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.

$$\mathrm{X} = \frac{R_S}{R_A} \tag{3.1}$$

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	$\mathrm{Y} = 0.1026\mathrm{X} + 0.0459$ [†]	$\mathrm{Y} = 0.1075\mathrm{X} + 0.0961$
0.2 - 0.4 m	$\mathrm{Y} = 0.2025\mathrm{X} - 0.0560$	$\mathrm{Y} = 0.5128\mathrm{X} - 0.5081$
0.4 - 0.6 m	$\mathrm{Y} = 0.1802\mathrm{X} - 0.0684$	$\mathrm{Y} = 0.0687\mathrm{X} + 0.1304$
0.6 - 0.8 m	$\mathrm{Y} = 0.134\mathrm{X} + 0.0496$	$\mathrm{Y} = 0.4819\mathrm{X} + 0.5321$
0.8 - 1.0 m	$\mathrm{Y} = 0.5759\mathrm{X} + 0.1136$	$\mathrm{Y} = 0.4563\mathrm{X} - 0.4882$
1.0 - 1.2 m	$\mathrm{Y} = 0.1211\mathrm{X} + 0.0656$	$\mathrm{Y} = 0.0936\mathrm{X} + 0.1168$

[†] **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).

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

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

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).

$$ET = P + I - (D + R) + (S_1 - S_2) \qquad (3.4)$$

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**

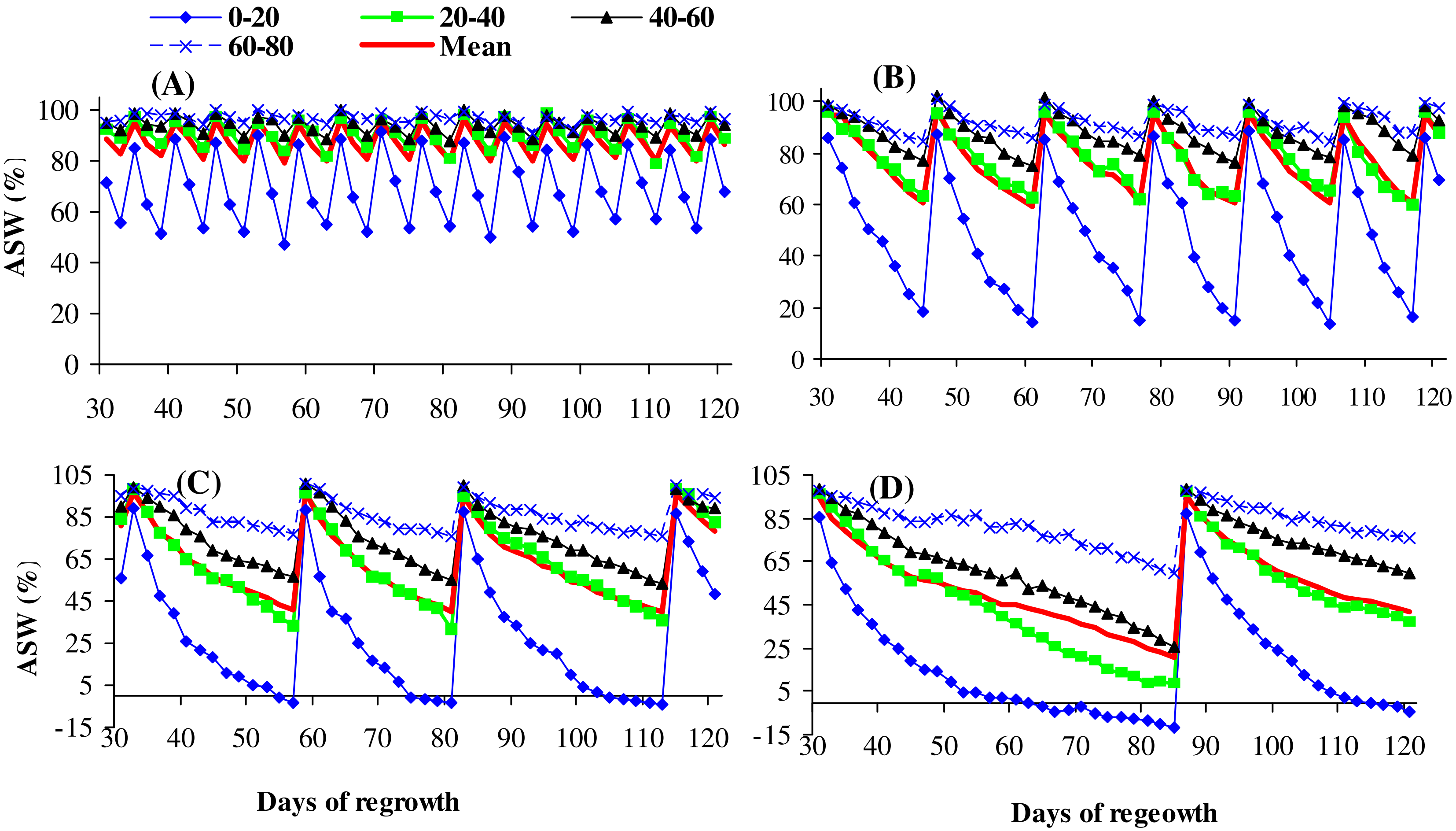

Figure 3.1: Temporal variations in plant available soil water (ASW) content in the layers of the root zone of rose-scented geranium in the open field trial: data for the 20 (A), 40 (B), 60 (C), and 80% (D) maximum allowable depletion (MAD) treatments for Harvest 1 (regrowth cycle during 12 May-11 September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

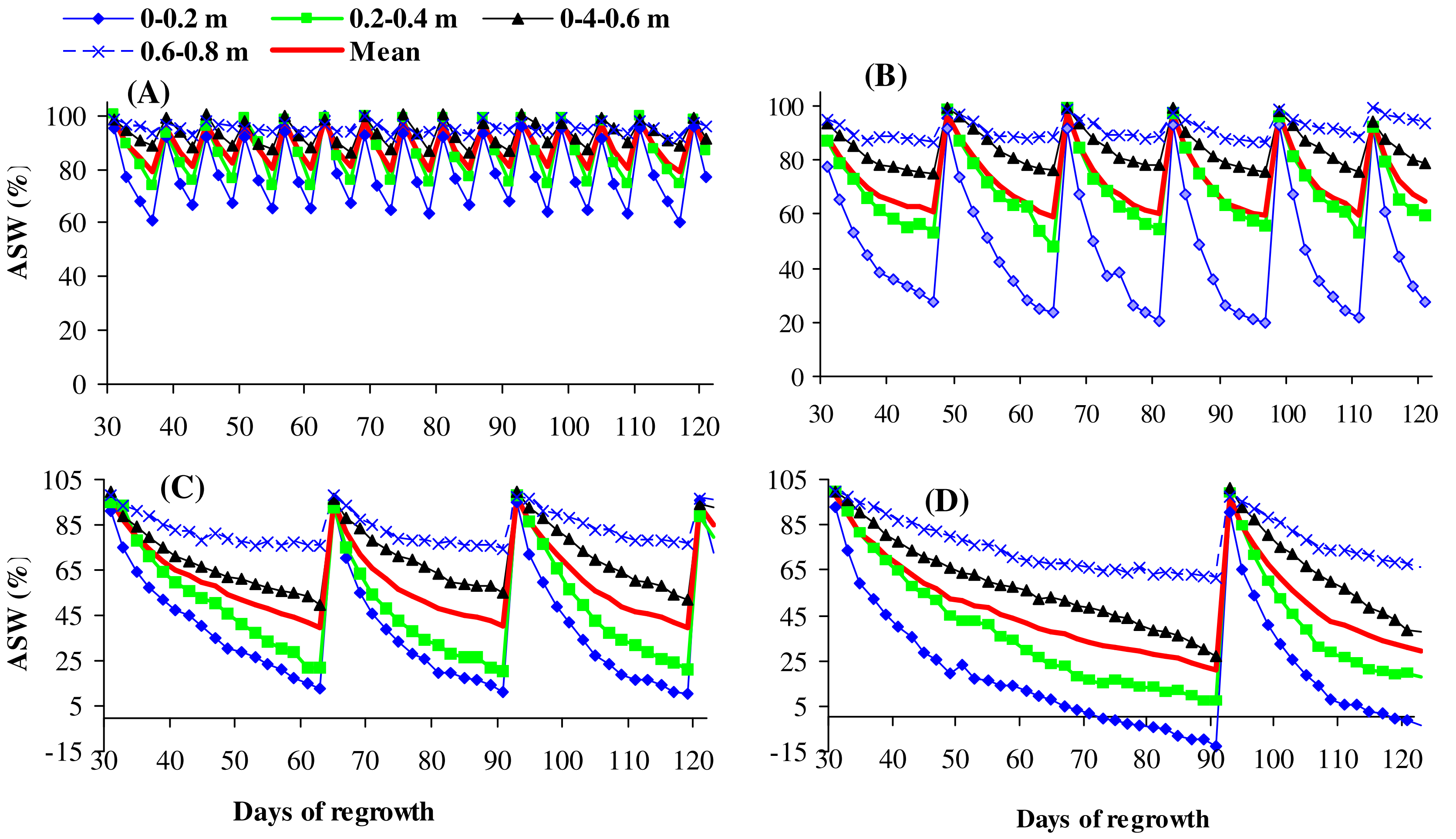

Figure 3.2: Temporal variations in plant available soil water (ASW) content in the layers of the root zone of rose-scented geranium in the rain-shelter trial: data for the 20 (A), 40 (B), 60 (C), and 80% (D) maximum allowable depletion (MAD) treatments in the rain shelter for Harvest 1 (regrowth cycle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

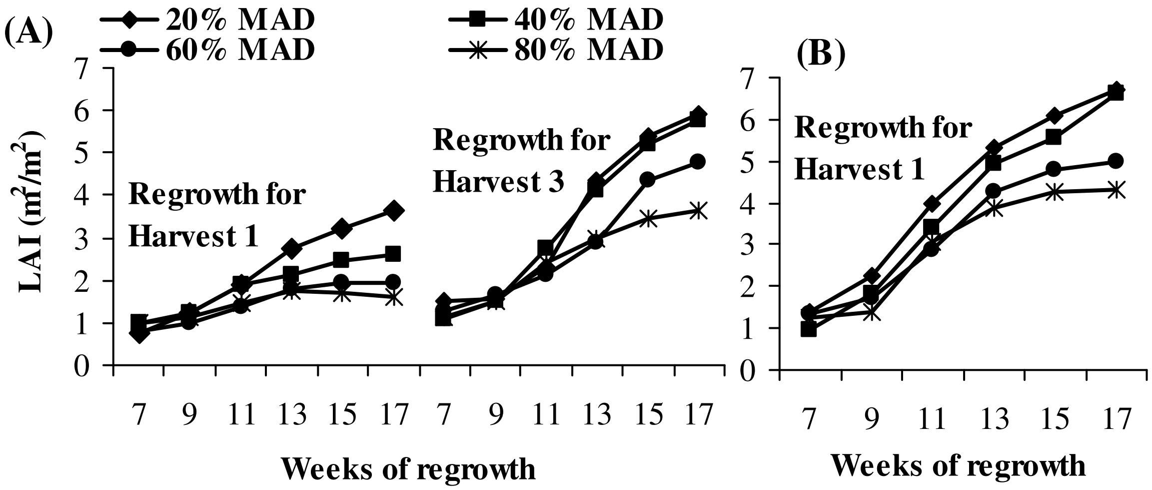

Figure 3.3: Leaf area index of rose-scented geranium as affected by different maximum allowable depletion (MAD) levels of plant available soil water. (A) Harvests 1 and 3 in the open, and (B) Harvest 1 in the rain shelter were conducted in July 2005, February 2006 and June 2006, respectively

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.

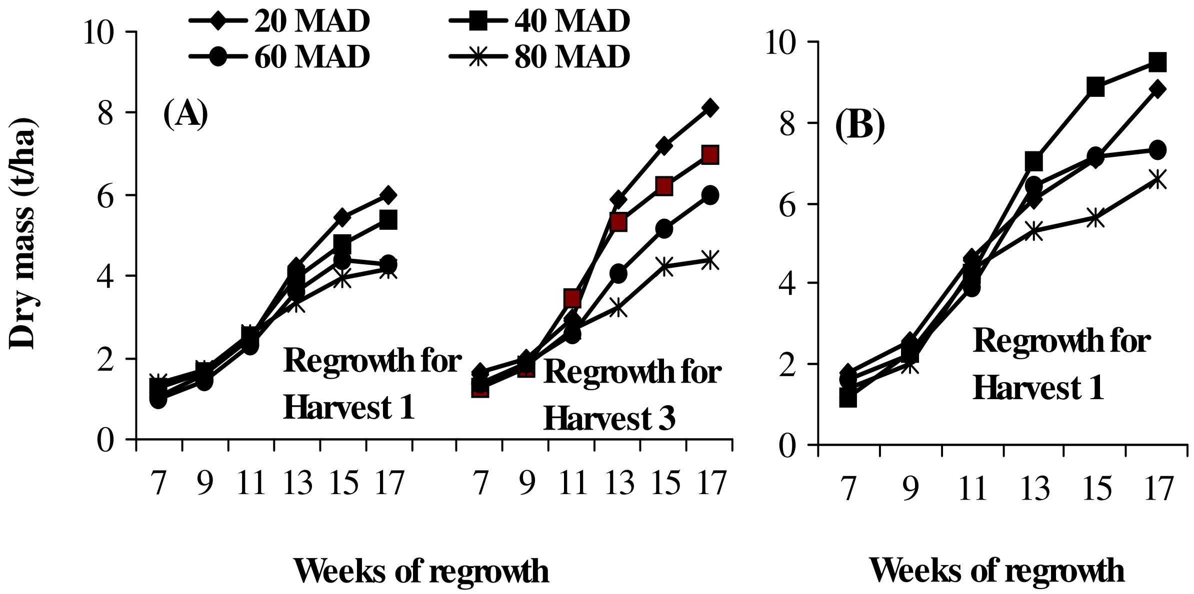

Figure 3.4: Herbage dry mass of rose-scented geranium grown at different maximum allowable depletion (MAD) levels of plant available soil water. (A) Harvests 1 and 3 in the open field and (B) Harvest 1 in the rain shelter were conducted in July 2005, February 2006 and June 2006, respectively

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).

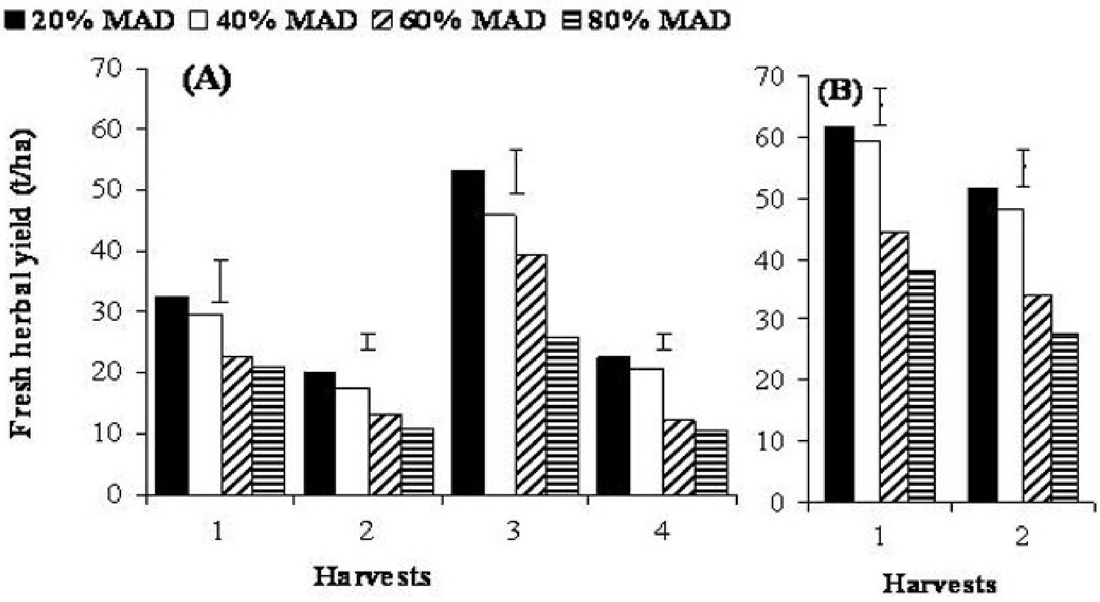

Figure 3.5: Fresh herbage yield of rose-scented geranium grown at different maximum allowable depletion (MAD) levels of plant available soil water. The vertical bars are LSD at α = 0.05; (A) Harvests 1, 2, 3 and 4 in the open field were conducted in July and September 2005, and January and October 2006,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

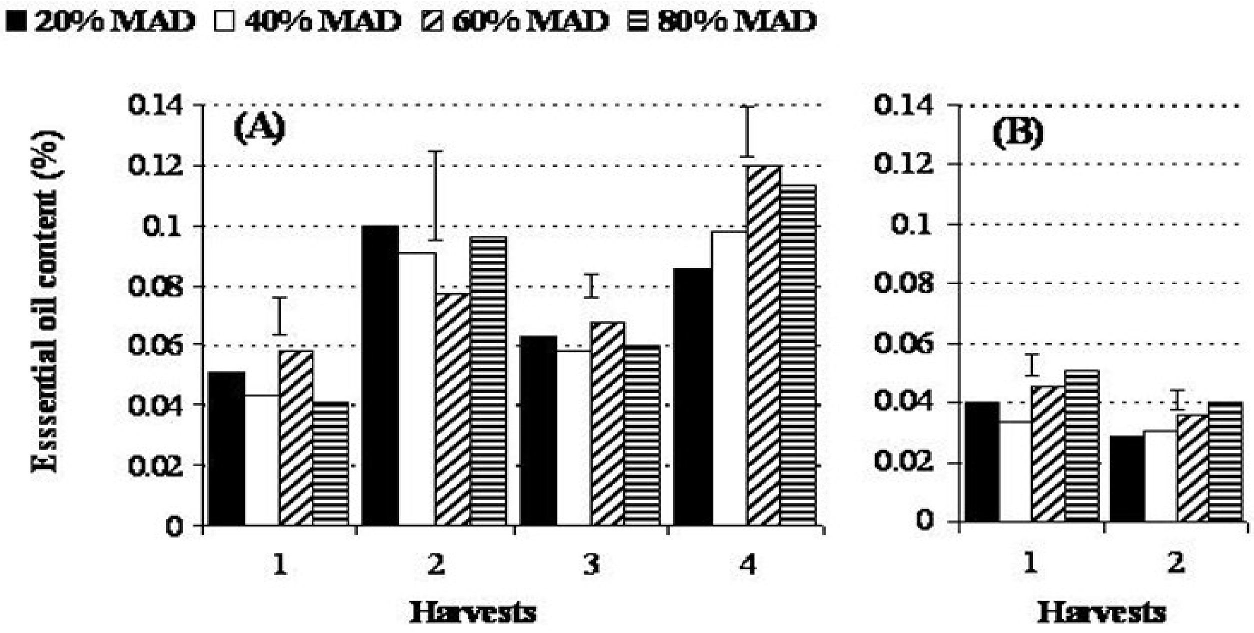

Figure 3.6: Essential oil content of rose-scented geranium grown at different maximum allowable depletion (MAD) levels of plant available soil water. The vertical bars are LSD at α = 0.05; (A) Harvests 1, 2, 3 and 4, in the open field, were conducted in July and September 2005, and January and October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

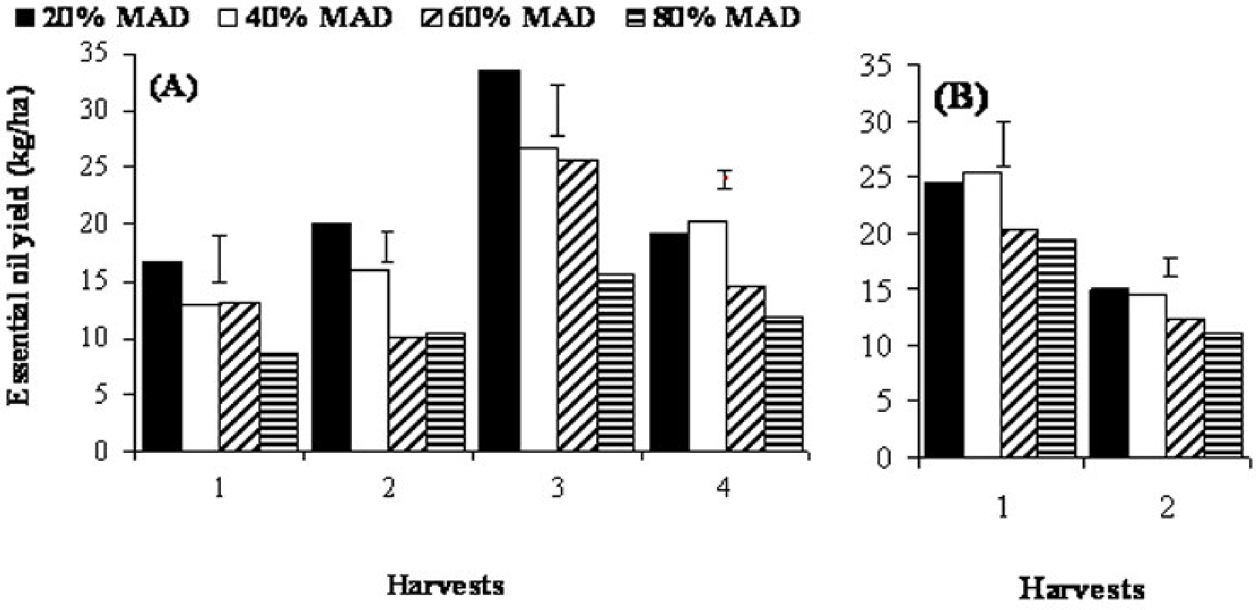

Figure 3.7: Essential oil yield of rose-scented geranium grown at different maximum allowable depletion (MAD) levels of plant available soil water. the vertical bars are LSD at α = 0.05; (A) Harvests 1, 2, 3 and 4, in the open field, were conducted in July and September 2005, and January and October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

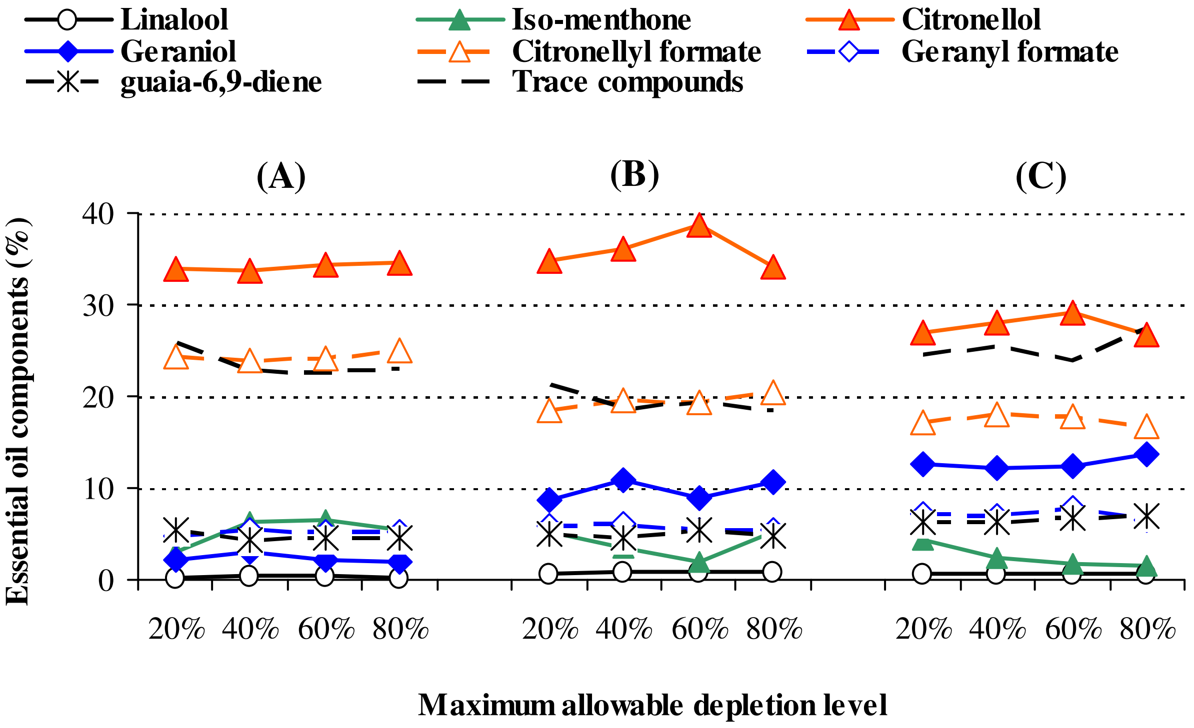

Figure 3.8: Major essential oil components (percentage of essential oil yield) of rose-scented geranium grown under different maximum allowable depletion levels of plant available soil water. (A) Harvest 1, (B) Harvest 2 and (C) Harvest 3, in the open field, were conducted in July and September 2005,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