranium (*Pelargonium* species) is a perennial herb that is cultivated for its high-value essential oil. Rose-scented geranium oil, commonly referred to as 'geranium' oil, is widely used in the perfumery, cosmetics and aromatherapy industries (Rajeswara Rao *et al.*, 1996). Trade in essential oils is expected to expand in the future as a result of a growing number of consumers and their preferences, and continuous discovery of new uses for the oil constituents (Lis-Balchin *et al.*, 1998; Dorman & Deans, 2000; Lis-Balchin & Roth, 2000; Sangwan *et al.*, 2001; Deans, 2002).

According to Weiss (1997), rose-scented geranium performs well in regions that receive an annual rainfall of 1 000 to 1 500 mm, with fairly good seasonal distribution. The author stated that long, dry seasons resulted in poor plant growth, low essential oil yield and changes in oil composition. Gauvin *et al.* (2004) also mentioned that on Réunion Island, the crop is successfully cultivated in areas that receive an annual rainfall of about 1500 mm. Similarly, Rajeswara Rao *et al.* (1996) found that higher rainfall seasons favoured vegetative growth and essential oil yield.

The available information also indicated that South African rose-scented geranium production is limited to the Mpumalanga Lowveld, KwaZulu-Natal and Limpopo provinces (SANDA, 2006), where annual rainfall is relatively high, about 510 to 1 000 mm in the summer season (Davies & Day, 1998). Since most arable land in South Africa falls within an arid or semi-arid climate, introducing rose-scented geranium production to those dry regions would only be possible under irrigation. Hence, searching for irrigation strategies, which could increase rose-scented geranium essential oil yield and maximise productivity of scarce irrigation water, is a foremost priority.

Under a deficit irrigation technique, Singh *et al.* (1996) suggested that applying 30 mm of irrigation when the cumulative pan evaporation reaches 50 mm could maximise irrigation water-use efficiency in rose-scented geranium fields. Subsequent irrigation trials by Singh (1999) confirmed that supplementary irrigation at 60% of IW:CPE ratio (irrigation water

applied to cumulative pan evaporation ratio) increases profitability of rose-scented geranium production in the semi-arid tropical climate of India.

Withholding irrigation during certain crop growth stages that are not sensitive to water stress is one of the several irrigation strategies often applied to improve water productivity (Jalota *et al.*, 2006). Kang *et al.* (2000) suggested that water stress at the seedling and stem-elongation stages of maize would be the best irrigation strategy in semi-arid areas. Research results reported by Çakir (2004) also revealed that water stress during the vegetative stage of corn reduced total biomass, without a significant reduction in grain yield.

Geranium oil is extracted mainly from leaves and, to a certain extent, from stems and flowers by hydrodistillation techniques (Rajeswara Rao *et al.*, 2002). Hence, severe reduction in herbage yield due to water stress could result in a significant decline in essential oil yield, as reported in aromatic compounds of tea plants (Panrong, Chunyan & Kebin, 2006). A certain water stress level could also trigger conversion of primary to secondary metabolites, such as essential oils (Simon *et al.*, 1992). In addition, it is known that essential oil yield and composition depend on the shoot age of aromatic plants (Marotti, Piccaglia & Giovanelli, 1994; Sangwan *et al.*, 2001; Kothari *et al.*, 2004; Lattoo *et al.*, 2006; Motsa *et al.*, 2006). Hence, it was hypothesised that the timing of water stress could influence rose-scented geranium essential oil yield, oil composition and water productivity. In the current work, therefore, the effects of withholding irrigation for a one-month period at different times of plant regrowth were investigated.

4.3 MATERIALS AND METHODS

4.3.1 Site description

The experiments were conducted in an open field and in a rain shelter at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa (latitude 25° 45'S and longitude 28° 16'E; altitude of 1372 m), from October 2004 to February 2007. The

experimental site is situated in a region with an average annual rainfall of 670 mm, mainly in the summer season (October to March). Highest long-term maximum and lowest long-term minimum temperatures are about 30°C in January and 1.5°C in July, respectively (Annandale *et al.*, 1999).

4.3.2 Plant culture

Rose-scented geranium (*Pelargonium capitatum* x *P. radens* cv. *Rose*) was used as plant material. About 45-day-old plantlets (raised from stem cutting by commercial nursery) were transplanted to the field on 28 October 2004. For the rain shelter trial, healthy stem cuttings (taken from the open-field trials) were planted in seedling trays (filled with peat) on 25 August 2005, and raised at high relative humidity (in a mist bed) in a greenhouse at the Hatfield Experimental Farm. Starting three weeks after planting, a complete nutrient solution was applied once a week. The plantlets were transplanted on 1 October 2005.

In the open field, the plants were allowed to grow for about seven months and on 3 June 2005 they were cut back to about 15 cm above the ground to start the irrigation treatments. Due to technical problems experienced in the rain shelter, irrigation treatments were applied only after one year.

4.3.3 Field layout and treatments

Field layout

In the open field, plots were 7.5 m long and 5 m wide. There was a buffer strip of 1.5 m between two adjacent blocks. Spacing between rows was 1 m and plants within a row were 0.62 m apart. Each experimental plot consisted of five rows. Data were recorded on plants in the three middle rows. In the rain shelter, seedlings were planted at narrower spacings of 0.75 m inter-row and 0.45 m intra-row due to limited space. Plastic sheets were installed

vertically to a depth of 80 cm to avoid lateral water movement and root growth between adjacent plots. Each experimental plot consisted of four rows of 6 m long. In both experiments, treatments were replicated four times and arranged in a randomised complete block design (RCBD).

Treatments

Rose-scented geranium has no definite phenological stages because the plant (1) is commonly established from stem cuttings, (2) is grown as a perennial crop, and (3) rarely flowers and does not bear fruits or seeds due to male sterility (Tokumasu, 1974; Demarne, 2002). A regrowth duration period of four months was, therefore, decided upon in accordance with local commercial farmers' practices. Motsa *et al.* (2006) also reported that a four-month regrowth cycle produced the highest essential oil yield per harvest in this region.

For the first month of regrowth (beginning of each experiment), plants were allowed to regenerate under full irrigation to ensure recovery after harvesting injury. Irrigation treatments, therefore, started from the 31st day of each regrowth cycle. The following predefined irrigation treatments were applied:

1. No water stress throughout the growth cycle (NNNN or control);
2. Withholding irrigation during the second month of regrowth cycles (NSNN);
3. Withholding irrigation during the third month of regrowth cycles (NNSN);
4. Withholding irrigation during the fourth month of regrowth cycles (NNNS);

4.3.4 Irritation monitoring

Since these trials and the maximum allowable depletion level experiments were carried out on the same site and shared common soil characteristics, for the detailed information on neutron probe calibration and irrigation-monitoring procedures, see Chapter 3.

Non-stressed treatments were irrigated to field capacity when about 20% of the available soil water was depleted. During the stress period, irrigation was withheld completely. Soil water status was monitored every second day using a neutron probe (Model 503 DR, CPN Corporation, CA, USA). Measurements were taken at 0.2 m depth increments from 0 to 1.2 m soil depth. A computer-controlled drip irrigation system (with water discharge rate of 1.6 ℓ/hr and at pressure range of 120-200 kPa) was used in both experiments. Dripper lines were spaced 0.5 m apart, and the distance between drippers (emitters) within a line was 0.3 m. Evapotranspiration (ET) for each regrowth cycle was calculated using Equation 3.4 (Chapter 3).

The water stress treatment during the last regrowth month (NNNS treatment) of Harvest 2 in the open field was disrupted by continuous heavy rainfall (248 mm) (Appendix B). Hence, plant water-use efficiency and total evapotranspiration of that particular regrowth period could not be determined. Regrowths of Harvests 1 and 3 (in the open field) were in a dry season (negligible effective rainfall), and in the rain shelter (Harvest 4), rainfall was successfully screened out. Hence, runoff and deep percolation of water in these harvests were assumed to be zero because the irrigation depth was always equal to the measured soil water deficit (ET loss), and application rate did not exceed soil infiltration rate.

4.3.5 Agronomic practices

During establishment, plants received 60 kg/ha nitrogen (N), 90 kg/ha phosphorus (P) and 60 kg/ha potassium (K). In the second week of each regrowth cycle, N, P and K were applied at rates of 30, 15 and 30 kg/ha, respectively. Hoeing was done during the first month of each regrowth cycle. Hand-weeding was practised, and standard pest and disease control measures were taken when necessary.

4.3.6 Data recorded

Data for three regrowth cycles from the open field, Harvest 1 (02 June to 1 Oct 2005), Harvest 2 (2 October 2005 to 1 February 2006), Harvest 3 (12 July to 11 November 2006), and for one growth cycle from the rain shelter, Harvest 4 (27 October 2006 to 26 February 2007) were collected. For further information on data collected, instruments used, procedures followed, and statistical analysis, see to Chapter 3.

4.4 RESULTS AND DISCUSSION

4.4.1 Soil water content during the irrigation-withholding periods

Soil water status during the irrigation withholding periods is depicted in Figure 4.1. The data showed that for all the treatments, plants extracted the most water from the top 0.4 m soil layer, indicating that the most active roots were concentrated in this soil layer. This highlights that the water below this soil layer was not readily available to the plants. The results imply that deep irrigation could be helpful only when it is intended to keep the plants alive during a prolonged drought condition. Based on similar observations, it was suggested that only the 0.45 m top root zone should be considered in irrigation scheduling for maize (Panda *et al*., 2003) and wheat (Panda *et a*l., 2004).

The soil water depletion rate, especially in the top 0.4 m root zone, tended to increase with increase in shoot age for which irrigation was withheld. Consequently, at the end of the irrigation-withholding period, the highest and lowest soil water contents were recorded for the NSNN and NNNS treatments, respectively. Higher soil water depletion levels during the later regrowth stages (e.g. in the fourth month of regrowth cycles) could be associated with larger foliar canopies since evapotranspiration loss is directly related to canopy size (Wright & Smith, 1983; Karam *et al*., 2005).

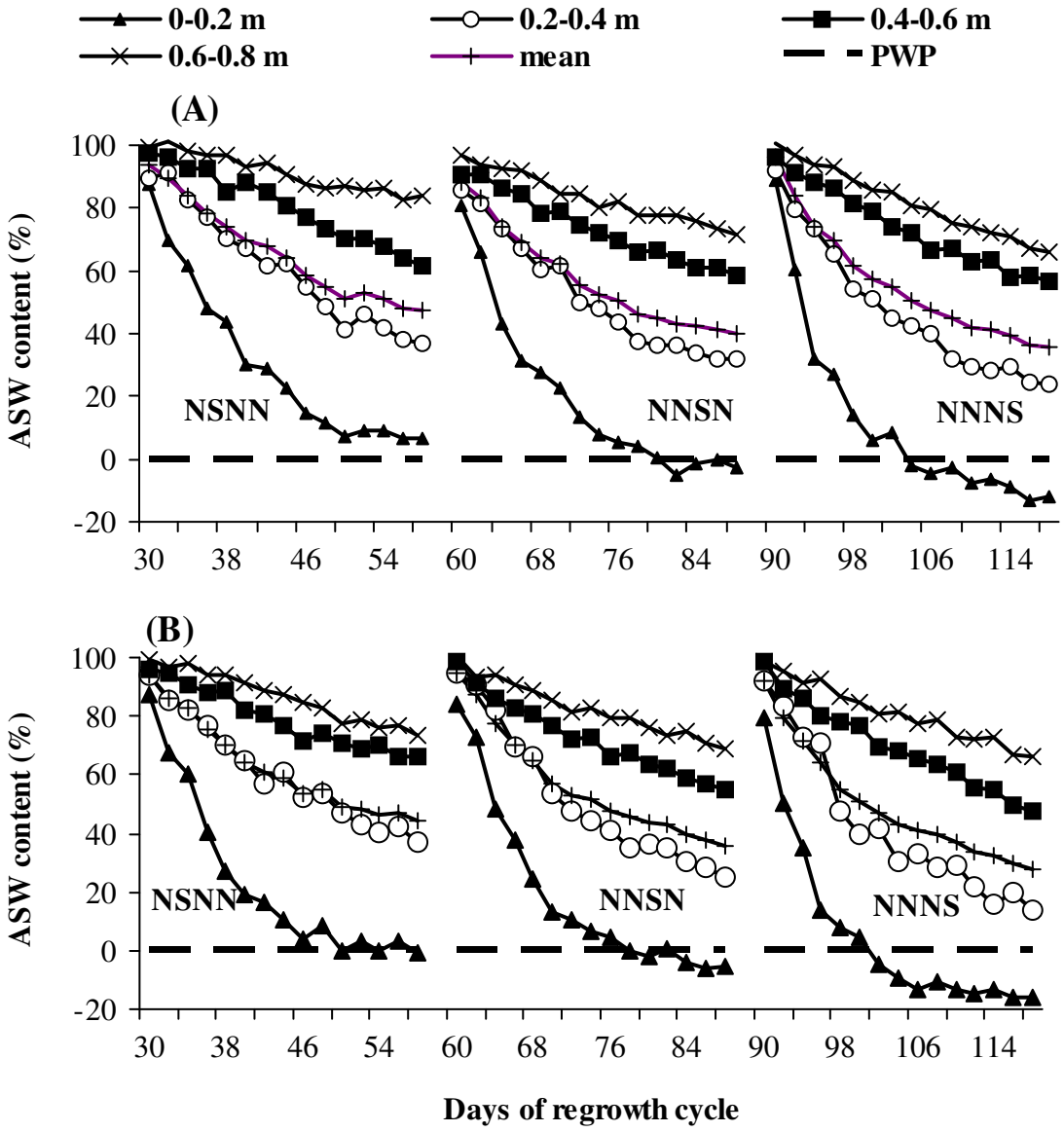

Figure 4.1: Available soil water content per soil layer in the root zone of rose-scented geranium during the one-month irrigation-withholding periods. NSNN, NNSN and NNNS represent irrigation-withholding treatments in the second, third and fourth month of regrowth cycles; (A) Harvest 1 and (B) Harvest 4 were conducted in October 2005 and February 2007, respectively

4.4.2 Herbage growth parameters

Leaf area index (LAI) accumulation during regrowth period

The data presented in Figure 4.2 show that the LAI values obtained differed substantially for the regrowth cycles of Harvests 1 and 4. The LAI in the regrowth cycle for Harvest 1 was very low compared to that of the regrowth cycle for Harvest 4. The major sources for this variation were probably difference in season and plant density. The regrowth for Harvest 1 was during a cool season (25 and 8°C mean maximum and minimum temperature, respectively). The regrowth cycle for Harvest 4, on the other hand, was during a warm to hot season (mean maximum and minimum temperatures of 30 and 16°C, respectively). Plant density was also lower (16000 plant/ha) for Harvest 1 (open field trial) than that for Harvest 4 (rain shelter trial, about 29600 plants/ha).

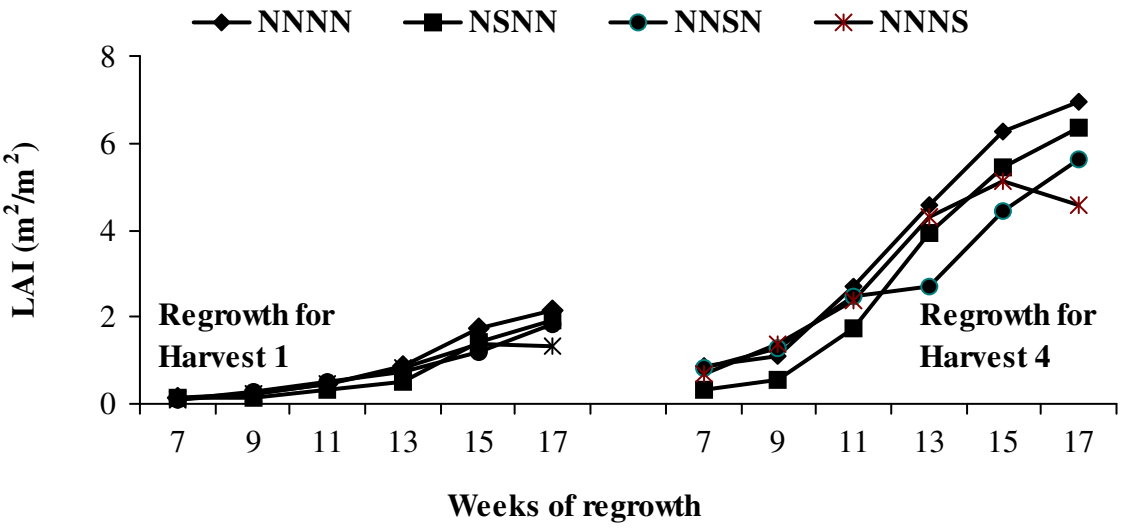

Figure 4.2: Leaf area index growth trends of rose-scented geranium that was water-stressed for one month at different regrowth stages. NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth regrowth months, respectively; Harvests 1 and 4 were conducted in October 2005 and February 2007, respectively

The effect of the irrigation-withholding treatments on LAI development could be clearly seen in the regrowth cycle for Harvest 4. The data show that the impact of the one-month irrigation-withholding period on LAI was affected by the shoot age at which the water stress was imposed. Water stress in the second month of the regrowth cycle resulted in a temporary decline in LAI development. In most cases, irrigation withholding in the third or fourth month of regrowth cycles resulted in a significant reduction in LAI per regrowth cycle (Table 4.1). The reduction in leaf area for the NNNS treatment (compared to the control, NNNN) for Harvests 1, 3 and 4 was 39, 36 and 34%, respectively.

Table 4.1: Maximum LAI of rose-scented geranium that was water-stressed for one month at different regrowth stages

Treatment	Open field		Rain shelter
	Harvest 1	Harvest 3	Harvest 4
NNNN	2.15 a[†]	4.61 a	6.96 a
NSNN	1.90 b	4.44 a	6.34 a
NNSN	1.83 b	3.09 b	5.64 ab
NNNS	1.32 c	2.96 b	4.57 b
Grand mean	1.80	3.778	5.89
CV (%)	6.41	11.58	17.38
LSD ($\alpha = 0.5$)	0.185	0.7	1.63

[†]Values with the same letter in a column are not significantly different; NNNN, NSNN, NNSN and NNNS represent control and irrigation withholding in the second, third and fourth month of regrowth cycles; Harvests 1, 3 and 4 were conducted in October 2005, November 2006 and February 2007, respectively

The severe negative effect of water stress imposed during the fourth month of regrowth could probably partially be attributed to hastened defoliation of older leaves (data not presented). The general LAI response to irrigation withholding is comparable to the results reported by Karam *et al.* (2005). According to the authors, lag in leaf area growth due to water stress in the

earlier growth stages could be compensated for by a stress-free period in the later growth stages of soybean.

Dry matter accumulation trends

The dry matter accumulation trends for the different regrowth cycles (Figure 4.3) were comparable with trends observed for LAI. The higher dry matter accumulation rate during the warmer season (regrowth for Harvest 4) confirms that rose-scented geranium favours warmer temperature regions (Kumar et *al.*, 2001; Lis-Balchin, 2002b; Motsa *et al.*, 2006).

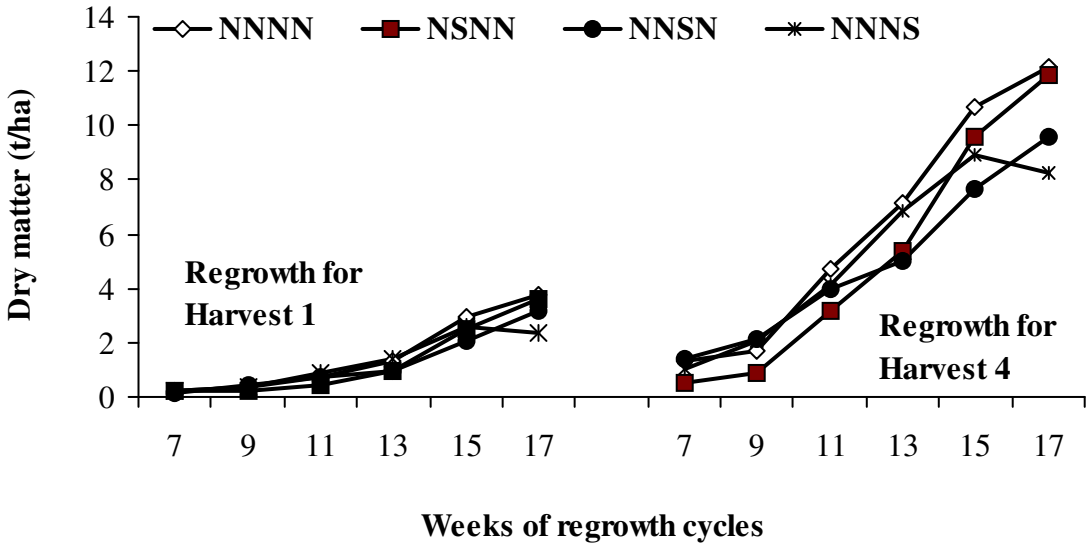

Figure 4.3: Dry matter accumulation of rose-scented geranium that was water-stressed for one month at different regrowth stages. NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; Harvests 1 and 4 were conducted in October 2005 and February 2007, respectively

The results presented in Table 4.2 indicate that leaf and stem dry matter contents were not affected by water stress applied during the second or third month of regrowth. In most cases, a significant increase in dry matter content was recorded only for the NNNS treatment, for

which the plants were harvested while still in a water-stressed condition. In addition, the data showed that lower dry matter content (%) was recorded for stems than for leaves in the same treatment and harvest.

Table 4.2: **Dry matter content (%) of rose-scented geranium that was water-stressed for one month at different regrowth stages**

Treatment	Open field				Rain shelter	
	Harvest 1		Harvest 3		Harvest 4	
	Dry leaf mass (%)	Dry stem mass (%)	Dry leaf mass (%)	Dry stem mass (%)	Dry leaf mass (%)	Dry stem mass (%)
NNNN	17.48 a[†]	14.16 a	18.14 c	15.22 a	16.27 c	13.96 b
NSNN	17.80 a	14.45 a	18.74 bc	15.95 a	17.15 b c	14.50 b
NNSN	17.92 a	15.55 a	19.00 b	16.42 a	17.64 b	14.79 ab
NNNS	18.31 a	16.07 a	20.09 a	16.5 a	19.54 a	16.32 a
Grand mean	17.87	15.06	18.99	16.02	17.65	19.89
CV	3.57	8.67	2.62	4.91	4.06	6.59
LSD (α = 0.5)	NS	NS	0.797	1.25	1.146	1.57

[†]Values followed by the same letter in a column are not significantly different; NNNN, NSNN, NNSN and NNNS represent control and irrigation withholding in the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 3 and 4 were conducted in October 2005, November 2006 and February 2007, respectively

Total herbage yield per regrowth cycle

The effects of a one-month irrigation-withholding period in different months of regrowth cycles on fresh herbage yield are illustrated in Figure 4.4. In general, withholding irrigation during any of the three regrowth months tended to reduce fresh herbage yield in all harvests, except for the NNNS treatment in Harvest 2. For this regrowth period (fourth month of Harvest 2) the irrigation-withholding period was interrupted by high (248 mm) and well-distributed rainfall (Appendix B). In Harvests 1, 3 and 4, significant fresh herbage mass

reductions were recorded for treatments NNSN and NNNS. The yield losses for treatment NNNS, compared to the non-stressed control (NNNN), were 25, 33 and 41%, in Harvests 1, 3 and 4, respectively.

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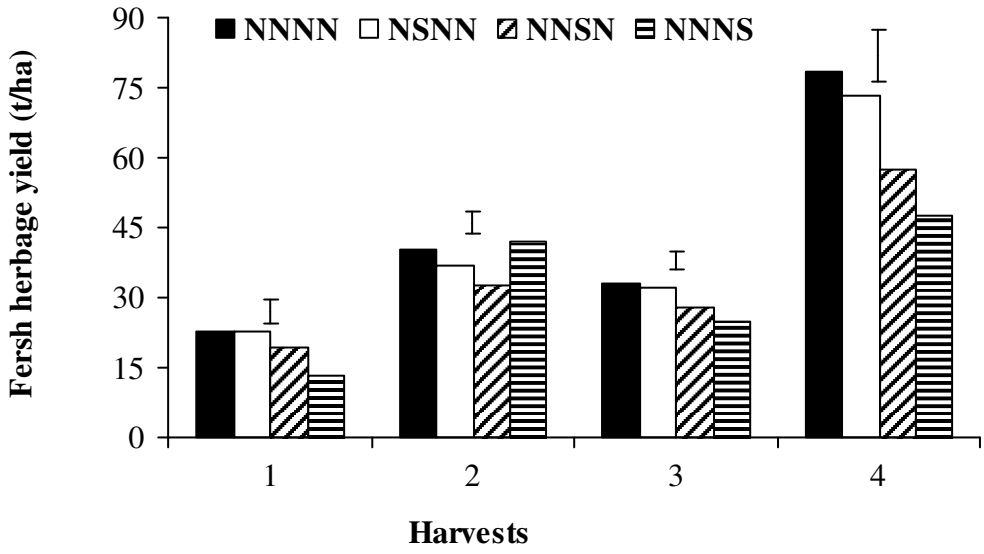

Figure 4.4: Fresh herbage yield of rose-scented geranium that was water-stressed for one month at different regrowth stages. The vertical bars are LSD at α = 0.05); NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 2, 3 (open field) and 4 (rain shelter) were conducted in October 2005, February 2006, November 2006 and February 2007, respectively

The minor reduction in herbage yield of plants that were water-stressed in the second month of regrowth (NSNN) could be explained by the relatively small canopy size during this early regrowth stage. In such a situation, transpiration rate was presumably low, which could have given the plants a better chance to readjust their physiological processes with relatively slow development of water stress. Withholding water in the later stages (when plants had well-developed canopies) had more serious consequences because transpiration demand was high (Brady & Weil, 1999; De Medeiros, Arruda, Sakai & Fujiwara, 2001). In such conditions, most of the readily available soil water probably was depleted within a short period of time, before the plants had a chance to make physiological adjustments to cope with the water stress

(Bray, 1997). This probably affected plant growth negatively. In agreement with the current results, studies conducted on *Cryptantha flava* revealed that larger plants were more sensitive to drought than smaller plants (Casper, Forseth & Wait, 2006).

The extremely high herbage yield in Harvest 4 (rain shelter) could possibly be explained by the higher plant density used in the rain shelter. In line with this observation, Rajeswara Rao (2002) reported that rose-scented geranium fresh herbage mass increased by 134.4% when it was planted at a 0.6 m x 0.3 m inter- and intra-row spacing, compared to a 1.2 m x 0.3 m inter- and intra-row spacing. In addition, the higher herbage yield from Harvest 2, a regrowth cycle during a warm season (mean maximum and minimum temperatures of 29 and 16°C, respectively), indicates that rose-scented geranium grows better in warm to hot seasons (Weiss, 1997; Motsa *et al*., 2006).

Contribution of leaves and stems to total fresh herbage yield was affected by the irrigation-withholding treatments (Table 4.3). The contribution of leaves to the total herbage yield increased as water stress was imposed later in the regrowth cycle. Thus, it became more noticeable when irrigation was withheld in the last regrowth month (NNNS treatment), except in Harvest 2 (where the NNNS treatment was not successfully applied).

Both the higher percentage fresh leaf mass (out of the total herbage yield) and higher dry matter content of leaves (compared to stems of the same treatment) (Table 4.3), at least partly, imply that rose-scented geranium plants have succulent stems. The extra water stored in the stems could possibly be reallocated to the leaves to balance the presumably lower water potential developed as a result of evapotranspiration losses. This might help plants to overcome brief water-stress conditions. The succulent nature of stems could also be among the long-term water-stress tolerating mechanisms in the *Pelargonium* species, which possibly enable members of the species to follow a crassulacean acid metabolism (CAM) in water-stressed conditions (Jones *et al.*, 2003).

Table 4.3: Fresh leaf mass to total fresh biomass ratio (%) of rose-scented geranium that was water-stressed for one month at different regrowth stages

Treatment	Open field			Rain shelter
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
NNNN	64.25 c†	65.50 b	58.40 c	65.40 b
NSNN	68.40 b	67.35 ab	61.02 b	66.04 b
NNSN	68.36 b	69.54 a	63.06 b	68.41 ab
NNNS	71.55 a	64.98 b	65.28 a	71.57 a
Grand mean	68.15	66.84	61.938	67.85
CV (%)	2.68	2.24	2.15	3.71
LSD (α = 0.05)	2.92	2.43	2.13	4.02

†**Values with the same letter in a column are not significantly different; NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation in the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 2, 3 and 4 were conducted in October 2005, February 2006, November 2006 and February 2007, respectively**

4.4.3 Essential oil yield and quality parameters

Essential oil content

Change in essential oil content (percentage oil on fresh herbage mass basis) was not consistent (Figure 4.5). The overall result, however, indicated that oil content tended to be higher in the water-stressed treatments. Except for Harvest 1 (essential oil content was highest in the NNNS treatment), maximum increase in essential oil content was observed when irrigation was withheld during the third month (the NNSN treatment).

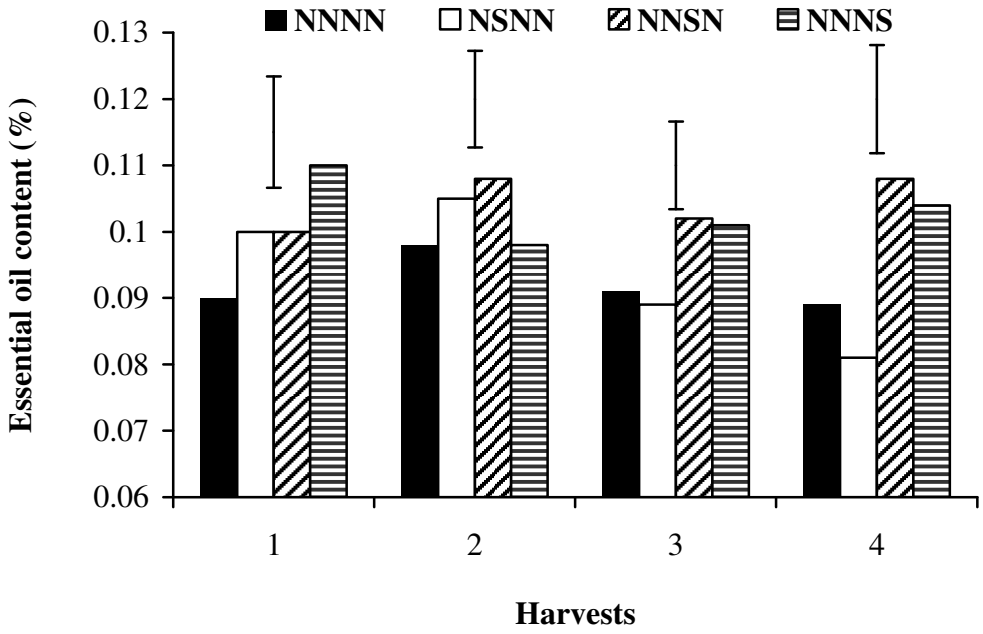

Figure 4.5: Essential oil content (% oil on fresh herbage mass basis) of rose-scented geranium that was water-stressed for one month at different regrowth stages. The vertical bars are LSD at α = 0.05; NNNN, NSNN, NNSN and NNNS represent control, and withholding irrigation during the second third and fourth regrowth months, respectively; Harvests 1, 2, 3 and 4 were conducted in October 2005, February 2006, November 2006 and February 2007, respectively

Similar to the present results, Weiss (1997) reported that essential oil content (percentage oil on fresh herbage mass basis) of rose-scented geranium for a harvests after a three-month wet period was lower than oil content obtained from plants harvested after a three-month dry period. Similarly, aromatic compounds of tea plants increased in water-stressed conditions (Panrong *et al.*, 2006)

Essential oil yield

Figure 4.6 shows the average essential oil yield (kg/ha) for the different treatments. The general response of essential oil yield was similar to that of fresh herbage yield. The present

results, therefore, support the report of Srivastava and Luthra (1993), which indicated that secondary metabolites such as essential oils are positively related to primary metabolites. The results of this research also agree with those results reported by Kumar *et al.* (2001) and Motsa *et al*. (2006), which indicated that higher vegetative growth resulted in higher total essential oil yield in rose-scented geranium, even if the percentage oil declined slightly under favourable growing conditions.

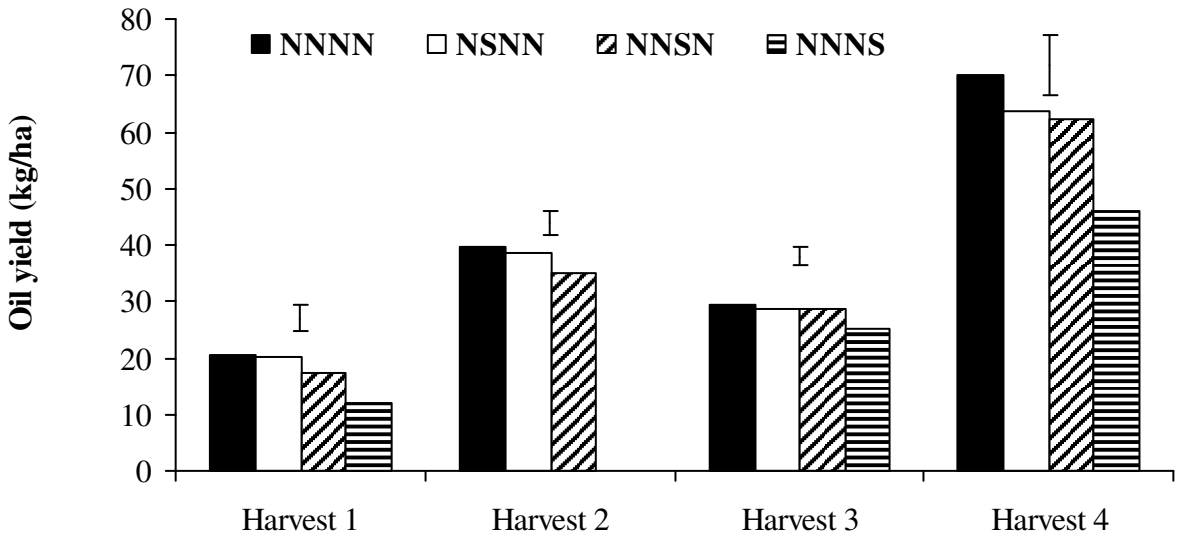

Figure 4.6: Essential oil yield (kg/ha) of rose-scented geranium that was water-stressed for one month at different regrowth stages. The vertical bars are LSD at α = 0.05; NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 2, 3 and 4 were conducted in October 2005, February 2006, November 2006 and February 2007, respectively

Compared to the fresh herbage yields, essential oil yield was less sensitive to water stress because the latter (essential oil yield) maintained or showed only a marginal reduction when irrigation was withheld in the second or third month of the regrowth cycles. Water stress during the fourth month of regrowth cycles (NNNS treatment) resulted in a significant essential oil yield loss. The losses in essential oil yield caused by irrigation withholding during

the fourth month of regrowth in Harvests 1, 2, and 3 (compared to the control, NNNN) were 41, 15, and 34%, respectively.

The increase in oil content (percentage oil on herbage fresh mass basis) and lower oil yield (kg/ha) in the water-stressed treatments suggest that the apparent increase in essential oil concentration in stressed conditions resulted from reduced leaf sizes and low leaf and stem water content. Such phenomena could lead to a reduction in fresh mass, the denominator in calculating percentage oil content. Similar to the present results, Panrong *et al.* (2006) reported that in water stressed conditions, the relative essential oil content increased, but total essential oil yield reduced due to a decline in herbage yield.

The current results contradict the general understanding that plant secondary metabolites, such as essential oils, are enhanced by water-stressed conditions (Yaniv & Palevitch, 1982; Sangwan *et al.*, 2001; Zobayed, Afreen & Kozai, 2007). Similarly, Simon *et al.* (1992) reported that mild to moderate water stress encouraged essential oil production in sweet basil. Weiss (1997) also documented that rose-scented geranium gave a slightly higher essential oil yield in a dry season than in a wet season, while the reverse was true for herbage yield.

Essential oil composition

Due to some technical problems, essential oil analysis for Harvests 3 and 4 could not be done. Gas chromatography (GC) results of the seven major and total trace essential oil constituents for Harvests 1 and 2 are presented in Figure 4.7. In all samples, regardless of irrigation treatment, citronellol was the highest component of the oils (32 ± 2.8%). Neither withholding irrigation nor the harvesting season affected linalool and guaia-6,9-diene concentrations. The overall result shows that the seven major essential oil constituents comprised 77.8% ± 3.1% of the total extracted oil.

In Harvest 1, there was no clear relationship between geraniol and citronellol. The mild increase in geraniol and citronellol contents in this regrowth cycle seemed to be paralleled by decreases in contents of the trace oil constituents. This could not be fully explained by the

stress treatments. It could be attributed to some reversible reaction undergone between alcohols (such as geraniol and citronellol) and their esters (part of the trace constituents) in the distillation processes (Babu & Kau, 2005).

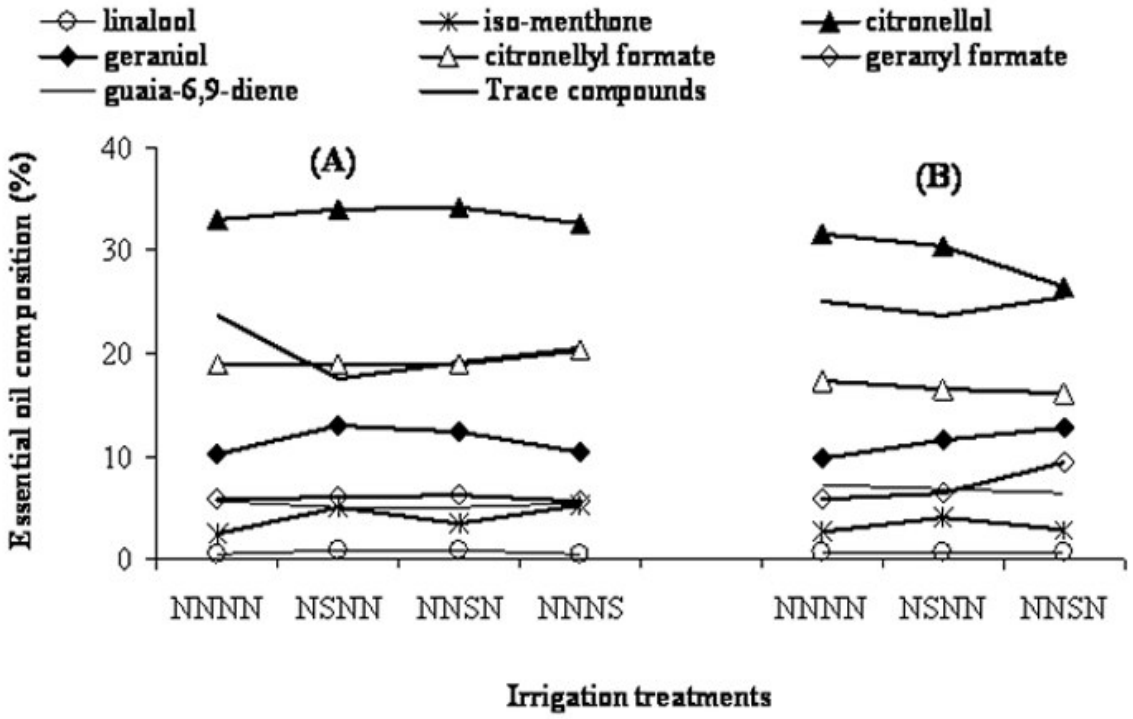

Figure 4.7: Essential oil composition of rose-scented geranium that was water-stressed for one month at different regrowth stages. NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; (A) Harvest 1 and (B) Harvest 2 were conducted in October 2005 and February 2006, respectively

In Harvest 2, a progressive increase in the concentration of geraniol and geranyl formate was accompanied by reductions in citronellol and citronellyl formate content in the treatments stressed towards the harvesting. The general relationship among these groups of compounds agree with previous reports (Rajeswara Rao *et al.*, 1996) which indicated that geraniol and geranyl formate were negatively related to citronellol and citronellyl formate. Contrary to the

patterns observed in the current results, however, Rajeswara Rao *et al.* (1996) indicated that water stress favoured citronellol and its ester concentrations.

Citronellol and geraniol levels and the ratio of these two components are usually primary indicators of oil quality. Although a C:G ratio in the range of one to three is acceptable, the most desirable in the perfumery and fragrance industries is a 1:1 ratio (Motsa *et al.,* 2006). In both harvests, the C:G ratio was consistently higher in the control (about 3.2) compared to that in the NSNN and NNSN treatments (which ranged between 2.1 and 2.8). The current results, therefore, indicate that water stress in the second or third month of regrowth improved oil quality by reducing the C:G ratio.

4.4.4 Water use and water-use efficiency (WUE)

Results of irrigation applied and evapotranspiration water losses (per harvest) are summarised in Table 4.4. Soil water data for Harvest 2 from the open field are not presented because the NNNS treatment was interrupted by intensive rainfall. Water applied was considerably higher in the non-stressed plots (NNNN treatment), and lowest when the irrigation was withheld in the last regrowth month (the NNNS treatment). These results support earlier reports, which associated evapotranspiration rate with high soil water (Wallace, 2000).

The amount of water applied was almost the same as the evapotranspiration for the NNNN, NSNN and NNSN treatments. In the NNNS treatment, a considerable difference was observed between the amount of water applied and used (evapotranspiration). For this treatment (NNNS), the amount of irrigation was substantially less, as the profile (root zone) was not refilled at the end of the season. The amount of irrigation water saved by withholding irrigation in the third month of regrowth (NNSN), with only marginal changes in essential oil yield, ranged between 33 and 46 mm (equivalent to 330 to 460 m^3 of water per hectare per growth cycle).

Table 4.4: Total irrigation applied and amount of water used by rose-scented geranium that was water-stressed for one month at different regrowth stages

Treatments	Open field		Rain shelter
	Harvest 1	Harvest 3	Harvest 4
	----------Irrigation applied (mm) ----------		
NNNN	346 a[†]	362 a	506 a
NSNN	316 a	329 a	467 a
NNSN	313 a	316 a	463 a
NNNS	268 b	259 b	392 b
Grand mean	310.8	316.5	457.0
CV (%)	7.1	9.0	7.8
LSD (α = 0.05)	35.2	45.8	57.0
	---------- Evapotranspiration loss ----------		
NNNN	355 a	361 a	502 a
NSNN	326 a	330 a	467 a
NNSN	319 a	321 a	457 a
NNNS	318 a	321 a	450 a
Grand mean	329.5	333.3	469.0
CV (%)	9.9	11.1	7.1
LSD (α = 0.05)	NS	NS	NS

[†]**Values with the same letter in a column are not significantly different; NNNN, NSNN, NNSN and NNNS represent control (no stress), stress during the second, third and fourth month of regrowth cycles; Harvests 1, 3 and 4 were conducted in October 2005, November 2006 and February 2007, respectively**

The results in Figure 4.8 indicate that the overall water-use efficiency (WUE) values for Harvests 1 and 3 (in the open field) were influenced by season. The WUE was higher in

Harvest 3, a regrowth cycle in higher temperatures (mean maximum and minimum of 28 and 12°C, respectively), than in Harvest 1 grown during lower temperatures (mean maximum and minimum of 25 and 8°C, respectively).

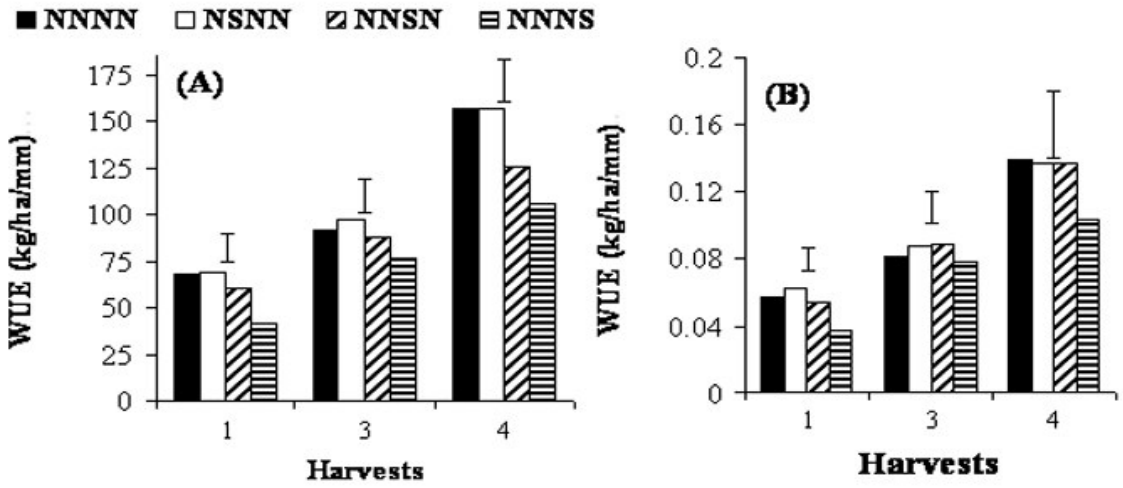

Figure 4.8: Water-use efficiency (WUE) (kg/ha/mm) of rose-scented geranium that was water-stressed for one month at different regrowth stages: (A) on fresh herbage mass and (B) on essential oil yield basis. The vertical bars are LSD at α = 0.05; NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 3 and 4 were conducted in October 2005, November 2006 and February 2007, respectively

The higher WUE (in terms of herbage yield) recorded for the NNNN and NSNN treatments (Figure 4.8a) was consistent with results reported for alfalfa (Saeed & El-Nadi, 1997) onion (Kadayifci *et al.*, 2005) and cucumber (Şimşek, Tonkaz, Kaçira, Çömlekçioğlu & Doğan, 2005). These findings support the ideas of Bessembinder *et al.* (2005), who stated that well-watered plants would result in higher water-use efficiency, provided that other factors such as soil nutrients are not limiting.

Results presented in Figure 4.8b indicated that WUE, in terms of essential oil produced, considerably reduced only when the water stress was applied in the fourth month of regrowth.

This observation, together with the marginal/negligible reduction in oil yield caused by water-stressed condition during the second and the third months of regrowth, implies that withholding irrigation during these regrowth stages would be possible without compromising essential oil yield. Such irrigation management strategy would save water, which could be used to avoid severe water stress in the fourth month of regrowth of the crop, to expand the irrigated land area or to alleviate water shortages in other economic and social service sectors, where freshwater is a limiting factor (Ali, Hoque, Hassan, & Khair, 2007; Bouman, 2007).

4.5 CONCLUSIONS AND RECOMMENDATIONS

The present study reveals that essential oil yield is positively related to biomass production. Essential oil concentration apparently increased in water-stressed conditions, but its contribution was not large enough to compensate for the essential oil loss as a result of reduction in herbage yield. A significant decline in essential oil yield was observed only when the crop was stressed in the fourth month of regrowth. Hence, farmers are advised to avoid severe water stress during the last month before harvest. In freshwater-scarce regions, withholding irrigation during the second and third months of regrowth of rose-scented geranium could improve water productivity, because the technique would save water that could be used to irrigate the crop during more water-stress-sensitive regrowth stages (fourth month of regrowth cycle), to expand the irrigated land area, or to alleviate freshwater shortage in other economic and social service sectors. Specifically, in cool weather conditions, when rose-scented geranium growth rate is relatively slow, this study suggests that increasing planting density could improve essential oil yield per hectare.

CHAPTER 5

RESPONSE OF ROSE-SCENTED GERANIUM GROWTH, ESSENTIAL OIL YIELD AND OIL COMPOSITIOM TO IRRIGATION FREQUENCY AND A ONE-WEEK WATER-WITHHOLDING PERIOD

5.1 ABSTRACT

Pot experiments were conducted to investigate the effects of irrigation frequency and withholding irrigation during the week prior to harvesting on rose-scented geranium growth, and essential oil yield and composition. A factorial experiment with three irrigation frequencies (twice a day, once a day and every second day) and two growth media (silica sand and sandy clay soil) was conducted in a tunnel. In a glasshouse, sandy clay soil was used as growing medium, and five irrigation frequencies (daily, and every second, third, fourth and fifth day irrigation to pot capacity) were applied as treatments. In both trials, irrigation was withheld on 50% of the plants in each main plot as a split. Herbage and essential oil yields were better in sandy clay soil than in silica sand. Essential oil content (percentage oil on fresh herbage mass basis) increased with decrease in irrigation frequency. Both herbage and total essential oil yields positively responded to frequent irrigation. A one-week stress period significantly increased essential oil content and total essential oil yield. Hence, the highest essential oil yield was obtained from a combination of high irrigation frequency and a one-week irrigation-withholding period. In the irrigation frequency treatments, citronellol and citronellyl formate tended to increase with an increase in the stress level, but the reverse was true for geraniol and geranyl formate contents.

Keywords: Citronellol, citronellyl formate, geraniol, geranyl formate, herbage yield, oil content

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5.2 INTRODUCTION

Soil water supply is one of the major abiotic factors that determine the biosynthetic processes in plants (Letchamo, Xu & Gosselin, 1995). Response of essential oil yield and composition to water stress varies with duration and severity of stress. According to literature, the production of primary metabolites and essential oil yield may decline when plants are exposed to sustained water stress (Panrong *et al.*, 2006). Letchamo *et al.* (1995) found positive correlations among photosynthesis, herbage yield and essential oil yield in thyme (*Thymus vulgaris* L.). Putievesky, Ravid and Dudai (1990) also reported that as irrigation intervals became more extended, herbage yield and essential oil yield were reduced in *Pelargonium graveolens*. Similarly, a report by Rajeswara Rao *et al.* (1996) indicated that a wet season encouraged vegetative growth of rose-scented geranium and resulted in higher essential oil yield.

Based on results Weiss (1998) obtained from his previous studies on rose-scented geranium, he suggested that climatic factors (wet season, for instance), which encourage herbage growth, would have a negative effect on essential oil yield. Similarly, Simon *et al.* (1992) reported that a moderate water stress imposed on sweet basil resulted in higher oil content and greater total oil yield. Furthermore, the authors indicated that water stress changed essential oil composition: water stress increased linalool and methyl chavicol, and reduced sesquiterpenes. Contrary to the above report, a short-term stress (withholding irrigation for eight days) did not change essential oil yield and oil composition of *Melaleuca alternifolia* (List *et al.*, 1995).

To an extent, research documenting the response of essential oil yield to soil water availability is contradictory, and the combined effects of long- and short-term water stress on the essential oil of rose-scented geranium have not been reported on. Therefore, the objective of this study was to investigate the effect of long- and short-term water stress on herbage yield, essential oil yield and essential oil composition of rose-scented geranium (*Pelargonium capitatum x P. radens* cv. Rose) grown in South Africa.

5.3 MATERIALS AND METHODS

5.3.1 Growth system description

Pot trials were conducted in a tunnel and in a glasshouse at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa, from January 2005 to December 2006. Shading effects of the walls/roofs of the tunnel (polyethylene plastic) and the glasshouse (glass) were in the range between 30 and 35% during the experimental period. In both greenhouses, temperature was regulated by fan and wet wall/pad system (controlled by computerised sensor). The cooling systems were set to regulate temperatures higher than 18°C. The highest maximum temperatures recorded in the tunnel and glasshouse during the experimental period were about 34 and 33°C, respectively (Appendix A).

5.3.2 Plant culture

In both greenhouses, *Pelargonium capitatum x P. radens* cv. Rose was used as planting material. For the tunnel, about 50-day-old plantlets regenerated from stem cuttings by a commercial nursery, were transplanted in 10-ℓ plastic pots [filled with either silica sand (with water holding capacity of 9.7% and 3.8% (v/v) at field capacity and permanent wilting point, respectively) or sandy clay soil (52: 8: 38 coarse sand, silt and clay content, respectively)] on 26 January 2005. For the glasshouse trial, healthy stem cuttings (taken from the tunnel trial) were raised in seedling trays filled with peat in a mist bed for 40 days (in a glasshouse, at the Hatfield Experimental Farm). The plantlets were transplanted in 10-ℓ plastic pots (filled with only sandy clay soil) on 29 October 2005. Water-holding capacity of the sandy clay soil (used as growing medium in both greenhouses) was about was 29% and 17% (v/v) at field capacity and permanent wilting point, respectively.

5.3.3 Treatments and experimental design

Treatments

Irrigation treatments in the tunnel were twice a day (IR1), once a day (IR2) and every other day (IR3), in either silica sand or a sandy clay soil. In the glasshouse five irrigation intervals, every day (T1), every second day (T2), every third day (T3), every third day (T4) and every fourth day (T5) irrigation, were applied as treatments. In both trials, a one-week irrigation-withholding period prior to harvesting was imposed on 50% of the plants in each plot.

The regrowth durations were three months ± one week, depending on the weather conditions during the brief stress treatment (on non-cloudy days). The plants appeared to be sensitive to water stress for some time after cutting. Hence, in the first month of regrowth, no water stress was applied. In addition, cultural practices (fertiliser application and some pest control measures) were done within that period. Irrigation treatments were applied during the second and third month of each regrowth cycle.

Experimental design

In the tunnel trial, the irrigation frequency by soil type treatment combinations were arranged in a randomised complete block design in four replications. Each plot consisted of two adjacent rows (75 cm apart) of 21 pots each (Figure 5.1). In the glasshouse trial, there were six rows of 42 pots each, representing the blocks/replications. The space between adjacent rows was 1 m, and the plants within a row were 0.30 m apart. In each row, each of the five irrigation treatments was randomly assigned to a group of eight pots (as a main plot), e.g. the irrigation treatments were arranged in complete randomised block design, six times replicated. In both greenhouses, irrigation was withheld on 50% of the plants in each main plot for the week prior to harvesting, as a split.

Figure 5.1: Plant arrangements in the tunnel trial

5.3.4 Irrigation monitoring

In the tunnel, a computer-regulated drip irrigation system (spaghetti water emitters with an average discharging rate of 2 ℓ/hr) was installed and used to monitor the irrigation intervals and amount of water given to each treatment refill to pot capacity. To minimise drainage, the amount of water applied was estimated by measuring water collected in water-collecting containers put in holes near pots, with gutters at the bottom.

In the glasshouse, the pots were put on top of parallel metallic/wooden bars, which were supported by bricks to give space for water-collecting cans (Figure 5.2). At each irrigation event, a measured amount of water was applied. The volume of water that was required to refill the pots to pot capacity (depleted water/evapotranspiration), at each irrigation event, was determined by subtracting the drained water from the applied water. To minimise nutrient losses, the drained water was recycled on the next irrigation event.

Figure 5.2: Drainage-collecting container in the glasshouse pot trials

5.3.5 Fertiliser application

During each regrowth period, each plant received 3 g nitrogen (N), 4.5 g phosphorus (P), and 3 g potassium (K) [in the form of 2:3:2 (22) NPK fertiliser granules] as a split in Week 1 and Week 7 of each regrowth cycle. In addition, 1 g N (as ammonium nitrate) and 1 g K (as potassium chloride) were applied to each pot in Week 9 of each regrowth cycle. To avoid salt accumulation, plants were over- irrigated on the first and second day of each regrowth cycle.

5.3.6 Data recorded

Data for four growth cycles, 22 June to 21 September 2005 (Harvest 1), and 25 January to 29 April (Harvest 2), 30 May to 29 August (Harvest 3) and 30 August to 30 November 2006 (Harvest 4), from the tunnel, and for two regrowth cycles, 26 March to 29 June (Harvest 1) and

30 June to 10 October 2006 (Harvest 2) from the glasshouse were recorded. Data captured, instruments used and procedures followed are described in Chapter 3. For technical reasons, essential oil data for Harvest 4 (tunnel trial) were not collected.

5.4 RESULTS AND DISCUSSION

5.4.1 Effects of irrigation frequency on herbage yield in the tunnel trials

The results indicated that herbage yield was sensitive to irrigation frequency (Table 5.1). Every reduction in irrigation frequency resulted in a significant decline in herbage yield. The herbage yield reduction rate was consistently higher between IR2 and IR3 (ranged from 42% to 58%) than between IR1 and IR2, where it ranged from 16% to 37%.

Table 5.1: Fresh herbage yield of rose-scented geranium grown under different irrigation frequencies in the tunnel

Irrigation	Fresh herbage mass (g/plant)				Dry matter (g/plant)	
frequency	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 1	Harvest 3
IR1	644.8 a[†]	895.5 a	1006.0 a	833.4 a	84.9 a	130.3 a
IR2	508.2 b	627.7 b	844.6 b	521.3 b	75.5 b	118.1 b
IR3	275.8 c	324.0 c	356.7 c	299.3 c	40.4 c	55.2 c
Grand mean	476.2	615.7	735.7	551.3	66.9	100.5
CV (%)	8.5	9.2	7.0	7.1	8.2	8.9
LSD ($\alpha = 0.05$)	30.5	43.2	41.8	41.5	3.7	5.8

[†]**Values followed by the same letters in a column are not significantly different at 5% level of probability; IR1, 2 and 3 are twice a day, once a day and every second day irrigation frequency, respectively; Harvests 1, 2, 3, 4 were conducted in September 2005, April, August and December 2006, respectively**

These results agree with results reported by Rajeswara Rao *et al.* (1996), who found that an increase in soil water availability encouraged vegetative growth in rose-scented geranium. Similarly, Singh (1999) found significant lower herbage yield of *Pelargonium graveolens* grown in 0.3 than in 0.6 irrigation water to cumulative pan evaporation (IW:CPE) ratio.

In addition, the data show that there was a clear impact of season on herbage yield. When plants experienced cold weather (mean maximum and minimum temperatures of 9 and 20°C, respectively) during regrowth for Harvest 1, the herbage yield was low. Although the regrowth period for Harvest 3 (August 2006) was also a winter season, the temperature-controlling system was switched off due to malfunctioning and the maximum temperature (during the day) inside the tunnel was higher (about 26°C) than the temperature outside (21°C). As a result, the herbage yield was as high as or even higher than the growth during spring/summer, Harvests 2 and 4 (regrowths under mean maximum and minimum temperatures of 18 and 34°C, respectively).

5.4.2 Effects of irrigation frequency on essential oil yield components in the tunnel trials

Compared to herbage yield, essential oil yield was less sensitive to the differences in irrigation frequencies (Table 5.2). Reducing the irrigation frequency from twice a day to once a day either maintained or enhanced essential oil yield per plant. Such a result was probably due to a tendency of essential oil content (percentage oil on herbage fresh mass basis) to increase with a decrease in irrigation frequency (Figure 5.3). Essential oil yield was significantly reduced when plants were subjected to relatively severe water stress in the every second day irrigation schedule.

These results are consistent with the research reports, which underlined that secondary metabolites, such as essential oils, were positively related to primary metabolites (Srivastavaand & Luthra, 1993; Letchamo *et al.*, 1995; Sangwan, *et al.*, 2001). Rajeswara Rao (2002) also reported that total essential oil yield of rose-scented geranium was positively related to fresh herbage yield, despite the inverse relationships between herbage yield and relative essential oil content. Similarly, Panrong *et al.* (2006) observed that essential oil content apparently increased, but the total essential oil yield decreased in *Lingtou dancong* tea plants grown under water-

stressed conditions, indicating that the relative increase in oil content was not sufficient to compensate the oil yield loss attributed to the reduced herbage growth in water stress conditions.

Table 5.2: Essential oil yield of rose-scented geranium as affected by irrigation frequency in the tunnel

Irrigation frequency	Essential oil yield (mg/plant)		
	Harvest 1	Harvest 2	Harvest 3
IR1	197.9 b[†]	761.6 a	408.6 a
IR2	238.7 a	764.5 a	409.7 b
IR3	145.3c	390.4 b	207.3 c
Grand mean	193.98	654.4	341.9
CV (%)	8.49	8.0	8.2
LSD ($\alpha = 0.05$)	23.1	49.5	22.6

[†]**Values followed by the same letters within a column are not significantly different at $\propto = 0.05$; IR1, 2 and 3 are twice a day, once a day and every second day irrigation frequency, respectively; Harvests 1, 2 and 3 were conducted in September 2005, and April and August 2006, respectively**

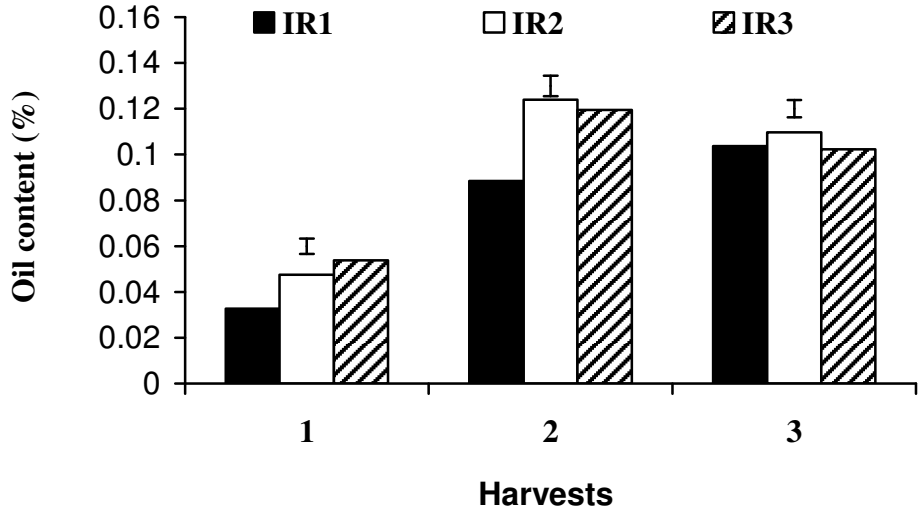

Figure 5.3: Essential oil content of rose-scented geranium as affected by irrigation frequency in the tunnel. The vertical bars are LSD (at $\alpha = 0.05$); IR1, 2 and 3 represent twice a day, once a day and every second day irrigation frequency, respectively; Harvests 1, 2 and 3 were conducted in September 2005, and April and August 2006, respectively

Essential oil yield performance among harvests was also affected by season. Despite the higher herbage yield recorded for Harvest 3, essential oil yield per plant was lower than that of Harvest 2 (Table 5.2). This result could be attributed to the lower night temperatures and/or the wider range between day and night temperatures in the winter season, during the regrowth period for Harvest 3 (Table 5.2), supporting a previous report (Motsa *et al.,* 2006), which indicated that rose-scented geranium essential oil content tended to decline with decrease in night temperatures.

5.4.3 Effects of irrigation frequency and withholding irrigation on herbage yield parameters in the tunnel trials

Figures 5.4 illustrates fresh and dry herbage yield as affected by irrigation frequency, one-week withholding irrigation and growth media (silica sand and sandy clay soil). The one-week withholding-irrigation period resulted in a significant decline in fresh herbage mass in IR1 (more often irrigated treatment), but not in IR2 and IR3, the less often irrigated treatments. This could be an indication that the plants in the lowest irrigation frequency had developed a water-conserving mechanism and/or had limited stored water that could be lost as evapotranspiration.

Herbage dry mass also reduced by the one-week irrigation withholding period, but the difference was not consistently significant. The data also showed that both fresh and dry herbage yields were lower in the silica sand than in the sandy clay soil, presumably due to the lower water retaining capacity of the silica sand. Thus, the overall result implies that high soil water results in high vegetative growth in rose-scented geranium (Weiss, 1997; Rajeswara Rao, 2002).

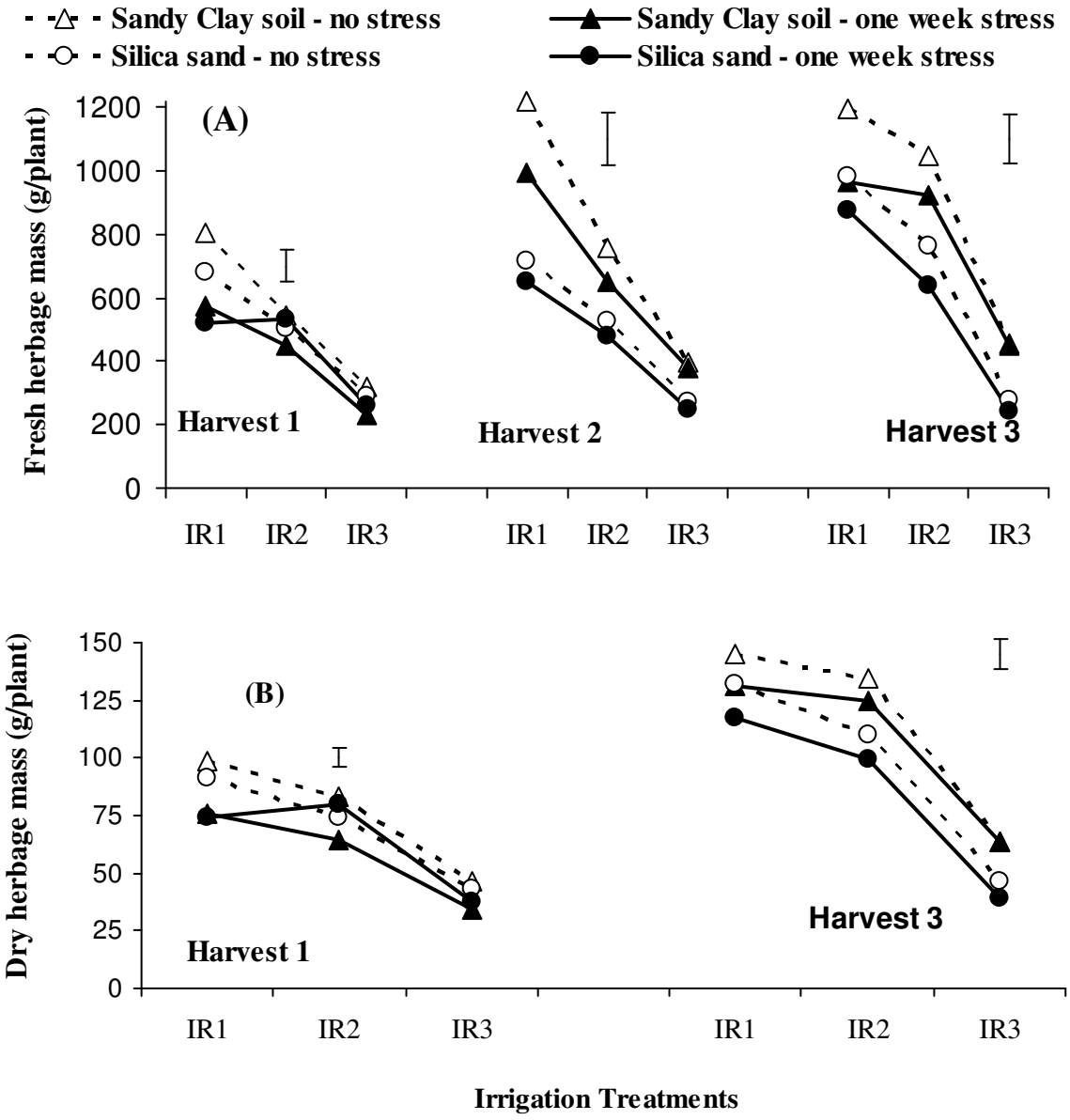

Figure 5.4: Rose-scented geranium fresh (A) and dry (B) herbage mass as affected by irrigation frequency and a one-week stress period before harvest in the tunnel. The vertical bars are LSD (at α = 0.05); IR1, 2, 3 represent twice a day, once a day and every second day irrigation frequency, respectively; Harvests 1, 2 and 3 were conducted in September 2005, and April and August 2006, respectively

5.4.4 Response of essential oil yield parameters to irrigation frequency, growing medium and withholding irrigation in the tunnel trials

The one-week irrigation-withholding treatment in most cases resulted in a significant increase in oil content (percentage oil on herbage fresh mass basis) (Figure 5.5). Thus, essential oil yield per plant improved (Figure 5.6) in spite of the general decline in fresh herbage yield observed (Figure 5.4). The highest essential oil yield was obtained from a combination of high-irrigation frequency (IR1 and IR2) and one-week stress in sandy clay soil.

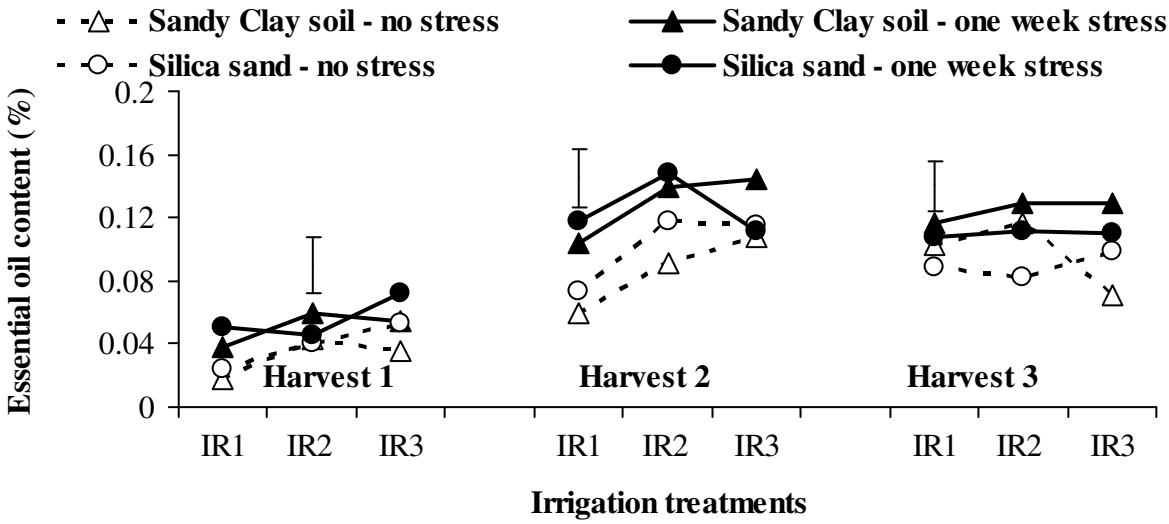

Figure 5.5: Rose-scented geranium oil content (percentage oil on fresh herbage mass basis) as affected by irrigation frequency and a one-week stress period before harvest in two growing media in the tunnel. The vertical bars are LSD (at α = 0.05); IR1, 2, 3 are twice a day, once a day and every second day irrigation frequency, respectively; Harvests 1, 2 and 3 were conducted in September 2005, and April and August 2006, respectively

In agreement with the current results, De Abreu and Mazzafera (2005) reported that several plant secondary metabolites in *Hypericum brasiliense* Choisy showed an increasing trend under water-stressed conditions. Simon *et al.* (1992) also reported that mild to moderate water stress imposed on sweet basil resulted in higher oil yield per plant. The authors stated that when plants were subjected to a mild or a moderate water stress, the relative essential oil content (per dry mass) was doubled. Weiss (1997) also mentioned that rose-scented geranium essential oil yield tended

to increase in water stressed conditions. A one-week irrigation-withholding period, however, did not affect essential oil yield of *Melaleuca alternifolia* (List *et al.*, 1995). This implies that different plant species may respond differently to duration and degree of water stress.

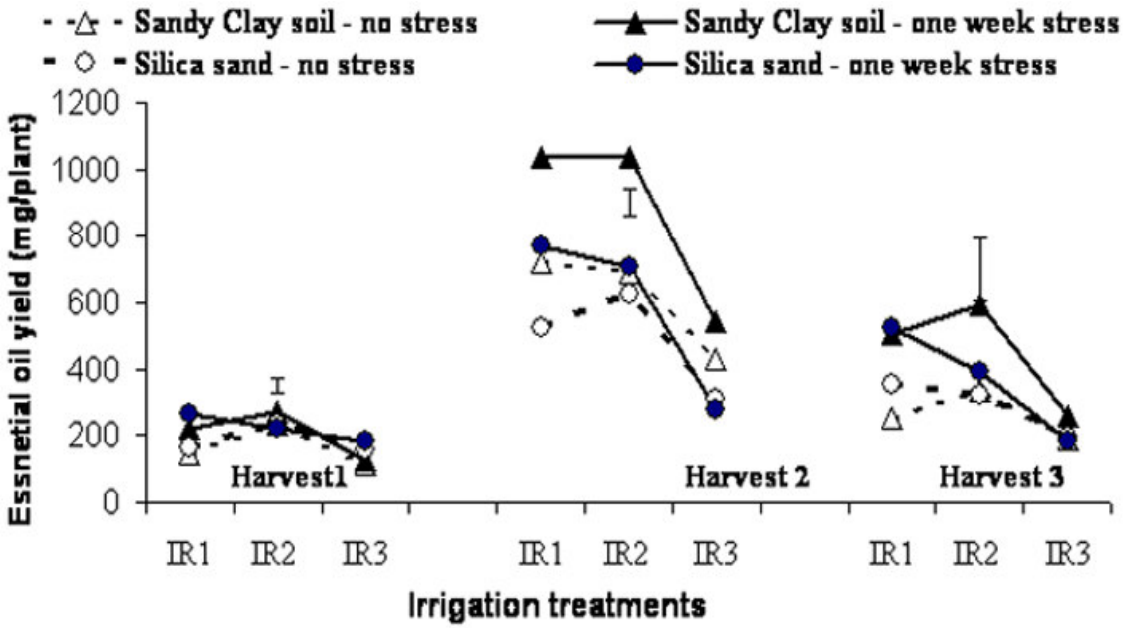

Figure 5.6: Essential oil yield of rose-scented geranium as affected by irrigation frequency, a one-week stress period before harvest and growing media in the tunnel. The vertical bars are LSD (at α = 0.05); IR1, 2, 3 represent twice a day, once a day and every second day irrigation frequency, respectively; Harvests 1, 2 and 3 were conducted in September 2005, and April and August 2006, respectively

In most cases, the effect of the one-week irrigation-withholding treatment on oil composition was limited (Figure 5.7). To a certain extent, the highest irrigation frequency (IR1) favoured geraniol content and a lower citronellol to geraniol ratio (C:G ratio). Citronellol and geraniol levels and the ratio of these two components are usually primary indicators of oil quality. A C:G ratio in the range of one to three is acceptable (Motsa *et al.*, 2006).

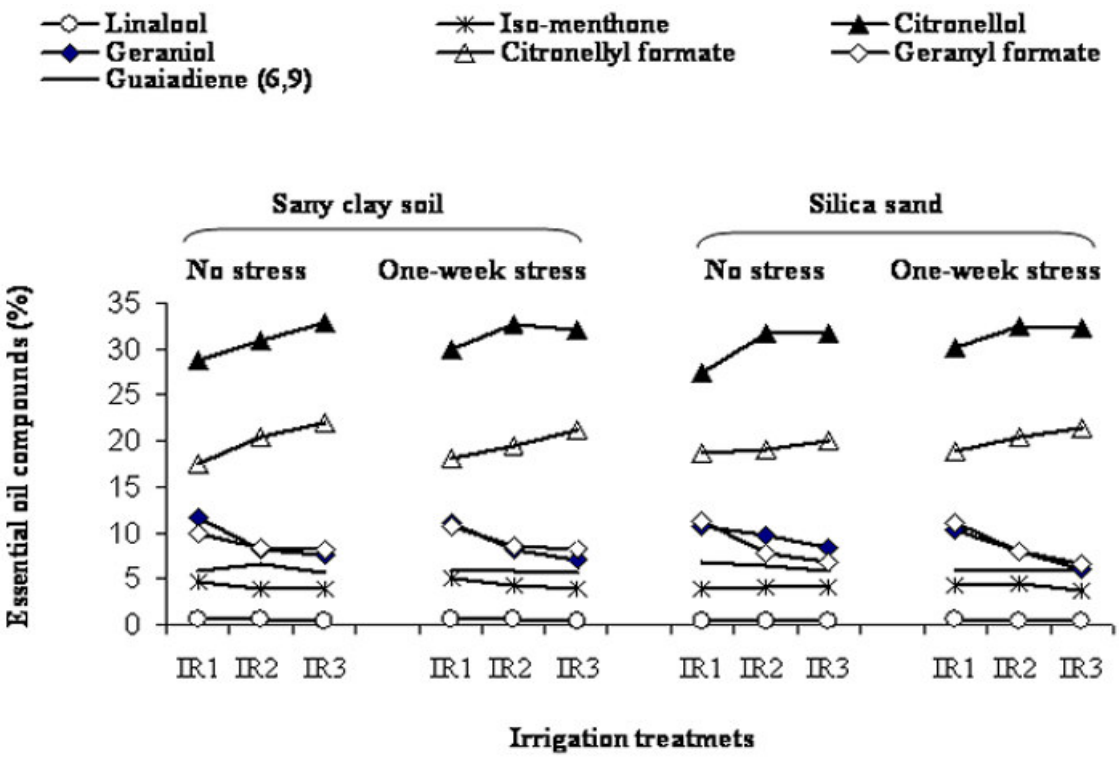

Figure 5.7: Chemical composition (%) of essential oil of rose-scented geranium as affected by irrigation frequencies and a one-week stress period in the tunnel. IR1, 2, 3 are twice a day, once a day and every second day irrigation frequency, respectively

The overall results show that geraniol and geranyl formate contents were negatively related with that of citronellol and citronellyl formate. The other three major essential oil components in rose-scented geranium (iso-menthone, guaia-6,9-diene, and linalool) did not show any response to the water irrigation levels. The relationship between geraniol and citronellol observed in the current experiments agrees with work of Rajeswara Rao *et al.* (1996), who suggested that water and thermal stress conditions could lead to conversion of some of the geraniol to citronellol in rose-scented geranium. Luthra *et al.* (1991), on the other hand, reported a positive correlation between geraniol and citronellol in *Cymbopogon winterianus*.

5.4.5 Response of herbage yield parameters to irrigation levels in the glasshouse trials

In the glasshouse, the response of herbage yield parameters to the irrigation frequency and the one-week irrigation-withholding treatments was more or less similar to that observed in the tunnel trials. Fresh herbage yield progressively decreased with a decrease in irrigation frequency (Figure 5.8).

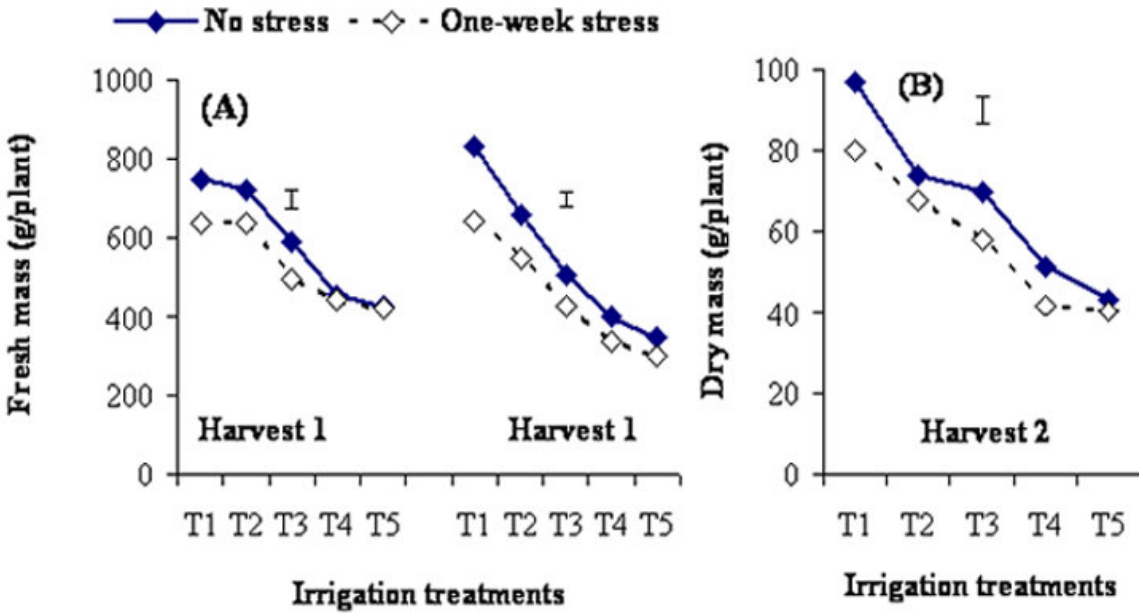

Figure 5.8: Fresh (A) and dry (B) herbage mass of rose-scented geranium as affected by irrigation frequency and a one-week irrigation-withholding period in the glasshouse. The vertical bars represent LSD (at $\alpha = 0.05$); Harvests 1 and 2 were conducted in June and October 2006, respectively; T 1, T2, T3, T4 and T5 are daily, and every second, third, fourth and fifth day irrigation treatments

The response of the plants to the treatment was slightly affected by season. During a relatively cool regrowth cycle (e.g. for Harvest 1, which had mean maximum and minimum temperatures of 26 and 12°C, respectively), herbage yield started to decline significantly when the irrigation frequency was extended to intervals of three or more days. In Harvest 2, a growth cycle in warm/hot season (mean minimum and maximum temperatures of 19 and 28°C, respectively), on the other hand a noticeable decline in fresh herbage yield started from the every second day

irrigation treatment (T2) . Consistent with the results that were recorded in the tunnel, the one-week irrigation-withholding period had a negative effect on both fresh and dry herbage yields. The impact, however, tended to decrease with irrigation frequency.

5.4.5 Response of essential oil yield parameters to irrigation levels in the glasshouse

Essential oil yield

In agreement with the results observed in the trials in the tunnel and previous reports (Rajeswara Rao, 2002; Singh, 1999), in the glasshouse trials, the essential oil yield positively responded to irrigation frequency (Figure 5.9). Thus, the results prove that essential oil is a function of primary metabolites or herbage yield (Srivastava & Luthra, 1993; Letchamo *et al.*, 1995; Sangwan *et al.*, 2001).

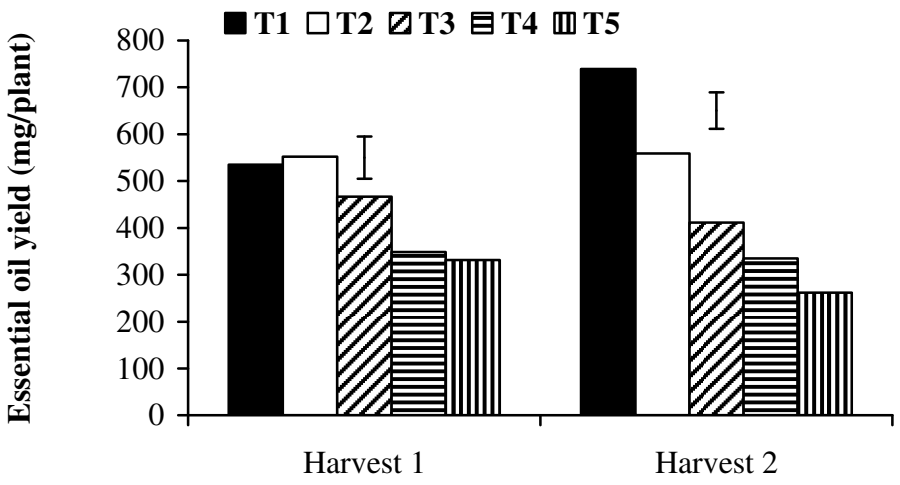

Figure 5.9: Effect of irrigation frequency on essential oil yield of rose-scented geranium in the glasshouse. The vertical bars represent LSD at $\alpha = 0.05$; Harvests 1 and 2 were conducted in June and October 2006, respectively; T 1, 2, 3, 4 and 5 represent daily, and every second, third, fourth and fifth day irrigation treatments

Essential oil content

In general, the one-week irrigation-withholding treatment increased essential oil concentrations, although the effect varied with the growing season (Figure 5.10). In Harvest 1, the response of essential oil content to the one-week irrigation-withholding period was not affected by the irrigation frequency treatment. The irrigation-withholding treatment for this harvest was imposed during relatively cool (minimum and maximum temperatures of 16 and 26°C, respectively) weather conditions. In Harvest 2, however, the impact of the one-week irrigation-withholding period declined with the irrigation frequency. This could be attributed to high water loss from the large herbage growth (Figure 5.8) of the plants grown under more frequent irrigation accompanied by the high minimum (20°C) and maximum (33°C) temperatures during the water-withholding period.

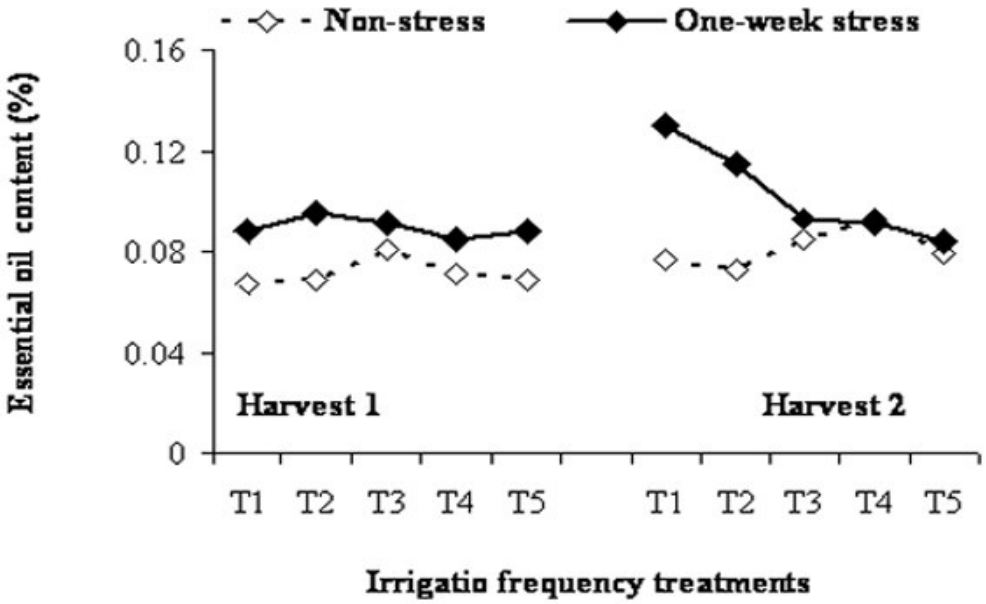

Figure 5.10: Essential oil content (percentage oil on fresh herbage mass basis) of rose-scented geranium as affected by irrigation frequency and one-week irrigation-withholding treatments. Harvests 1 and 2 were conducted in June and October 2006 in the glasshouse; T1, 2, 3, 4 and 4 represent daily, and every second , third, fourth and fifth day irrigation treatments

Consistent with the results obtained from the tunnel trials, the increase in essential oil content induced by the one-week irrigation-withholding treatment resulted in a significant increase in oil yield per plant (Figure 5.11). In both harvests, the combinations of high irrigation frequency (daily and/or the every second day irrigation) and one-week withholding-irrigation treatments performed the best in essential oil yield. In general, the effects of irrigation frequency and the one-week irrigation-withholding period were more prominent in Harvest 2 (regrowth cycle during a warm season) than in Harvest 1 (a regrowth during a cool season).

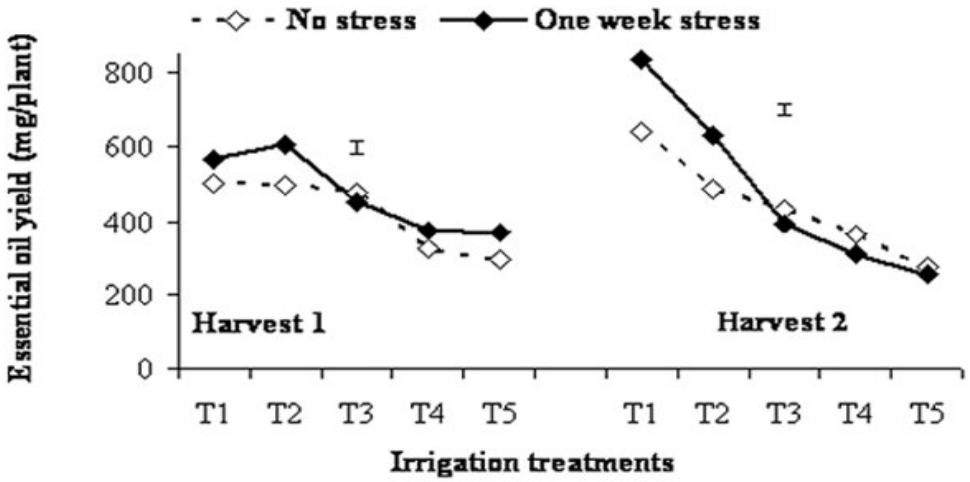

Figure 5.11: Essential oil yield of rose-scented geranium grown in different irrigation frequencies and a one-week water stress period. The vertical bars are LSD (at α = 0.05); Harvests 1 and 2 in the glasshouse were conducted in June and October 2006, respectively; T 1, 2, 3, 4 and 5 represent daily, and every second , third, fourth and fifth day irrigation treatments, respectively

Essential oil composition

Responses of essential oil composition to the long term (irrigation frequency) and brief stress (withholding water for one week) supported the results obtained from the trials in the tunnel (Figure 5.12). There was no clear indication that the one-week irrigation-withholding period affected oil composition. Less often irrigation favoured citronellol and citronellyl formate

contents. Every increase in these compounds was associated with a decrease in geraniol and geranyl formate levels in the oil. Citronellol to geranium ratio ranged between 2.4 (in higher irrigation frequency) and 4.8 (in the less often irrigated treatments). The ratio remained in the acceptable range for the T1 and T2. The increase in C:G ratio in the less frequently irrigated treatments (T3, T4 and T5) could negatively affect the oil preference in the perfume industry (Motsa *et al.* (2006).

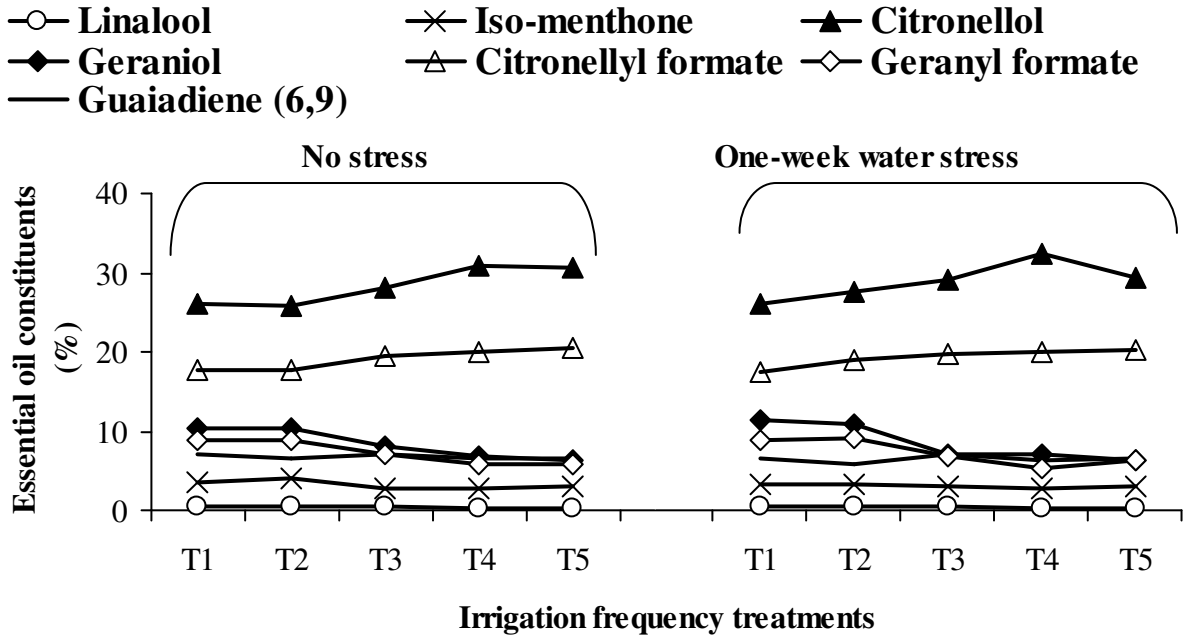

Figure 5.12: Composition (percentage of essential oil) of rose-scented geranium as affected by irrigation frequency and irrigation withholding for the week prior to harvesting treatments in the glasshouse. T1, T2, T3, T4 and T5 represent daily and every second, third, fourth and fifth day irrigation, respectively

5.4.7 Water use and water-use efficiency (WUE)

The data presented in Table 5.3 indicate that water usage decreased with a decrease in irrigation frequency. The higher water use in the more often irrigated treatments could be attributed to high evapotranspiration rate associated with large canopy and high water availability. In agreement with this observation, Şimşek *et al*. (2005) reported that crop evapotranspiration rate of cucumber

(*Cucumbis sativus*) decreased with a decrease in irrigation level. Wallace (2000) also indicated that more often irrigation encourages water loss/use. In Harvest 2, the water usage was higher than in Harvest 1, probably caused by the higher temperature and improved plant growth in Harvest 2.

Table 5.3: Water use and water-use efficiency (on essential oil yield basis) of rose-scented geranium grown under different irrigation frequencies and a one-week irrigation-withholding period

Treatments	Harvest 1		Harvest 2	
	Total water (litre/plant)	WUE (mg/litre)	Total Water (litre/plant	WUE (mg/litre)
T1	42.79 a[†]	12.76 ab	48.15 a	15.68 a
T2	39.07 b	14.30 a	41.43 b	13.76 b
T3	34.26 c	13.82 a	36.18 c	11.51 c
T4	28.08 d	12.59 ab	30.97 d	10.91cd
T5	27.65 d	12.03 b	28.73 d	9.29 d
Grand mean	34.37	13.10	37.09	12.10
CV (%)	11.95	15.78	10.68	13.3
LSD ($\alpha = 0.05$)	3.50	1.761	3.48	1.47

[†]**Values followed by the same letters within a column are not significantly different at ∝ = 0.05; Harvest 1 and 2 were conducted in June and October 2006 in the glasshouse; T1, 2, 3, 4 and 5 represent daily, every second, third, fourth and fifth irrigation treatments**

Effect of irrigation frequency on WUE was influenced by growing season. The result obtained in Harvest 1 shows that extending the irrigation interval to every second and third day slightly improved WUE indicating that a certain amount of the water applied to the daily irrigated treatment was not productive. This result to a certain extent supports the general understanding that a certain water stress level improves WUE (Kirda, 2000; Liang, Zhang, Shao & Zhang, 2002). The data recorded in Harvest 2, on the other hand, showed that the WUE tended to increase with irrigation frequency. These results agree with the ideas of Bessembinder *et al.*

(2005), who argued that WUE increases with soil moisture provided that other factors such as the essential nutrients are available at the required levels.

5.5 CONCLUSIONS

The current study indicates that long-term water stress brings about parallel reduction in primary (herbage yield) and secondary metabolites (essential oil). Herbage yield seems to be an indicator of essential oil yield, i.e. essential oil yield is a function of primary metabolites. Less frequent irrigation resulted in increase in citronellol and citronellyl formate contents and the reverse was true for geraniol and geranyl formate levels in the oil. A brief period of water stress following high irrigation frequency reduced herbage yield, but enhanced both relative essential oil content and essential oil yield. This could be an indication of reallocation of primary metabolites to secondary metabolites at certain water stress levels and/or duration. At field level, applying a one-week irrigation-withholding period on a full soil profile may not result in sufficient stress on rose-scented geranium because the plants may get enough water from deeper soil layers. The author suggests that, for the one-week withholding period to effectively improve geranium oil yield, certain deficit irrigation techniques (FAO, 2000) might have to be adopted to keep the subsoil as dry as possible, probably by applying shallower but more frequent irrigation during the regrowth period.

CHAPTER 6

RESPONSE OF ROSE-SCENTED GERANIUM LEAF PHYSIOLOGY AND MORPHOLOGY TO IRRIGATION FREQUENCY

6.1 ABSTRACT

Understanding physiomorphological responses of plants to water stress could be a base for developing suitable crop varieties and/or irrigation strategies for arid and semi-arid regions. Leaf morphological and physiological responses of rose-scented geranium (*Pelargonium capitatum x P. radens* cv. Rose*)* to irrigation frequency were investigated in a glasshouse study at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa. Daily, and every second, third, fourth and fifth day irrigation were applied as treatments. One week before harvesting, leaves were sampled for electron-microscopic observations. All plants were rewatered at the same time and irrigation was withheld for the last week prior to harvesting. Progressive physiological changes were recorded on a daily basis. Upon rewatering, stomatal conductance (G_s) and transpiration rate (R_t) were significantly lower in the less often irrigated than in the more often irrigated treatments, while leaf water potential (ψ_w) and relative water content (RWC) were the same for all plants. With progress in days of the irrigation withholding period, all the parameters in the more often irrigated treatments dropped at faster rates. Hence, at the end of the stress period, G_s, R_t, ψ_w and RWC were lower in the plants from the more often irrigated than from the less often irrigated treatments. Water stress reduced leaf size, and apparently increased trichome density, while the total number of trichomes per leaf remained more or less the same, indicating that total essential oil yield is mainly affected by leaf number. Stomatal closure was the main water stress avoidant/adaptation mechanism. These results imply that imposing certain water stress levels could enhance water-saving mechanisms and improve water-use efficiency of the crop.

Keywords: Irrigation withholding; leaf water potential; relative water content; rose-scented geranium; stomatal conductance; transpiration rate; trichomes

6.2 INTRODUCTION

Water stress is the most limiting factor in agricultural productivity in arid and semi-arid regions of the world (Chartzoulakis, Patakas, Kofidis, Bosabalidis & Nastou, 2002; Shi-wei *et al.*, 2006). Crop yield losses caused by water stress are estimated to exceed the total yield loss associated with other biotic and abiotic environmental factors (Boyer, 1985). To adapt to or avoid water stress, plant species make a series of physiological, biochemical and morphological adjustments (Chartzoulakis *et al.*, 2002; Lei, Tong & Shengyan, 2006).

Some of the common responses of plants species to water-stressed conditions are increased root depth (Singh & Singh 1995; Niu, Jiag, Wan, Liu, Gao & Li, 2005), reduced cell and leaf sizes, increased cell density (Bosabalidis & Kofidis, 2002; Martínez, Silva, Ledent & Pinto, 2007), and decreases in stomatal conductance, transpiration rate (Chartzoulakis *et al.*, 2002; Heschel & Riginos, 2005) and leaf water potential (Lei *et al.*, 2006). Some plant species also adapt to water stress environments by changing the levels of certain secondary metabolites (Bosabalidis & Kofidis, 2002).

Different plant species or genotypes may use different combinations of the above-mentioned water stress adaptation mechanisms (Wright & Smith, 1983; Gutschick, 1999). Singh and Singh (1995), for instance, reported that because of differences in root water absorbing capacity from the different soil depths, plant growth and yield of pearl millet, sorghum and maize varied with soil water status. In water-stressed conditions, maize extracted more water from the top 45 cm soil depth; sorghum was best in extracting water from soil profiles between 45 and 135 cm. Pearl millet, on the other hand, showed a tendency of taking the same amount of water from all soil layers in the root zone.

Rose-scented geranium (*Pelargonium* species) is an aromatic plant cultivated for its essential oil, which is mainly extracted from leaves by steam or water plus steam distillation-techniques (Rajeswara Rao *et al.*, 1996). Several reports indicated that essential oil yield of rose-scented geranium positively correlates to herbage yield (Rajeswara Rao *et al.*, 1996; Singh, 1999; Motsa *et al.*, 2006). Reducing vegetative growth as a water-stress-avoiding mechanism could be

counterproductive, as was observed in peppermint under severe osmotic stress conditions (Charles, Joly, & Simon, 1990), unless major trade-offs of physiological and morphological change, which would increase yield and/or quality, would take place. A report by Simon *et al*. (1992), for instance, indicated that chemical composition of essential oil of sweet basil was affected by soil water levels. There is also a general understanding that water-stressed conditions favour the production of plant secondary metabolites such as essential oils (Sangwan *et al.*, 2001).

Knowledge of leaf morphological and physiological response of essential oil crops such as rose-scented geranium (commonly cultivated for their herbage extracts) to soil water levels is limited. Studying leaf physiological and morphological responses associated with water stress could be helpful to avoid soil water levels that could result in irreversible damage to the crop. Such knowledge could also be an indispensable contribution to the process of developing irrigation protocols that would increase water productivity by encouraging building up of certain productive crop physiological and morphological adaptation mechanisms to water stress (Liang *et al.*, 2002). Hence, the main objective of these experiments was to examine rose-scented geranium leaf physiological and morphological changes associated with different irrigation levels.

6.3 MATERIALS AND METHODS

The leaf physiological and morphological data were collected from plants grown in glasshouses under five irrigation frequency treatments, namely daily (T1) , every second day (T2), every third day (T3), every fourth day (T4) and every fifth day (T5), followed by one week of withholding of irrigation prior to harvesting. Information on planting material, irrigation management and fertiliser application is presented in Chapter 5. On the morning of Day 0 of the one-week irrigation-withholding period all plants were rewatered and data collection started after three to four hours on the same day.

6.3.1 Data recorded

Leaf physiological data

Transpiration rate (R_t), stomatal conductance (G_s) and relative water content (RWC) were monitored on young fully opened leaves on a daily basis during the one-week irrigation-withholding periods. R_t and G_s were measured on the abaxial and adaxial sides of none-detached leaves while in their natural orientation, using an LI-1600 steady-state porometer (LI-COR, Inc. USA) and a leaf porometer (Decagon Device, Washington, USA). Leaf water potential was measured with a portable pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA, USA). To avoid water loss, the leaves were mounted to the pressure chamber within 30 seconds after they were detached from the mother plants. The pressure readings were taken when water film/meniscus started to appear on the incised petiole surface protruding from the pressure chamber lid (Lambers *et al.*, 1998).

Relative water content (RWC) was determined gravimetrically. Ten leaf discs of 1 cm^2 per replication were cut (from progressively stressed plants). After fresh mass was recorded, the leaf discs were floated in distilled water for about 12 hours in the dark to achieve full turgor. Excess water on leaf surface was removed with blotting paper, and the turgid mass for each disc was recorded. For the next 72 hours, the leaf discs were oven-dried at 70°C for dry mass determination. The RWC values were calculated using Equation 6.1 (Barrs & Weatherly, 1962).

$$RWC\ (\%) = \frac{Fresh\ leaf\ mass - Dry\ leaf\ mass}{Turgid\ leaf\ mass - Dry\ leaf\ mass} \times 100 \qquad (6.1)$$

Leaf morphological data

For the electron-microscopic leaf morphological study, fresh samples of about 1 x 1 cm^2 were cut with surgery blades and fixed in 3% (wt/v) aqueous solution of gluteraldehyde (in 0.05 M phosphate buffer, pH 7.0). After repeatedly being immersed in distilled water, the samples were

post-fixed in osmium tetraoxide (1% wt/v) for about two hours and dehydrated in a series of ethanol concentrations [30, 50, 70, 90 and twice 100% (wt/v) for 15 min each]. The samples were then dried in a critical point drying apparatus (Bio-Rad E300, Watford, England), mounted on aluminium stabs with double-sided adhesive tape, and coated with gold under a vacuum unit (Polaron E5200C, Watford, England). The specimens were examined under a JSM-840 scanning electron microscope (JEOL, Tokyo, Japan) at different magnifications, depending on the clarity of the targeted leaf appendage/surface. Stomatal and trichome counting and other measurements were done on digital photos obtained from a computer connected to the scanning electron microscope, by opening the saved photo files in Photoshop 7 Savvy (Sybex, San Francisco, USA), and making specific two-dimensional selections in accordance with the scanning electron-microscopic scales printed on the photos.

For light microscopic observations, samples (of 1x 1 cm^2) were fixed in FAA (formalin/acetic-acid/alcohol) for 24 hours. After dehydration in series concentrations of alcohol [once in 30, 50, 70, 90 and twice in 100% (v/v) ethanol for 24 hours each], the samples were immersed in a series of xylene concentrations [once in 30, 50, 70, 90 and twice in 100% (v/v) for 24 hours each]. Following embedding in paraffin wax, the samples were sectioned to about 8 µm using a rotary microtone (Reichert-Jung-2040, Reichert-Jung, Germany). The sections were mounted on glass slides, thereafter, stained in safranin and counterstained in Fast Green. Specimens were covered with cover glass over a film of transparent glue. Pictures were taken using an Olympus digital camera (Olympus SZX7, Olympus Optical Co. Ltd, Japan) fitted on a light microscope (Olympus SZX-TR30, Olympus Optical Co. Ltd, Japan).

6.4 RESULTS AND DISCUSSION

6.4.1 Leaf physiological response to water stress

Stomatal conductance

Results obtained during the one-week irrigation-withholding period revealed that irrigation frequency induced changes in stomatal behaviour (Figure 6.1). On Day 0 of the irrigation-withholding period the plants from the less often irrigated treatments (T4 and T5) had lower stomatal conductance rates than the plants in the more often irrigation treatments (T1 and T2).

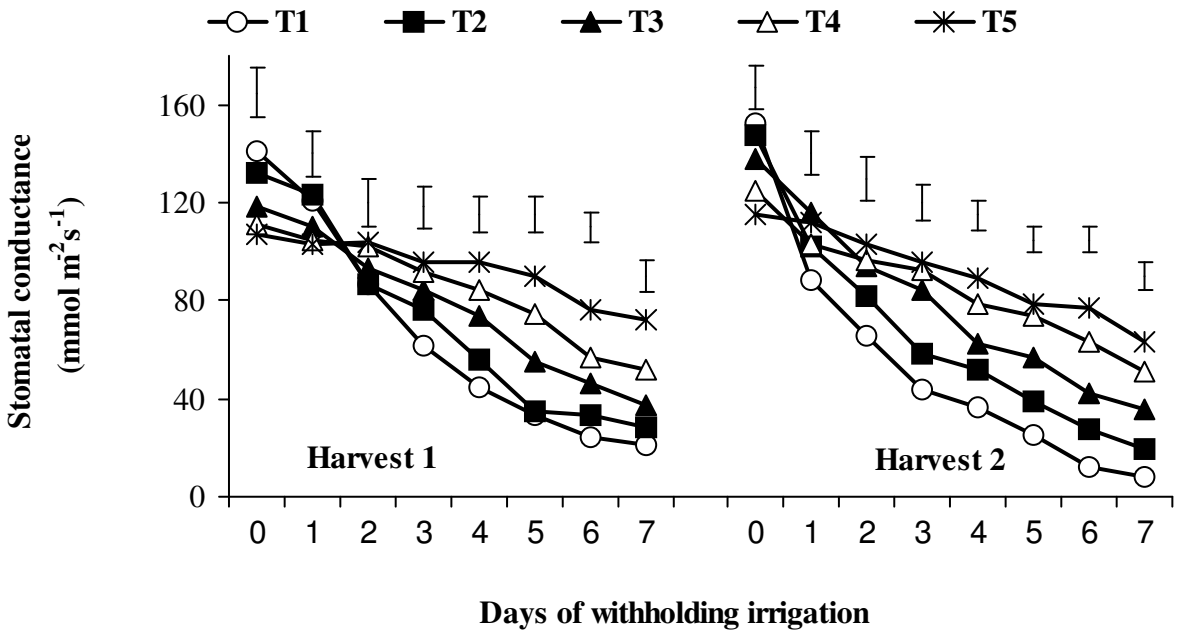

Figure 6.1: Effect of irrigation frequency on stomatal conductance of rose-scented geranium recorded during a one-week irrigation-withholding period. The vertical bars are LSD (at ∝ = 0.05); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth day irrigation, respectively

During the first and the second day of the irrigation-withholding period, the stomatal conductance of the plants from more often irrigated treatments declined at a higher rate. Hence, the stomatal conductance ranking order observed upon rewatering was reversed on Day 2 or 3 of the irrigation-withholding period. These results as a whole indicate that water stress induced long term changes in stomatal conductance as an adaptation mechanism. To a certain extent, the results agree with results reported by Liang *et al.* (2002), which showed that in wheat (*Triticum aestivum*) which was relieved from water stress, stomata reopened late. A recent report also characterises reduced stomatal conductance to be a main drought-avoidance mechanism used by *Medicago truncatula* cv. Jemalong plants (Nunes, Araújo, Silva, Fevereiro & Da Silva, 2008).

Transpiration rate

The response of transpiration rate of the plants from the different irrigation frequency treatments to water withholding was similar to that for stomatal conductance (Figure 6.2). At high soil water status (on Day 0 and Day 1 of the irrigation-withholding period), the plants from the high irrigation frequency (T1 and T2) lost water at a higher rate than those from the less frequent irrigation, while the reverse was true after Day 1 (in Harvest 1) or Day 3 (in Harvest 2).

The initial faster declining transpiration rate in T1 and T2 could be attributed to the higher soil drying rate, which resulted from initially higher stomatal conductance (Gutschick, 1999) accompanied by a large canopy size (Figure 6.3). Similarly, Xue, Zhu, Musick, Stewart and Dusek (2006) stated that higher soil water status/irrigation frequency increased evapo-transpiration rate in winter wheat.

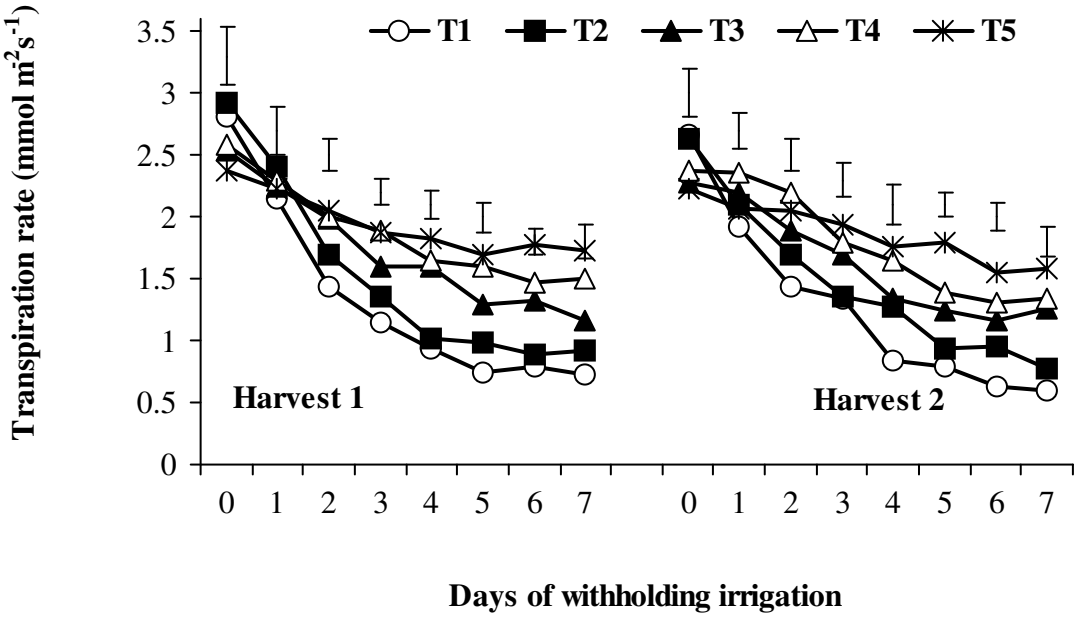

Figure 6.2: Effect of irrigation frequency on transpiration rate of rose-scented geranium leaves recorded during a one-week irrigation withholding period. The vertical bars are LSD (at ∝ = 0.05); Harvests 1 and 2 were conducted in June and October 2006, T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments, respectively

Figure 6.3: Rose-scented geranium canopy size as affected by irrigation frequency. Plants from every fifth day (A) and daily (B) irrigation treatments, in Harvest 2 (October 2006)

Relative leaf water content

The results presented in Figure 6.4 show that irrigation frequency did not have a significant effect on leaf water status on Day 0 of the irrigation-withholding period. All plants, regardless of how frequently they were irrigated, had the same relative water content (RWC). With a progress in the days of withholding irrigation, the RWC of the plants from the frequently irrigated treatments showed a faster declining tendency compared to that of the plants from the les often irrigated treatments. The overall results support the declining tendency in RWC with progress in soil depletion levels observed in sunflower (Pankovič, Sakač, Keveršan & Plesničar, 1999) and wheat (Liang *et al.*, 2002).

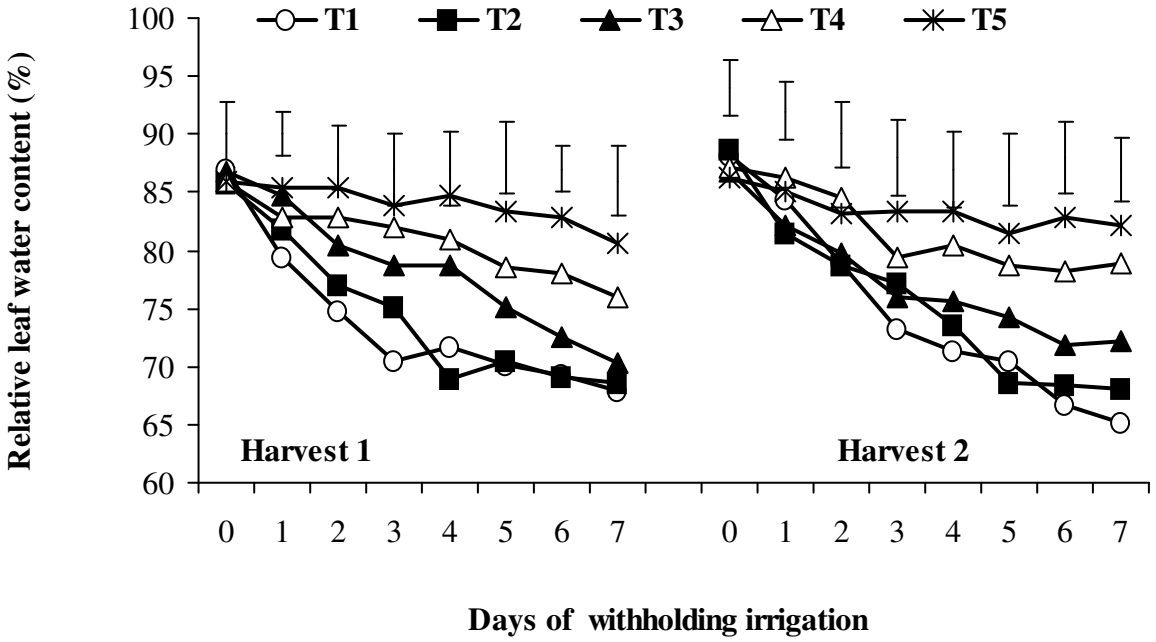

Figure 6.4: Effect of irrigation frequency on relative water content of rose-scented geranium leaves observed during a one-week irrigation-withholding period. The vertical bars are LSD (at ∝ = 0.05); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth day irrigation treatments, respectively

Leaf water potential

On Day 0 of the one-week irrigation-withholding period, the magnitudes of the leaf water potential (ψ_W) were the same for all the plants grown under the different irrigation frequencies (Figure 6.5), which could be an indication that irrigation frequency had no long-term effect on leaf water potential. These results are consistent with a previous report (Liang *et al.*, 2002), which stated that water-stress-relieved wheat plants managed to have the same ψ_W as that of control plants within a short time. Similar observations were also reported in avocado (Chartzoulakis *et al.*, 2002) and soybean (Lei *et al.*, 2006).

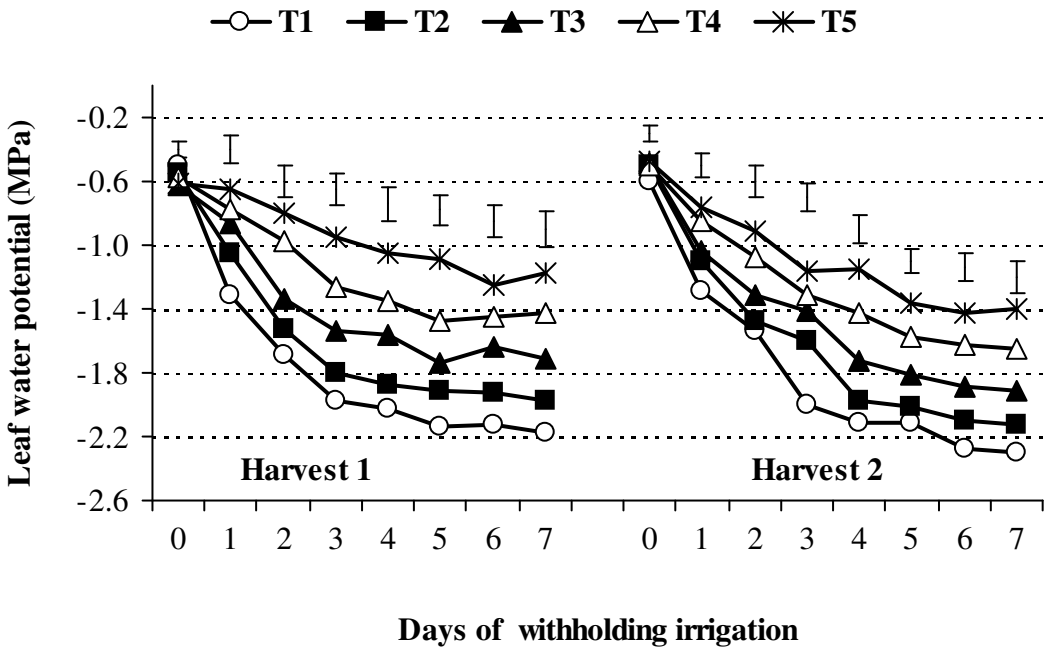

Figure 6.5: After-effect of irrigation frequency on leaf water potential of rose-scented geranium leaves recorded during a one-week irrigation-withholding period. The vertical bars are LSD (at ∝ = 0.05); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth day irrigation treatments, respectively

The data recorded on Day 1 show that the ψ_W started to decline in all treatments. The declining rate increased with increase in irrigation frequency. As a result, at the end of the water stress period (on the seventh day of withholding irrigation), the highest and lowest ψ_W were recorded for the T5 (every fifth day of irrigation) and T1 (every-day irrigation) treatments, respectively. In relation to that of the control treatment (T1), the mean improvements in ψ_W induced by the irrigation frequency in both harvests were 8.8, 19, 31, and 42% for T2, T3, T4 and T5, respectively.

The physiological data, as a whole, highlight that in water-stressed conditions the rose-scented geranium plants developed some water-saving mechanisms: water induced a long-term decline in stomatal conductance and transpiration rate, which enabled the plants to maintain higher relative water content and leaf water potential under prolonged water stress. These findings support the general understanding that certain deficit irrigation techniques could induce some physiological adjustments in plants that would contribute to boosting water productivity (Kirda, 2000).

6.4.2 Leaf morphological response to water stress

Trichome morphology

Regardless of irrigation treatments, two groups of glandular (different in shape and size) and one type of non-glandular trichomes were observed in both abaxial and adaxial surfaces of the leaves (Figure 6.6). The small glandular trichomes had nearly a columnar shape with a slightly bent terminal (apical) cell pointing towards the leaf tip.

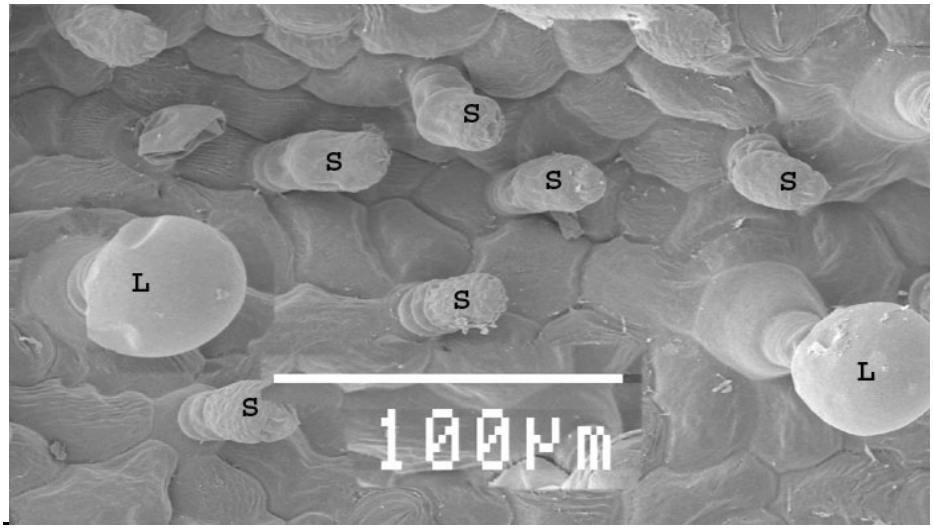

Figure 6.6: Large (L) and small (S) glandular trichomes on a leaf surface viewed under a scanning electron microscope

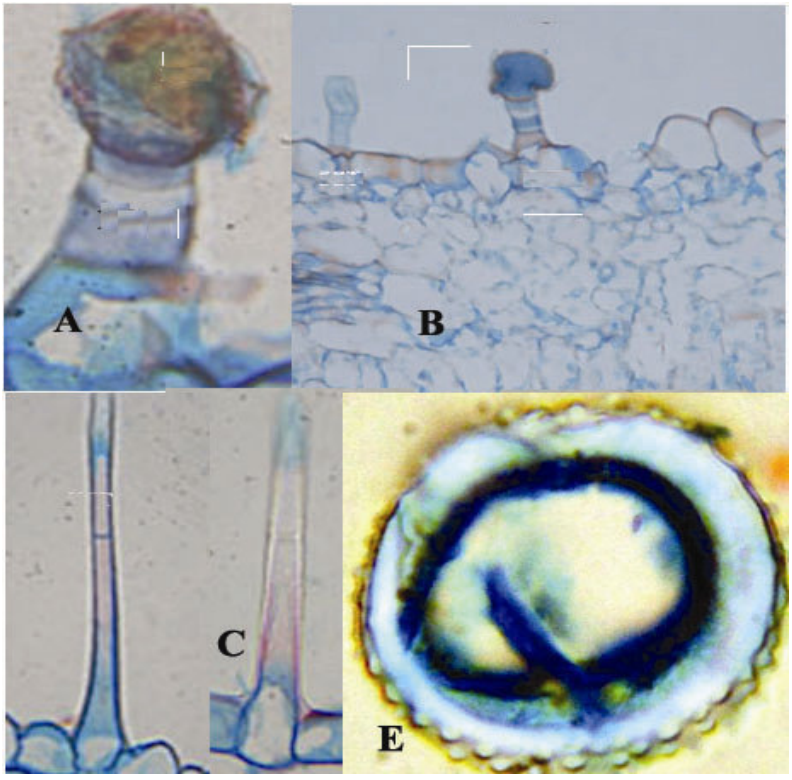

Figure 6.7: Leaf trichomes observed under a light microscope: (A) large glandular trichome, (B) large and small glandular trichomes, (C) non-glandular trichomes and (E) cross-sectional view of a non-glandular trichome

Both groups of glandular trichomes were morphologically of the peltate type, consisting of five cells, one basal, three stalk and one apical (head) cell (Figure 6.7), as previously reported for *Pelargonium scabrum* (Oosthuizen & Coetzee, 1983). It is not clear whether the two groups of glandular trichomes are different. The small trichomes could be miniature trichomes (the same as the larger glandular trichomes) but failed to attain full growth to secret and/or store essential oils, since the glandular cells looked as if they were shrivelled or lacking stored oil in their sub-cuticular spaces.

The two groups of trichomes may also be different types of glands as was described for *P. scabrum* (Oosthuizen & Coetzee, 1983) and P. *graveolens* and *P. radens* (Van der Walt & Dermane, 1988). *P. radens* is one of the parents of the cultivar used in the present investigations. The small glandular trichomes also look like the columnar glandular trichomes observed in leaves of *Cucurbit pepo* subspecies *pepo* var. Styrica (Kolb & Müller, 2004). Consistent with the previous reports (Turner *et al.*, 2000; Sharma, Sangwan & Sangwan, 2003), all the trichomes observed in the current investigation arose from a single epidermal cell (Figure 6.7 B and C).

On average, the diameter of fully expanded apical cells of the large glandular trichome was about 50 µm. Ruptured glandular trichome head cells show that the sub-cuticular space, in which essential oils are stored (Turner *et al.*, 2000; Werker, 2000), is relatively small (Figure 6.8 C and D). Most of the trichomes' globular heads are occupied with solid-like material, presumably the secretory cell (Werker, 2000).

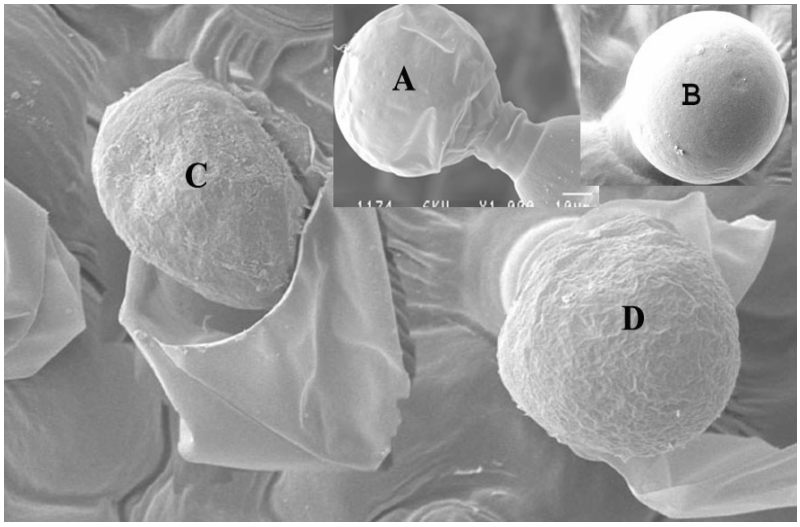

Figure 6.8: Morphology of glandular trichomes observed under a scanning electron microscope: (A) shrivelled, (B) fully expanded and (C and D) ruptured glandular trichomes

Figure 6.9 shows glandular trichomes and stomata on surfaces of an immature leaf (unopened, about seven mm in length) and a mature (open and fully expanded) leaf. Trichome growth remained uniform in size but the density was reduced in the mature leaf. This indicates that trichomes and stomata, which are in the same part of the leaf, were initiated simultaneously. The trichomes appeared fully developed and have their sub-cuticular space turgid (probably filled with oil) even before the leaf opens, whereas the stomata becomes functional later on (Figure 6.9 B and C) as mentioned by Werker (2000). The uniform size of glandular trichomes in the leaves of different age groups implies that there was no further formation of new glands in the later leaf expansion processes.

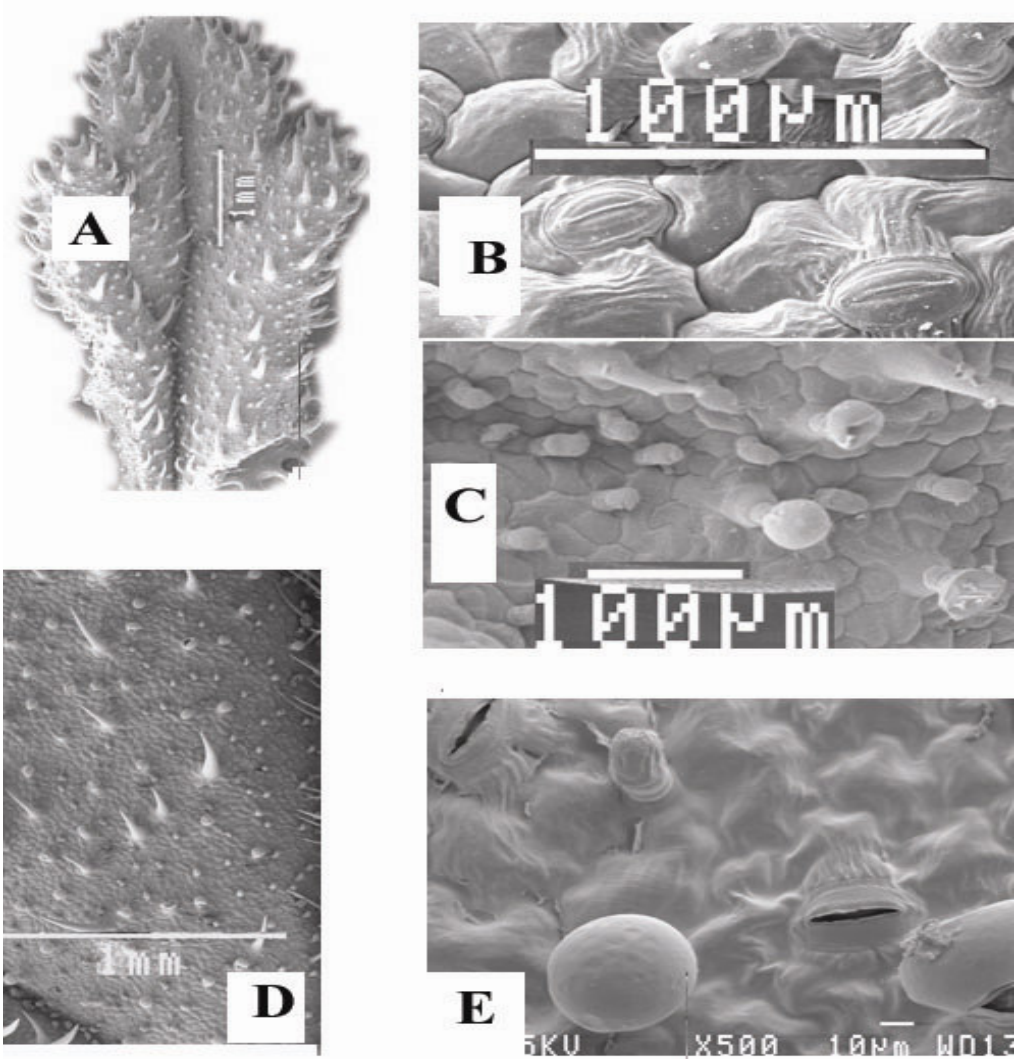

Figure 6.9: Glandular trichomes and stomata in immature leaves (A, B and C) and in mature leaves (D and E)

These observations support a previous report (Werker, Putievsky, Ravid, Dudai & Katzir, 1993), which indicated that trichomes were formed during early leaf formation in *Ocimum basilicum*. In addition, Werker (2000) suggested that formation of glandular trichomes takes place before cell multiplication ceases. Similarly, Valkama, Salminen, Koricheva and Pihlaja (2004) reported that final number of trichomes is reached some time during the early leaf developmental stage in *Betula* species. In contrasting to the above findings and views, formation of glandular trichomes in menthol mint was observed to be non-synchronous and happened throughout the leaf growth phases (Sharma *et al.*, 2003).

Stomatal and non-glandular trichome density as affected by irrigation frequency

In all leaf sections observed under the electron microscope, both leaf hair (non-glandular trichomes) and stomatal densities were higher in the abaxial than in the adaxial leaf surface (Table 6.2.). Irrigation treatments did not have a significant effect on stomatal density on the adaxial surface of the leaf.

Table 6.1: Non-glandular trichome and stomatal density from leaves of rose-scented geranium grown under different irrigation frequencies (for Harvest 2, October 2006)

Treatment	Stomatal number (per mm^2)		Leaf hair number (per mm^2)	
	Adaxial	Abaxial	Adaxial	Abaxial
T1	36.6 a[†]	101.8 b	5.3 c	28.3 b
T2	37.4 a	110.3 b	7.5 c	31.2 b
T3	43.5 a	119.3 ab	10.6 b	37.8 ab
T4	40.5 a	151.9 a	14.0 a	39.3 a
T5	42.0 a	149.6 a	15.3 a	38.8 a
Grand mean	40.0	126.4	10.6	35.1
CV (%)	20.0	22.2	21.0	17.4
LSD (α = 0.05)	NS	33.9	2.7	7.4

[†]**Values followed by the same letters in a column are not significantly different at 5% level of probability; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments**

On the abaxial leaf surface, significant increases in stomatal and non-glandular trichome densities were observed in the less often irrigated treatments (T4 and T5). The increase in non-glandular trichome density seems to be consistent with the general understanding that in water-stressed conditions, leaf hair density increases to minimise transpiration rate (Lambers *et al.*, 1998) and/or to reflect solar radiation, particularly the ultraviolet wavebands (Holmes & Keiller, 2002). The apparent increase in leaf hair and stomatal densities could be associated with a decrease in epidermal cell size, which could have led to an increase in cell density (Bosabalidis & Kofidis, 2002; Martínez *et al.*, 2007).

The increased stomatal density of rose-scented geranium in less often irrigated treatments is contrary to the behaviour normally observed in succulent plants when adapting to dry environments (Sayed, 1998). The present results indicate that in *Pelargonium* species, the most remarkable water stress adaptation mechanism was partial stomatal closure (Figure 6.10). A study by Bañon, Fernandez, Franco, Torrecillas, Alarcón and Sánchez-Blanco (2004) also revealed that water stress increases stomatal density in *Lotus creticus*.

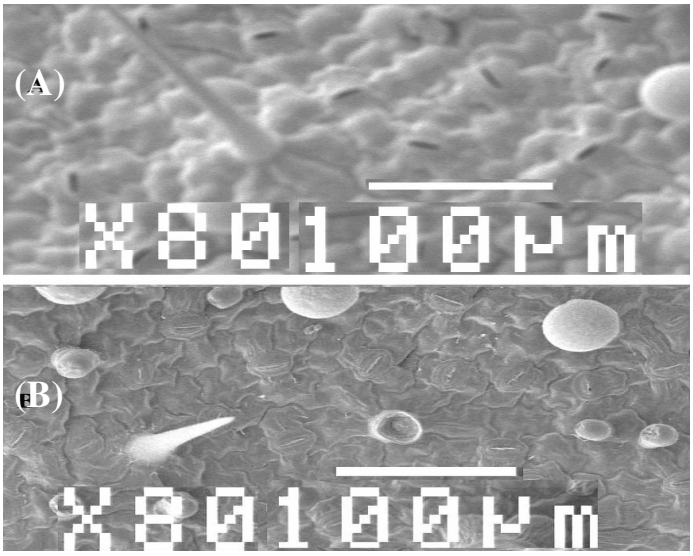

Figure 6.10: Effect of irrigation frequency on stomatal opening observed under a scanning electron microscope: (A) Leaves from the daily irrigated treatment (T1) open on Day 0 and (B) closed stomata on Day 7 of the one-week irrigation withholding period of Harvest 2 (October 2006)

These results also agree with research findings, which indicated that in water-stressed conditions, stomatal density increased but their apertures were reduced in olive cultivars (Bosabalidis & Kofidis, 2002). Niu *et al.* (2005) also described stomatal closure as the major drought-tolerance mechanism used by plant species in semi-arid sandland.

Glandular trichome density

Irrespective of the irrigation treatments, on both leaf surfaces the number of small glandular trichomes was higher than that of the large trichomes (Table 6.2). The abaxial leaf surface was the major site for glandular trichomes. The density of small glandular trichomes was negatively affected by irrigation frequency in both leaf surfaces. On the abaxial surface of the leaves, the density of the large glandular trichomes was significantly higher on the less frequently irrigated plants.

Table 6.2: Glandular trichome density on the abaxial and adaxial surfaces of rose-scented geranium leaves (for Harvest 2, October 2006)

Treatment	Adaxial (per mm^2)		Abaxial (per mm^2)	
	Large	Small	Large	Small
T1	6.5 a[†]	10.4 b	18.2 b	31.9 b
T2	7.8 a	13.7 b	20.0 b	36.5 ab
T3	9.3 a	16.2 ab	25.2 ab	43.0 a
T4	9.0 a	18.0 a	31.7 a	46.7 a
T5	9.7 a	19.3 a	32.4 a	46.3 a
Grand mean	8.0	15.5	25.5	40.9
CV (%)	27.0	18.3	24.0	21.1
LSD (α = 0.05)	NS	3.4	7.4	10.3

[†]**Values followed by the same letters in a column are not significantly different at 5% level of probability; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments**

The increase in glandular trichome density in the stressed treatments could have resulted from a decrease in epidermal cell size (Bosabalidis & Kofidis, 2002). After exploring the effects of environmental factors on leaf hair density, Roy, Stanton and Eppley (1999) suggested that unlike leaf area, trichome number per leaf is less sensitive to environmental stresses, implying that the apparent increase in trichome density observed in water-stressed conditions mainly arose from a reduction in leaf size. In agreement with these reports, in the current experiments

the size of individual leaves of rose-scented geranium appears reduced with a reduction in irrigation frequency (Table 6.3 & Figure 6.11). These findings highlight that leaf number is the major contributor to total essential yield per plant (hectare).

Table 6.3: Response of petiole and leaf length to irrigation frequency

Treatment	Harvest 1		Harvest 2	
	Petiole length (mm)	Leaf length (mm)	Petiole length (mm)	Leaf length (mm)
T1	137.3 a†	89.4 a	110.5 a	68.8 a
T2	127.9 b	91.7 a	100.8 b	64.3 ab
T3	104.2 c	78.7 b	92.0 bc	59.5 bc
T4	86.2 d	70.9 bc	84.0 cd	57.0 c
T5	83.8 d	68.1 c	82.0 d	54.5 c
Grand mean	107.9	79.8	93.9	60.8
CV (%)	5.7	8.9	8.6	6.9
LSD (α = 0.05)	7.3	8.6	9.7	5.1

†Values followed by the same letters in a column are not significantly different at 5% level of probability; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments; Harvests 1 and 2 were conducted in June and October 2006, respectively

Figure 6.11: Leaf size of rose-scented geranium as affected by irrigation frequency: Mature leaves sampled from daily (T1), and every second (T2), third (T3) fourth (T4) and fifth (T5) day irrigation treatments in Harvest 2 (October 2006)

6.5 CONCLUSIONS

The current study provides evidence that rose-scented geranium makes physiological and morphological modifications to avoid severe damage from water stress. Less often irrigation induced a long-term decline in stomatal conductance and transpiration rate, which enabled the plants to maintain higher relative water content and leaf water potential under prolonged water stress. Stomatal density apparently increased with a decrease in irrigation frequency implying that stomatal closure is a major water-loss-controlling mechanism in rose-scented geranium. Irrespective of the irrigation frequency, two types of glandular (small and large) and one non-glandular trichome groups were observed in both the adaxial and abaxial surfaces of the leaves. Both glandular trichome sizes showed a synchronised development, indicating that trichomes, at least in the same part of a leaf, are initiated or formed at the same time. The number of small glandular trichomes was higher than that of the large ones in both leaf sides. Trichome density apparently increased with a decrease in irrigation frequency, the opposite was true for leaf size. The trade-offs between leaf size and glandular trichome indicates that leaf number contributes more than leaf size to total essential oil yield.

CHAPTER 7

GENERAL DISCUSSION

Rose-scented geranium (*Pelargonium* species) is an aromatic plant cultivated for its essential oil, which is commonly used in the perfumery, aromatherapy and cosmetic industries. The demand for essential oils is on the increase with population growth and widening of the uses and preferences for the essential oil components. Recent studies indicate that rose-scented geranium oil could contribute to the food-processing (Lis-Balchin *et al.*, 1998; Lis-Balchin & Roth, 2000) and pharmaceutical industries (Dorman & Deans, 2000). The crop is commonly produced under rainfed agriculture and oil yield level per annum is low, 5 to 20 kg/ha (Weiss, 1997). Despite being the centre of origin and diversity for the *Pelargonium* species, the South African geranium oil industry's contribution to international markets is still low (Weiss, 1997), about 20 tons per year (R.A. Learmonth, personal communication).

The available information on the relationship between soil water level and production of secondary metabolites such as essential oils in plants appears contradictory. There is a general understanding that water-stressed conditions increase secondary metabolite production in plants (Yaniv & Palevitch, 1982; Sangwan *et al.*, 2001). Similarly, Weiss (1997) reported that a dry season resulted in a mild increase in rose-scented geranium essential oil yield. Other studies on rose-scented geranium (Rajeswara Rao *et al.*, 1996; Singh, 1999), on the other hand, indicated that irrigation/high soil water improved essential oil yield. Hence, it was hypothesised that rose-scented geranium essential oil yield could be improved though introducing innovative irrigation practices in arid and semi-arid regions such as found in South Africa. The general approach of the study was to grow the crop under different irrigation managements, which would bring certain physiological changes in favour of essential oil yield and/or quality, at the same time increasing productivity of the scarce freshwater resources.

Rose-scented geranium was grown under different maximum allowable depletion (MAD) levels of plant available soil water (Chapter 3). Relatively little water depletion from the root zone between 0.4 and 0.8 m depth indicated that water uptake by the rose-scented geranium root

system was almost limited to the top 0.4 m soil layer. Increasing the soil water depletion level to 60% of the plant available soil water (ASW) and higher resulted in a significant reduction in herbage mass and essential oil yield. An increase in MAD level apparently increased the essential oil concentration (percentage oil on fresh herbage mass basis), but its contribution to total essential oil yield (kg/ha oil) was limited. Up to 28% of irrigation water could be saved by increasing maximum allowable depletion level of ASW from 20 to 40%, without a significant reduction in essential oil yield.

The MAD treatments did not bring significant variations in essential oil composition. Remarkable differences in oil composition among harvests seem to be related to plant age (starting from transplanting date) or the season in which regrowth cycles took place, since the seasonal temperatures were in the order of Harvest 1< Harvest 2 < Harvest 3. It could be realised that combined geraniol and geranyl format contents were negatively correlated to the combined citronellol and citronellyl formate contents (R^2 = 0.75) in the essential oil extracted by the steam-distillation method.

A one-month irrigation-withholding period at different shoot ages (Chapter 4) demonstrated that a significant decline in herbage yield occurs when a water stress period was imposed during the third or fourth month of the regrowth cycles. Essential oil yield was, on the other hand, reduced remarkably only when water stress was imposed during the fourth month of regrowth. The results imply that at certain water stress levels, there could be a trade-off between vegetative growth and essential oil yield (Weiss, 1997), or else essential oil yield has a higher tolerance to water stress. The tendency of essential oil yield to reduce with a decrease in herbage mass confirms that primary and secondary metabolites are positively related (Letchamo *et al.*, 1995; Rajeswara Rao, 2002). With a marginal oil yield loss, it was possible to save about 330 to 460 m^3/ha of irrigation water by withholding irrigation during the third month of each regrowth cycle. The overall results highlight that in water-scarce regions, withholding irrigation either during the second or the third month of regrowth in rose-scented geranium could improve water productivity.

Responses of herbage and essential oil yields, and essential oil composition to irrigation frequency and a one-week irrigation-withholding period were investigated in semi-controlled greenhouses (Chapter 5). Essential oil content (percentage oil on fresh herbage mass basis) apparently increased with a decrease in irrigation frequency. Total essential oil yield/ha, however, increased with an increase in herbage yield and irrigation frequency, as was observed in the MAD level of ASW and the one-month irrigation-withholding trials.

Unlike the results recorded for the long-term water stress treatments (irrigation frequency, MAD level and a one-month irrigation withholding period), an increase in essential oil content induced by the one-week irrigation withholding period was high enough to improve essential oil yield per plant. The overall results show that the combination of a high irrigation frequency and a terminal one-week irrigation-withholding period could improve essential oil yield.

The one-week water-withholding period did not affect essential oil composition. Irrigation frequency, on the other hand, affected citronellol, citronellyl formate, and geraniol and geranyl formate levels. The levels of citronellol and its ester (citronellyl formate) consistently increased with a decrease in irrigation frequency, while the opposite was true for the levels of geraniol and its ester (geranyl formate). These results are consistent with the results obtained from the MAD trial in the different harvests. Such a relationship was also true for the one-month irrigation-withholding trial, particularly in Harvest 2 (Figure 4.7B), although in opposite directions, e.g. in the irrigation frequency trials high soil water favoured geraniol and geranyl format levels, while the reverse was true for the one-month irrigation-withholding trials.

The physiological and morphological studies (Chapter 6) revealed that rose-scented geranium adapts to or avoids water stress by making certain changes in leaf physiology and morphology. In these studies, stomatal conductance (G_s), transpiration rate (R_t), leaf water potential (ψ_w) and relative water content (RWC) of plants grown under different irrigation frequencies were investigated during a one-week irrigation-withholding period prior to harvesting. Data recorded on Day 0 (upon rewatering) show that irrigation frequency had an after-effect on G_s and R_t because the magnitudes of these parameters were significantly lower in the plants that were less irrigated compared to the more frequently irrigated plants. Leaf water potential and RWC were

the same for all plants regardless of the irrigation level. With a progress in days of the irrigation-withholding period, all the parameters in the more frequently irrigated treatments declined at higher rates.

The lower G_s, R_t,, ψ_w and RWC in the plants from the more frequently irrigated than the less frequently irrigated treatments at the end of the stress period, highlight that by reducing stomatal conductance, the plants from less irrigated plants managed to minimise transpirational water loss. Thus, they maintained their ψ_w and RWC at higher levels for a longer period in the course of the irrigation-withholding period. From such observation, it could be concluded that stomatal conductance is the main physiological mechanism used by rose-scented geranium to adapt to or avoid water stress conditions. Similar results were also reported for *Triticum aestivum* (Liang *et al.*, 2002) and *Medicago truncatula* (Nunes *et al.*, 2008).

Microscopic observations reveal that irrespective of the irrigation frequency, one type of non-glandular and two types (slightly different in shape and remarkably different in size) of glandular trichomes were observed. Uniform growth and a declining tendency in the density of the glandular trichomes with an advance in leaf expansion imply that new trichome formation stopped with epidermal cell specialisation/division at an early leaf developmental stage. Such observations are consistent with those reported for *Ocimum basilicum* (Werker *et al.*, 1993) and *Brich* species (Valkama, Salminen, Koricheva & Pihlaja, 2003). These findings infer that the total number of glandular trichomes per plant is determined by the total number of leaves, and not by leaf size.

The impacts of the different irrigation treatments considered in the current trials (discussed in Chapters 3, 4, and 5) on water-use efficiency (WUE) were not consistent, and probably affected by seasonal differences (Saeed & El-Nadi, 1997). In the glasshouse trials (Chapter 5), for instance, the highest WUE was recorded for the T2 and T3 treatments in Harvest 1, a growth cycle during the cool season. This result supports the results that indicate that WUE was improved by certain soil water stress conditions in potatoes (Onder, Caliskan, Onder & Caliskan, 2005; Zhang *et al.*, 2006) and in sesame (Uçan, Killi, Gençoğlan & Merdun, 2007). In warm to hot seasons (Harvest 2), on the other hand, WUE increased with an increase in soil

water level, proving the suggestions made by Bessembinder *et al.* (2005) that WUE increases with an increase in soil water level, provided that other environmental factors are in the required range for optimum plant growth.

The results of the water management trials, as a whole, indicate that irrigation increases essential oil yield by boosting vegetative growth. For water saving without a significant reduction in essential oil yield, 40% depletion of the available soil water could be allowed. Withholding irrigation for one month between the 30th and the 90th day of regrowth cycles could also have similar water-saving and possibly energy and labour cost minimising advantages. It is, however, advisable to avoid severe water stress in the fourth month of a regrowth cycle.

At field level, soil water had no remarkable impact on essential oil composition. Despite the fluctuations observed, the citronellol to geraniol ratio (C:G ratio) were relatively high (ranged between 1.9 and 16). Similarly, in data recorded for the same cultivar grown under different nitrogen levels, the lowest and highest C:G ratios were 2.4 and 6.8, respectively (Araya *et al.*, 2006). This slightly contradicts the general understanding that South African geraniol oil is comparable to the geranium oil produced on Réunion Island, namely the Bourbon-type oil (SANDA, 2006), with a C:G ratio of one or close to one (Gupta *et al*, 2001b; Gauvin *et al.*, 2004). According to Rodolfo, Koroch, Simon, Hitimana, Daka, Ranarivelo and Langenhoven, (2006), South African geranium oil shares similar characteristics with Chinese geranium oil, particularly for the high citronellol and cironellyl formate contents.

From a perfumery point of view, essential oil of this cultivar will not be among the most preferred quality, because of the high C:G ratio. However, C:G ratio may not affect market value for geranium oil in the future because promising results have been discovered in the field of pharmacology (Dorman & Deans, 2000; Deans, 2002) and food-processing industries (Lis-Balchin *et al.*, 1998; Lis-Balchin & Roth, 2000).

The herbage and essential oil yields obtained from the rain shelter were consistently higher than those from the open field of regrowths that experienced similar climatic conditions [e.g.

Harvest 4 from the open field versus Harvest 2 from the rain shelter (Chapter 3) and Harvest 2 from the open field versus Harvest 4 from the rain shelter (Chapter 4)]. These differences, at least partly, resulted from the difference in plant density (16 000 and 30 000 plants/ha in the open field and rain shelter, respectively). These results are consistent with results reported by Rajeswara Rao (2002), which indicated that herbage and essential oil yield consistently increased with increase plant density. The current results together with findings in literature indicate that optimizing the agronomic practices such as plant density, nutrient supply (Araya *et al*., 2006) and cultivar selection (Gupta *et al*., 2001b) could enhance oil yield and/or quality, thereby improve water productivity.

This investigation should be followed up by a study that could explain the controversial results obtained from the one-month (Chapter 4) and one-week (Chapter 5) irrigation-withholding trials. Such investigation could help to develop certain irrigation practices by which the high yield at pot level could be achieved in the field.

In addition, results obtained from the trials in the field and in the greenhouse show that responses of geranium oil composition to irrigation level were affected by other factors, most probably by temperature. Irrigation had a clear impact on the greenhouses where temperature was partly regulated, while in the open field (with high diurnal temperature fluctuation), essential oil composition did not respond to soil water levels. Hence, studying the combined effect of temperature and soil water level under controlled conditions could be helpful for further geranium oil quality-improving efforts.

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APPENDIX A

SOIL AND WEATHER DATA FOR THE EXPERIMENTAL SITES

1. SOIL DATA

Table A1: Soil chemical properties of the top 30 cm soil depths of the sites

Parameter	Open field	Rain shelter
Electrical resistance (Saturation paste method) (Ohm)	2000.0	2200.0
pH (1:2.5 soil: water)	5.1	5.2
Iron (Fe) (EDTA extraction method) (mg/kg)	71.1	67.2
NH_4^+ (1:2 soil :1M KCl) (mg/kg)	4.0	3.5
NO_3^- ((50 g soil :100 ml 1M KCl) (mg/kg)	6.1	5.3
Phosphorus (P) (Bray I method) (mg/kg)	28.2	29.0
Potassium (K) (Ammonium acetate extractable) (mg/kg)	73.0	65.0
Sodium (Na) (Ammonium acetate extractable) (mg/kg)	31.0	21.0
Calcium (Ca) (Ammonium acetate extractable) (mg/kg)	190.0	186.0
Magnesium (Mg) (Ammonium acetate extractable) (mg/kg)	42.0	44.0

Table A2: Physical properties for the different soil layers of the experimental sites

Experimental	Soil depth	Particle size distribution (%)			Bulk density	Water content (m^3/m^3)	
	(cm)	Clay	Silt	Sand	(Mg/m^3)	Field capacity	Permanent wilting point
Open field	0-20	20.1	13.1	66.8	1.42	0.202	0.102
	20-40	24.2	12.5	63.3	1.64	0.251	0.110
	40-60	26.6	10.3	63.1	1.48	0.240	0.119
	60-80	28.4	14.5	57.1	1.49	0.267	0.130
Rain shelter	0-20	23.2	10.3	56.5	1.45	0.261	0.130
	20-40	28.2	11.3	60.5	1.61	0.298	0.150
	40-60	27.1	13.2	59.7	1.43	0.270	0.140
	60-80	26.5	12.2	61.3	1.49	0.268	0.142

2. WEATHER DATA

Table 1: Mean monthly maximum and minimum temperatures, and total rainfall recorded during regrowth cycles for the maxim allowable soil water depletion trials

	----------Open field trials----------									
Parameters	Harvest 1					Harvest 2				
	12 Mar- 11 July 2005					14 May-13 Sep 2005				
Months (part of a month)	Mar†	Apr	May	June	July	May	June	July	Aug	Sep
Maximum temperature (°C)	25.5	23.4	24.3	23.2	20.8	24.2	23.2	22.7	24.8	28.9
Minimum temperature (°C)	14.6	12.7	8.6	6.0	4.8	7.5	6.0	5.0	9.4	11.6
Rainfall (mm)	9.3	7.2	0.2	0.1	0.0	0.2	0.1	0.0	1.4	0.0
Maximum RH (%)	98.4	99.6	87.9	77.9	75.5	87.9	77.9	75.5	75.8	63.6
Minimum RH (%)	52.0	66.3	30.3	22.4	19.4	30.3	22.4	19.4	23.5	14.9
Parameters	Harvest 3					Harvest 4				
	14 Sept- 13 Jan 2006					26 June-25 Oct 2006				
Month (part of month)	Sep	Oct	Nov	Dec	Jan	June	July	Aug	Sep	Oct
Maximum temperature (°C)	30.2	30.3	29.5	29.4	27.4	16.9	22.2	21.3	25.8	29.6
Minimum temperature (°C)	12.3	14.2	15.3	15.5	17.5	2.4	5.4	6.0	9.1	14.3
Rainfall (mm)	0.0	15.2	79.2	65.4	95.7	0.0	0.0	36.1	0.5	9.0
Maximum RH (%)	70.2	76.2	85.1	86.0	97.1	82.3	78.8	79.6	70.2	76.2
Minimum RH (%)	18.1	21.6	35.7	32.0	60.4	23.6	22.2	26.2	18.1	21.6
Parameter for	----------Rain shelter trials----------									
	Harvest 1					Harvest 2				
	27 Feb-26 June 2006					27 June-26 Oct 2006				
Month (part of a month)	Feb	Mar	Apr	May	June	June	July	Aug	Sep	Oct
Maximum temperature (°C)	21.8	26.0	24.9	21.2	22.3	15.5	22.2	21.3	25.8	29.8
Minimum temperature (°C)	16.9	14.7	11.2	5.5	4.7	2.7	5.4	6.0	9.1	14.4
Rainfall (mm)	62.8	103.9	30.2	2.1	0.0	0.0	0.0	36.1	0.5	9.9
Maximum RH (%)	96.2	92.7	92.8	86.4	82.3	82.3	78.8	79.6	70.2	76.2
Minimum RH (%)	51.5	45.7	39.1	27.2	23.6	23.6	22.2	26.2	18.1	21.6

†**Data include only the part of the month within the specific regrowth period; For Harvest 4 (rain shelter) the rain was out-screened; For the trials in the rain shelter the rain was out-screened; RH resents relative humidity**

Table 2 Mean monthly maximum and minimum temperatures and total rainfall recorded during the regrowth cycles for the one-month irrigation withholding trials

Parameters	Harvest 1					Harvest 2				
	03 June- 02 Oct 2005					03 Oct 2005-01 Feb 2006				
Months (part of a month)	June†	July	Aug	Sep	Oct	Oct	Nov	Dec	Jan	Feb
Maximum temperature (°C)	23.0	22.7	24.8	29.6	26.7	30.5	29.5	29.4	27.3	27.1
Minimum temperature (°C)	6.0	5.0	9.4	12.1	9.1	14.6	15.3	15.5	17.7	16.9
Rainfall (mm)	0.1	0.0	1.4	0.2	0.0	15.2	79.2	65.4	248.0	0.0
Maximum RH (%)	77.9	75.5	75.3	63.6	73.6	73.6	82.9	30.2	97.1	96.2
Minimum RH (%)	22.4	19.4	23.5	14.9	20.1	20.1	28.0	87.6	60.4	51.5
Parameters	Harvest 3					Harvest 4				
	12 July- 11 Nov 2006					27 Oct 2006-26 Feb 2007				
Month (part of month)	July	Aug	Sep	Oct	Nov	Oct	Nov	Dec	Jan	Feb
Maximum temperature (°C)	22.9	21.3	25.8	29.7	28.4	28.7	27.8	27.8	31.0	32.6
Minimum temperature (°C)	6.3	6.0	9.1	14.6	14.1	15.7	14.8	14.8	15.8	16.3
Rainfall (mm)	0.0	3.6	0.5	19.0	10.1	9.0	91.6	117.2	56.4	38.3
Maximum RH (%)	78.8	79.6	70.2	76.2	85.1	76.2	85.1	86.0	86.8	79.8
Minimum RH (%)	22.2	26.2	18.1	21.6	35.7	21.6	35.7	32.09	27.8	21.3

†**Data include only the part of the month within the specific regrowth period; For Harvest 4 (rain shelter) the rain was out-screened; RH represents relative humidity**

Table A3: Average minimum and maximum temperatures and radiant energy inside and outside of the tunnel and glasshouse during each regrowth period

Growth system	Harvest†	Minimum (°C)		Maximum (°C)		Light energy ($MJm^{-2}d^{-1}$)	
		Inside	Outside	Inside	Outside	Inside	Outside
Tunnel	Harvest 1	9.1	8.1	20.2	24.8	10.2	15.7
	Harvest 2	18.2	15.1	33.4	26.4	10.3	15.9
	Harvest 3	10.4	4.6	26.4	21.4	8.3	12.8
	Harvest 4	19.3	14.6	34.2	29.1	11.6	17.9
Glasshouse	Harvest 1	12.4	10.0	26.3	19.3	12.8	20.7
	Harvest 2	19.2	9.2	33.2	22.4	13.8	21.3

†**Harvests 1, 2, and 3 in the tunnel were conducted in September 2005, and April, August and December 2006, respectively; Harvests 1 and 2 in the glasshouse were done in June and October 2006, respectively**

APPENDIX B

NEUTRON PROBE CALIBRATION PROCESSES

1. OPEN-FIELD TRIALS

Table B1: Neutron probe calibration data the open-field trials (maximum allowable depletion and the one-month irrigation-withholding experiments)

Spot	Soil layer (m)	Neutron probe reading from soil		Standard reading in air	Standard ratio		Gravimetric soil water content		Average oil bulk density (Mg/m^3)	Volumetric soil water content (m3/m3)		
		Spot 1	Spot 2		Spot 1	Spot 2	Spot 1	Spot 2		Spot 1	Spot 2	average
Wet	0.0-0.2	16311	15861	10611	1.537	1.495	0.151	0.134	1.42	0.214	0.190	0.202
	0.2-0.4	16053	17209	10611	1.513	1.522	0.146	0.164	1.62	0.237	0.265	0.251
	0.4-0.6	16262	17835	10611	1.533	1.881	0.134	0.190	1.48	0.199	0.281	0.240
	0.6-0.8	17527	16587	10611	1.708	1.493	0.189	0.170	1.49	0.281	0.253	0.267
	0.8-1.0	18682	17475	10611	1.811	1.647	0.219	0.174	1.39	0.304	0.242	0.273
	1.0-1.2	19567	18477	10611	1.874	1.701	0.209	0.187	1.42	0.297	0.265	0.281
Dry	0.0-0.2	6228	7340	10748	0.58	0.683	0.083	0.072	1.42	0.119	0.102	0.110
	0.2-0.4	13623	12451	10748	1.27	1.158	0.124	0.110	1.62	0.201	0.178	0.190
	0.4-0.6	15048	13482	10748	1.65	1.354	0.146	0.127	1.48	0.215	0.187	0.201
	0.6-0.8	15807	14739	10748	1.47	1.371	0.157	0.161	1.49	0.234	0.240	0.237
	0.8-1.0	16232	16958	10748	1.67	1.578	0.190	0.176	1.39	0.265	0.244	0.254
	1.0-1.2	16807	16043	10748	1.53	1.493	0.171	0.181	1.42	0.243	0.257	0.250

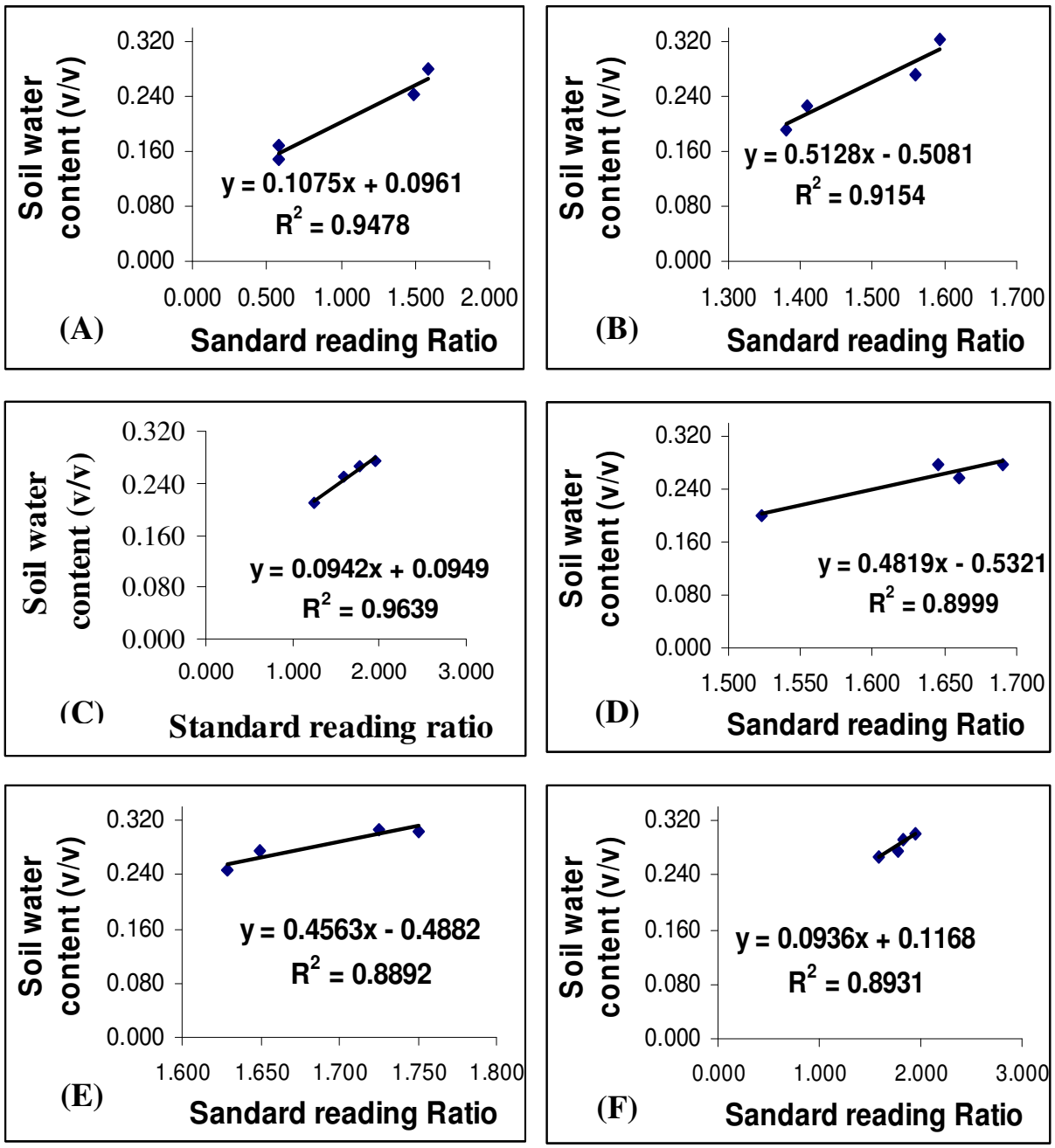

Figure B1: Regression equations for the 0-20 cm (A), 20-40 cm (B), 40-60 cm (C), 60-80 cm (D), 80-100 cm (E) and 100-120 cm (F) soil layers (for the open-field trials)

2. RAIN-SHELTER TRIALS

Table B2: Neutron probe calibration data for the rain-shelter (maximum allowable depletion and the one-month irrigation-withholding experiments)

Spot	Soil layer (m)	Neutron probe reading from soil		Probe reading in air	Standard ratio		Gravimetric soil water content		Average oil bulk density (g/cm3)	Volumetric soil water content (m3/m3)		
		Spot 1	Spot 2		Spot 1	Spot 2	Spot 1	Spot 2		Spot 1	Spot 2	average
Wet	0.0-0.2	16864	15787	10595	1.592	1.490	0.193	0.167	1.45	0.280	0.242	0.2610
	0.2-0.4	17369	16528	10595	1.593	1.560	0.201	0.169	1.61	0.324	0.272	0.2980
	0.4-0.6	17796	18672	10595	1.963	1.762	0.192	0.186	1.43	0.274	0.266	0.270
	0.6-0.8	17909	17588	10595	1.690	1.660	0.187	0.173	1.49	0.278	0.258	0.2680
	0.8-1.0	18550	16846	10595	1.751	1.649	0.188	0.169	1.62	0.304	0.274	0.2890
	1.0-1.2	19352	20660	10595	1.826	1.950	0.203	0.210	1.43	0.291	0.301	0.2960
Dry	0.0-0.2	6228	6145	10934	0.588	0.580	0.103	0.117	1.45	0.1495	0.1695	0.1595
	0.2-0.4	14622	15243	10934	1.380	1.409	0.119	0.141	1.61	0.192	0.2266	0.2093
	0.4-0.6	15348	16839	10934	1.249	1.589	0.146	0.174	1.43	0.2088	0.2488	0.2288
	0.6-0.8	16137	17430	10934	1.523	1.645	0.134	0.186	1.49	0.199	0.2778	0.2384
	0.8-1.0	16832	18283	10934	1.629	1.726	0.151	0.189	1.62	0.2454	0.3054	0.2754
	1.0-1.2	16806	18808	10934	1.586	1.775	0.187	0.193	1.43	0.2677	0.2757	0.2717

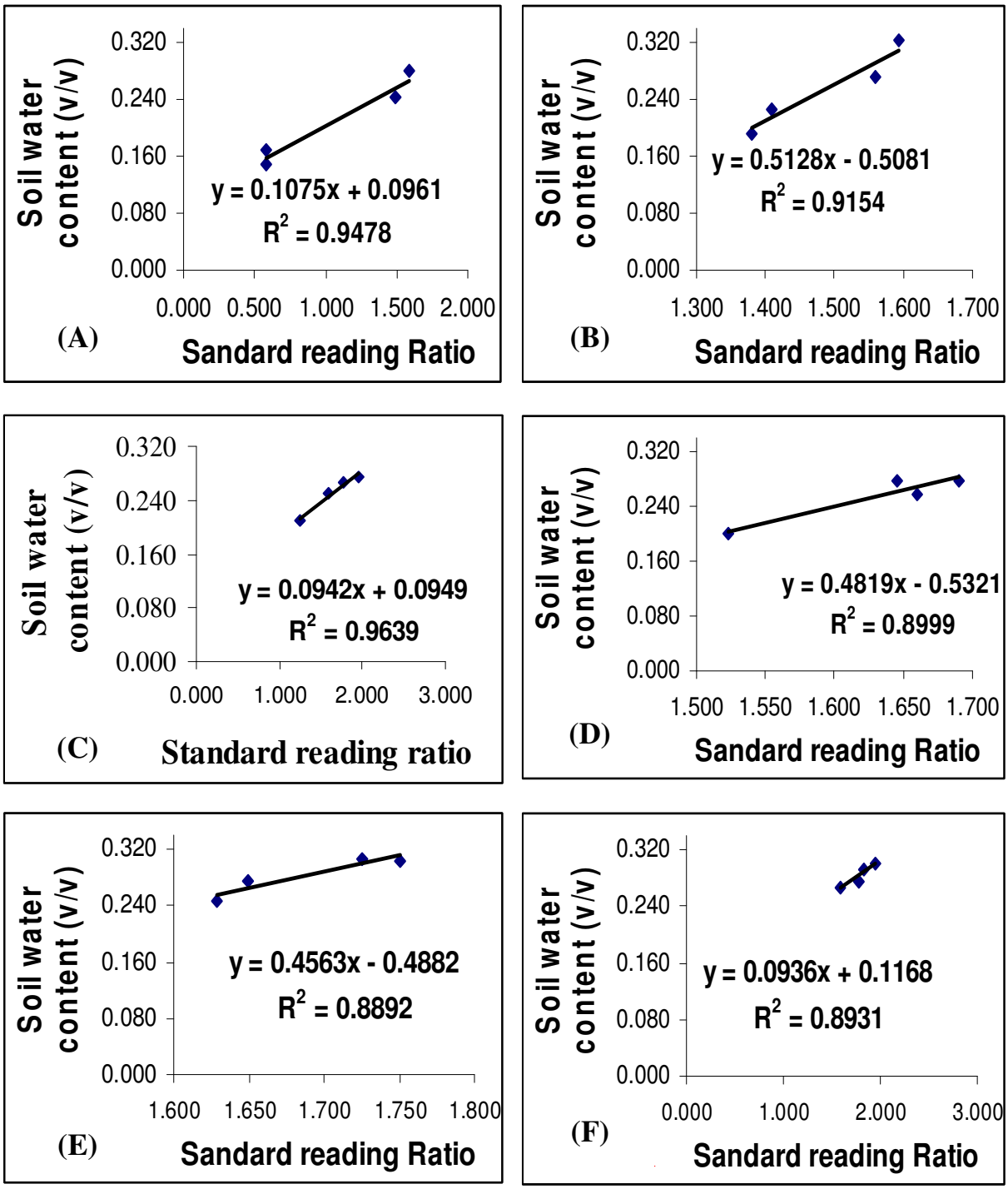

Figure B2: Regression equations for the 0-20 cm (A), 20-40 cm (B), 40-60 cm (C), 60-80 cm (D), 80-100 cm (E) and 100-120 cm (F) soil layers (for the rain-shelter trials)

APPENDIX C

SUMMARISED ANALYSIS OF VARIANCE (ANOVA) TABLES

Table C1: Summary of ANOVA table for the maximum allowable soil water depletion experiments (open-field trial)

Harvest	Source of variation	Degree of freedom	†F-probability levels for							
			Fresh yield	Dry yield	Leaf area	Leaf fresh proportion (%)	Dry matter content		Oil yield	Oil content
							Leaf	Stem		
Harvest 1 (July 2005)	Block	3	0.458^{NS}	0.128^{NS}	0.709^{NS}	0.753^{NS}	0.649^{NS}	0.503^{NS}	0.565^{NS}	0.305^{NS}
	Treatment	3	0.017*	0.009*	0.005*	0.000*	0.000**	0.000**	0.008**	0.035*
	Error	9	-	-	-		-	-	-	-
Harvest 2 (September 2005)	Block	3	0.691^{NS}	0.649^{NS}	0.632^{NS}	0.80^{NS}	0.792^{NS}	0.503	0.665^{NS}	0.142^{NS}
	Treatment	3	0.000**	0.001**	0.001**	0.000**	0.002**	0.000**	0.000**	0.033*
	Error	9	-	-	-		-	-	-	-
Harvest 3 (January 2006)	Block	3	0.746^{NS}	0.115^{NS}	0.837^{NS}	0.0926^{NS}	0.814^{NS}	0.880^{NS}	0.417^{NS}	0.925^{NS}
	Treatment	3	0.000**	0.001**	0.000**	0.000**	0.021*	0.014*	0.000**	0.069^{NS}
	Error	9	-	-	-		-	-	-	-
Harvest 4 (October 2006)	Block	3	0.103^{NS}	0.853^{NS}	0.072^{NS}	0.140^{NS}	0.765^{NS}	0.453^{NS}	0.0177*	0.105^{NS}
	Treatment	3	0.000**	0.000**	0.000**	0.001**	0.000**	0.001**	0.000**	0.004**
	Error	9	-	-	-		-	-	-	-

†**Not significant (NS), significant at α = 0.05 (*) and significant at α = 0.01 (**)**

Table C2: Summary of ANOVA table for the maximum allowable soil water depletion experiments (rain-shelter trial)

Harvest	Source of variation	Degree of freedom	†F-probability levels for							
			Fresh yield	Dry yield	Leaf area	Leaf fresh proportion (%)	Dry matter content		Oil yield	Oil content
							Leaf	Stem		
Harvest 1 (July 2005)	Block	3	0.103 ^{NS}	0.410 ^{NS}	0.058 ^{NS}	0.192 ^{NS}	0.985 ^{NS}	0.525 ^{NS}	0.112 ^{NS}	0.276 ^{NS}
	Treatment	3	0.000**	0.002**	0.000**	0.000**	0.000**	0.000**	0.002**	0.003**
	Error	9	-	-	-		-	-	-	-
Harvest 2 (September 2005)	Block	3	0.269 ^{NS}	0.123 ^{NS}	0.219 ^{NS}	0.067 ^{NS}	0.525 ^{NS}	0.985 ^{NS}	0.274 ^{NS}	0.091 ^{NS}
	Treatment	3	0.000**	0.000**	0.000**	0.000**	0.0173*	0.024*	0.002**	0.007**
	Error	9	-	-	-	-	-	-	-	-

†**Not significant (NS), significant at α = 0.05 (*) and significant at α = 0.01 (**)**

Table C3: Summary of ANOVA table for the one-month irrigation-withholding trials

Harvest	Source of variation	Degree of freedom	†F-probability levels for						
			Fresh yield	Dry yield	Leaf area index	Leaf fresh proportion (%)	Dry matter content (%)		Oil yield
							Leaf	Stem	
Harvest 1 (October 2005)	Block	3	0.728 ^{NS}	0.562 ^{NS}	0.204 ^{NS}	0.012*	0.085 ^{NS}	0.619 ^{NS}	0.730 ^{NS}
	Treatment	3	0.008**	0.008**	0.000**	0.003**	0.084 ^{NS}	0.200 ^{NS}	0.009**
	Error	9	-	-	-	-	-	-	-
Harvest 2 (September 2005)	Block	3	0.063 ^{NS}	-	-	0.146 ^{NS}	-	-	0.123 ^{NS}
	Treatment	3	0.004**	-	-	0.002**	-	-	0.051 ^{NS}
	Error	9	-	-	-	-	-	-	-
Harvest 3 (November 2006)	Block	3	0.215 ^{NS}	-	0.392 ^{NS}	0.0279*	0.396 ^{NS}	0.033*	0.223 ^{NS}
	Treatment	3	0.002**	-	0.000**	0.000**	0.003**	0.290 ^{NS}	0.590 ^{NS}
	Error	9	-	-	-	-	-	-	-
Harvest 4 (February 2007)	Block	3	0.451 ^{NS}	0.748 ^{NS}	0.935 ^{NS}	0.712 ^{NS}	0.215 ^{NS}	0.840 ^{NS}	0.479 ^{NS}
	Treatment	3	000**	0.003**	0.045*	0.027*	0.001**	0.000**	0.004**
	Error	9	-	-	-	-	-	-	-

†Not significant (NS), significant at α = 0.05 (*) and significant at α = 0.01 (**)

Table C4: Summary of ANOVA table for the effects of irrigation frequency, growing medium and a one-week irrigation-withholding trials in the tunnel (for Harvest 1, September 2005)

Source of variation	Degree of freedom	Fresh herbage yield	Dry yield	Leaf fresh mass %	Dry matter content (%)		Oil yield	Oil content
					Leaf area	Stem		
Block	3	0.029*	0.0462*		0.022*		0.016*	0.363 NS
Irrigation frequency	2	0.000**	0.0000**		0.000**		0.000**	0.000**
Soil type (B)	1	0.059 NS	0.682 NS		0.8.21 NS		0.000**	0.005**
AB	2	0.003**	0.112 NS		0.010**		0.000**	0.000**
Error	15	-		-	-	-	-	-
One-week stress (C)	1	0.000**	0.000**		0.000**		0.000**	0.000**
AC	2	0.000**	0.004**		0.000**		0.000**	0.004**
BC	1	0.001**	0.001**		0.003**		0.925 NS	0.921 NS
ABC	2	0.029**	0.032*		0.321 NS		0.005**	0.034 NS
Error	18	-	-	-	-	-	-	-

†**Not significant (NS), significant at α = 0.05 (*) and significant at α = 0.01 (**)**

Table C5: Summary of ANOVA table for the effects of irrigation frequency, growing medium and a one-week irrigation-withholding trials in the tunnel (for Harvest 2, April 2006)

Source of variation	Degree of freedom	†F-probability levels for			
		Fresh herbage yield	Leaf area	Oil yield	Oil content
Block	3	0.213 NS	0.892 NS	0.216 NS	0.871 NS
Irrigation frequency (A)	2	0.000**	0.000**	0.000**	0.000**
Soil type (B)	1	0.000**	0.000**	0.000**	0.189NS
AB	2	0.000**	0.122 NS	0.000**	0.030**
Error	15	-	-	-	-
One-week stress (C)	1	0.001**	0.046*	0.000**	0.000**
AC	2	0.013*	0.675 NS	0.001**	0.001**
BC	1	0.223 NS	0290 NS	0.564 NS	0.003**
ABC	2	0.055 NS	0.924 NS	0.000**	0.031*
Error	18	-	-	-	-

†**Not significant (NS), significant at α = 0.05 (*) and significant at α = 0.01 (**)**

Table C6: Summary of ANOVA table for the effects of irrigation frequency, growing medium and a one-week irrigation-withholding trial in the tunnel (for Harvest 3, August 2006)

Source of variation	Degree of freedom	†F-probability levels for					
		Herbage yield		Leaf fresh mass %	Leaf area	Oil yield	Oil content
		Fresh	Dry				
Block	3	0.346 NS	0.025*	0.014*	0.742 NS	0.641 NS	0.687 NS
Irrigation frequency (A)	2	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Soil type (B)	1	0.000**	0.000**	0.000**	0.000**	0.006**	0.189 NS
AB	2	0.009**	0.031*	0.492 NS	0.000**	0.000**	0.030*
Error	15	-	-	-	-	-	-
One-week stress (C)	1	0.000**	0.002**	0.924 NS	0.000**	0.000**	0.000**
AC	2	0.002**	0.352 NS	0.008**	0.026*	0.000**	0.001**
BC	1	0.313 NS	0.697 NS	0.169 NS	0.424 NS	0.000**	0.003**
ABC	2	0.105 NS	0.721 NS	0.658 NS	0.624 NS	0.022*	0.031*
Error	18		-	-	-	-	-

†**Not significant (NS), significant at α = 0.05 (*) and significant at α = 0.01 (**)**

Table C7: Summary of ANOVA table for the effects of irrigation frequency and the one-week irrigation-withholding trials in the glasshouse

Harvest	Source of variation	Degrees of freedom	†F-probability levels for				
			Fresh herbage mass	Percentage fresh leaf mass	Leaf area	Essential oil yield	Essential oil content
Harvest 1 (June 2006)	Block	5	0.178 NS	0.143 NS	0.026*	0.186 NS	
	Irrigation Frequency (A)	4	0.000**	0.000**	0.000**	0.000**	
	Error (A)	20	-	-	-	-	
	One-week stress (B)	1	0.000**	0.001**	0.000**	0.000**	
	AB	4	0.000**	0.1045 NS	0.019*	0.000**	
	Error (AB)	25	-	-	-	-	-
Harvest 2 (October. 2006)	Block	5	0.012*	-	-	0.009**	
	Irrigation Frequency (A)	4	0.000**	-	-	0.000**	
	Error (A)	20	-	-	-	-	
	One-week stress (B)	1	0.000**	-	-	0.000**	
	AB	4	0.000**	-	-	0.006**	
	Error (AB)	25	-	-	-	-	-

†**Not significant (NS), significant at α = 0.05 (*) and significant at α = 0.01 (**)**

Table C8: Summary of ANOVA table for the leaf physiological parameters recorded during the one-week irrigation-withholding period prior to harvesting in the glasshouse (for Harvest 1, June 2006)

Parameter	Source of variation	Degree of freedom	†F-probability levels for							
			Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Relative water content	Block	5	0.545 NS	0.290 NS	0.790 NS	0.744 NS	0.352 NS	0.701 NS	0.329 NS	0.865 NS
	Irrigation frequency (A)	4	0.986 NS	0.031*	0.006**	0.002**	0.000**	0.001**	0.000**	0.001**
	Error (A)	20	-	-	-	-	-	-	-	-
Leaf water potential	Block	5	0.142 NS	0.316 NS	0.791 NS	0.924 NS	0.861 NS	0.671 NS	0.155 NS	0.249 NS
	Irrigation frequency (A)	4	0.088 NS	0.000**	0.000**	0.000**	0.000**	0.000**	0.029*	0.008**
	Error	20	-	-	-	-	-	-	-	-
Transpiration rate	Block	5	0.443 NS	0.468 NS	0.094 NS	0.078 NS	0.798 NS	0.493 NS	0.779 NS	0.855 NS
	Irrigation frequency (A)	4	0.143 NS	0.682 NS	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
	Error (A)	20	-	-	-	-	-	-	-	-
Stomatal conductance	Block	5	0.766 NS	0.625 NS	0.991 NS	0.685 NS	0.942 NS	0.430 NS	0.571 NS	0.826 NS
	Irrigation frequency (A)	4	0.014*	0.014*	0.131 NS	0.239 NS	0.005**	0.000**	0.000**	0.000**
	Error (A)	20	-	-	-	-	-	-	-	-

†**Not significant (NS), significant at α = 0.05 (*) and significant at α = 0.01 (**)**

Table C 9: Summary of ANOVA table for the leaf physiological parameters recorded during the one-week irrigation-withholding period prior to harvesting in the glasshouse (for Harvest 2, October 2006)

Parameter	Source of variation	Degree of freedom	†F-probability levels for Day 0	Day 1	Day 2	Day3	Day 4	Day 5	Day 6	Day 7
Relative water content	Block	5	0.455 NS	0.275 NS	0.622 NS	0.991 NS	0.504 NS	0.235 NS	0.324 NS	0.029 NS
	Irrigation frequency	4	0.817 NS	0.289 NS	0.159 NS	0.037*	0.006**	0.001**	0.000**	0.000**
	Error	20	-	-	-	-	-	-	-	-
Leaf water potential	Block	5	0.219 NS	0.354 NS	0.954 NS	0.958 NS	0.856 NS	0.640 NS	0.235 NS	0.255 NS
	Irrigation frequency	4	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
	Error	20	-	-	-	-	-	-	-	-
Transpiration rate	Block	5	0.555 NS	0.828 NS	0.531 NS	0.394 NS	0.747 NS	0.076 NS	0.716 NS	0.695 NS
	Irrigation frequency	4	0.826 NS	0.056 NS	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
	Error	20	-	-	-	-	-	-	-	-
Stomatal conductance	Block	5	0.458 NS	0.793 NS	0.327 NS	0.205 NS	0.0941 NS	0.085 NS	0.210 NS	0.2919 NS
	Irrigation frequency	4	0.007**	0.034**	0.002**	0.000**	0.000**	0.000**	0.000**	0.000**
	Error	20	-	-	-	-	-	-	-	-

†Not significant (NS), significant at α = 0.05 (*) and significant at α = 0.01 (**)