

CHAPTER 5

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

5.1 ABSTRACT

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

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

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5.2 INTRODUCTION

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

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

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

5.3 MATERIALS AND METHODS

5.3.1 Growth system description

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

5.3.2 Plant culture

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

5.3.3 Treatments and experimental design

Treatments

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

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

Experimental design

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



Figure 5.1: Plant arrangements in the tunnel trial

5.3.4 Irrigation monitoring

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

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



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

5.3.5 Fertiliser application

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

5.3.6 Data recorded

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

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

5.4 RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

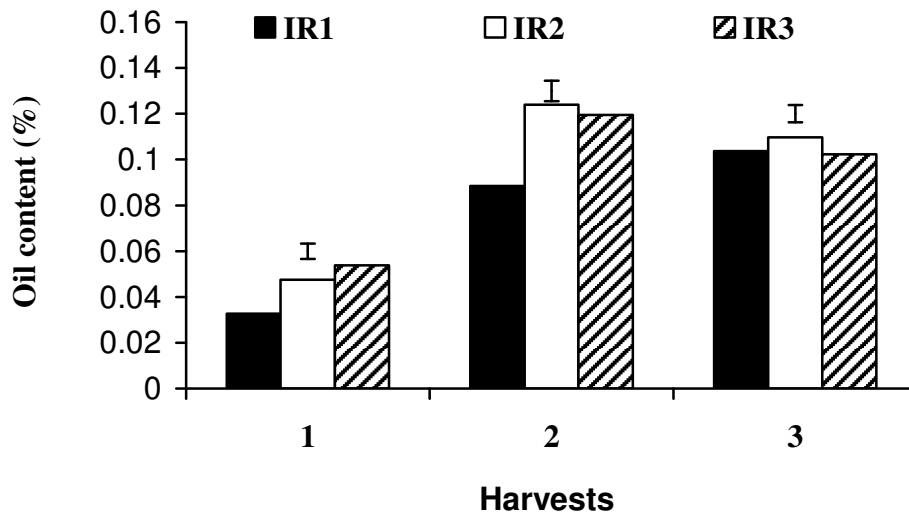


Figure 5.3: Essential oil content of rose-scented geranium as affected by irrigation frequency in the tunnel. The vertical bars are LSD (at $\alpha = 0.05$); IR1, 2 and 3 represent twice a day, once a day and every second day irrigation frequency, respectively; Harvests 1, 2 and 3 were conducted in September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

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

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

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

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

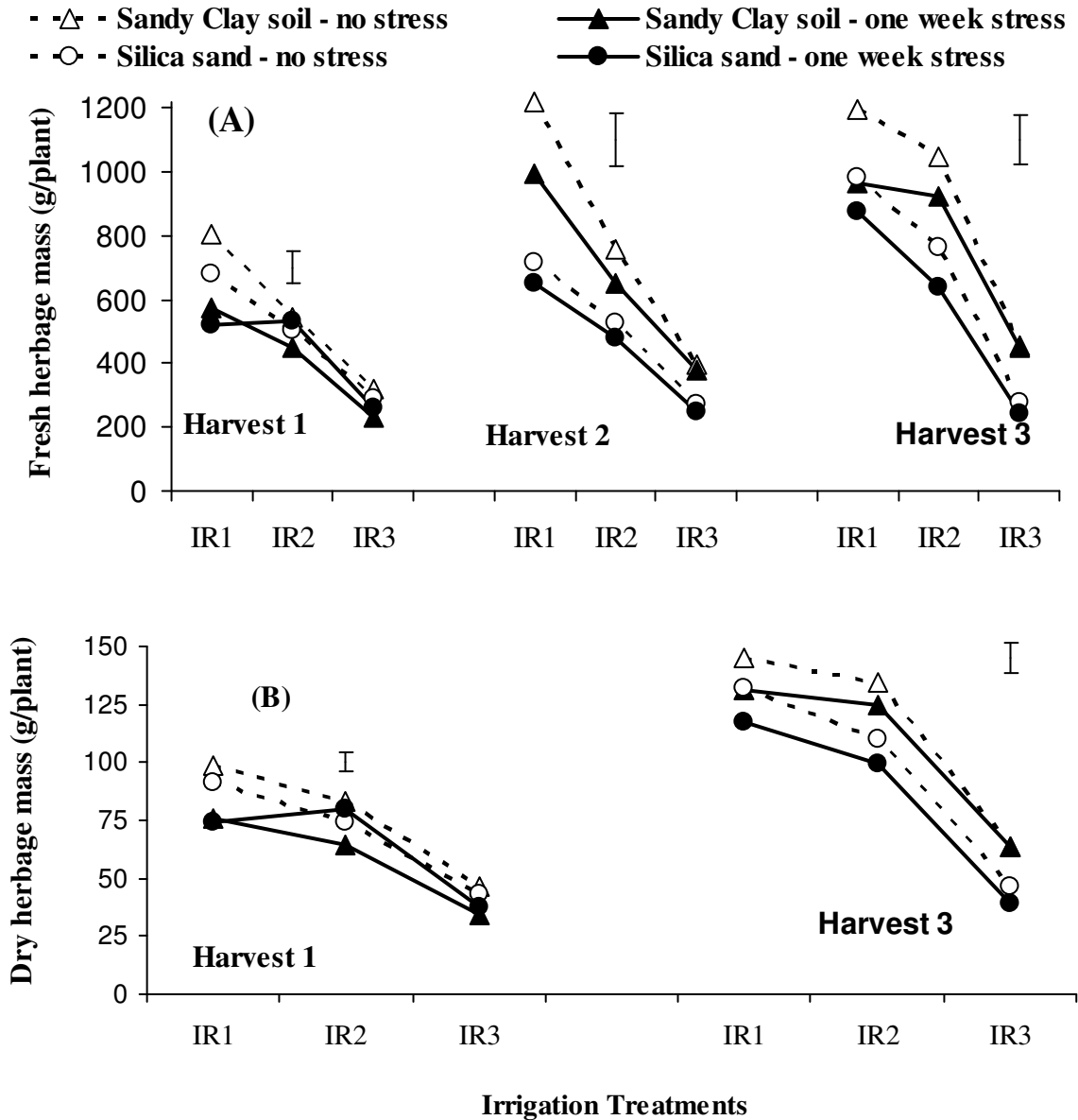


Figure 5.4: Rose-scented geranium fresh (A) and dry (B) herbage mass as affected by irrigation frequency and a one-week stress period before harvest in the tunnel. The vertical bars are LSD (at $\alpha = 0.05$); IR1, 2, 3 represent twice a day, once a day and every second day irrigation frequency, respectively;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

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

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

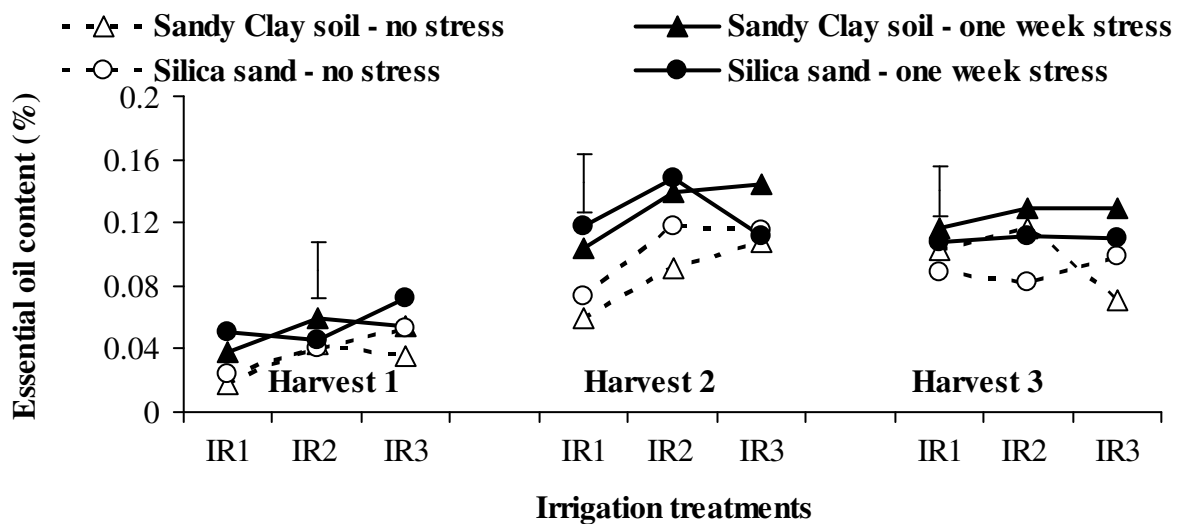


Figure 5.5: Rose-scented geranium oil content (percentage oil on fresh herbage mass basis) as affected by irrigation frequency and a one-week stress period before harvest in two growing media in the tunnel. The vertical bars are LSD (at $\alpha = 0.05$); IR1, 2, 3 are twice a day, once a day and every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

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

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

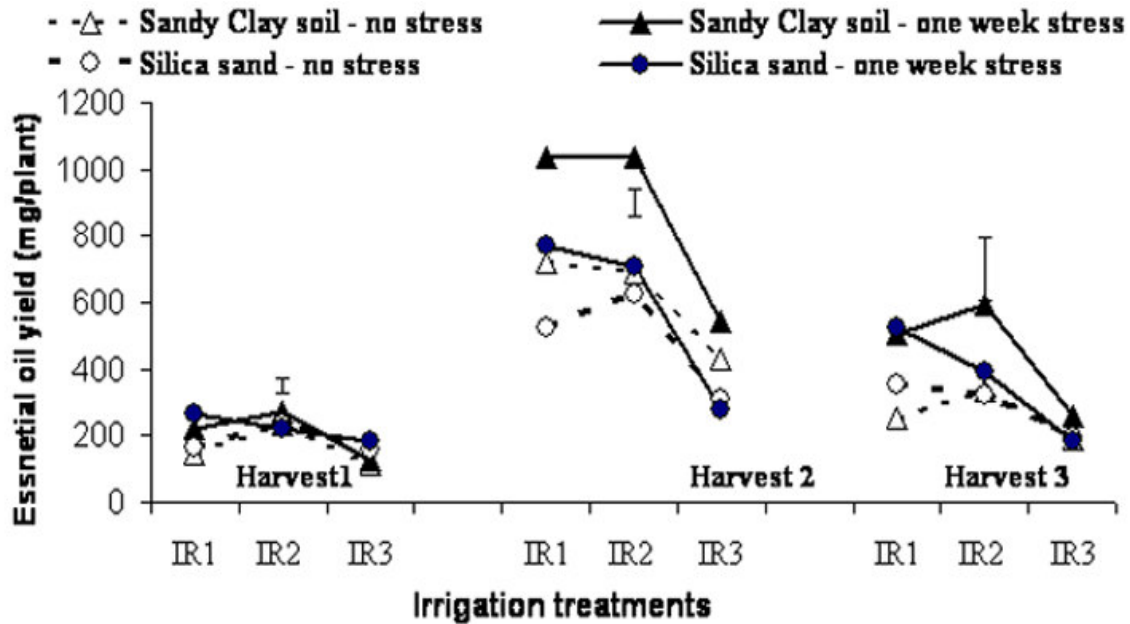


Figure 5.6: Essential oil yield of rose-scented geranium as affected by irrigation frequency, a one-week stress period before harvest and growing media in the tunnel. The vertical bars are LSD (at $\alpha = 0.05$); IR1, 2, 3 represent twice a day, once a day and every second day irrigation frequency,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

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

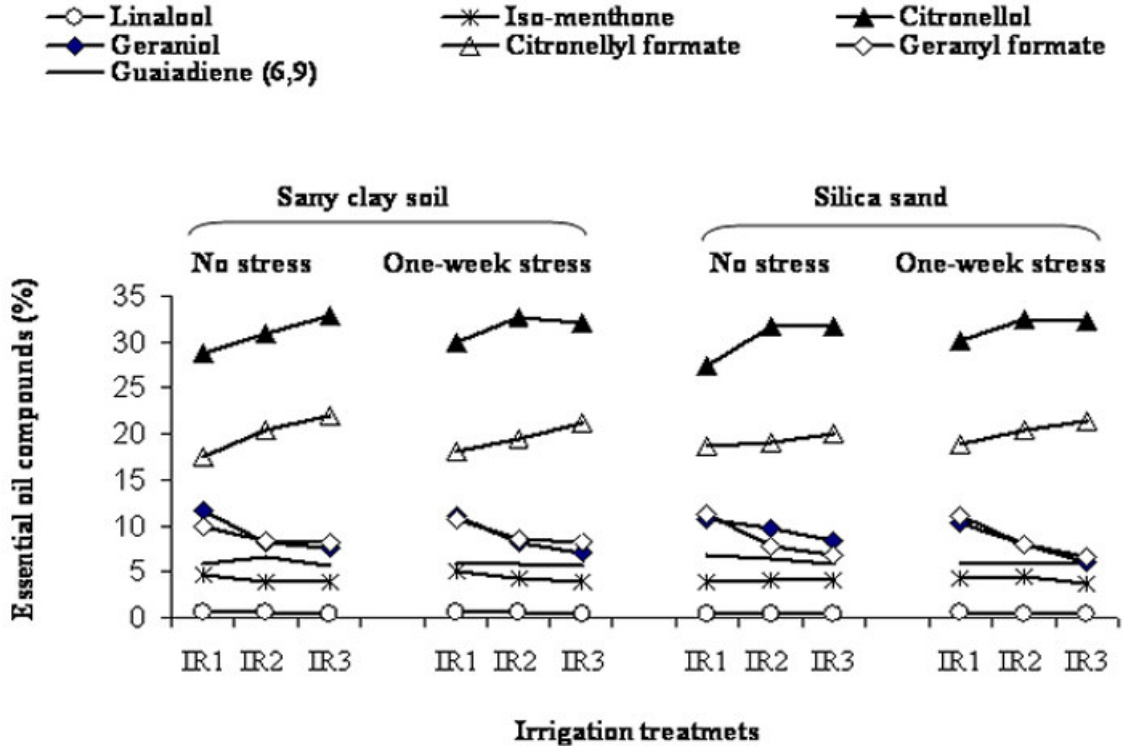


Figure 5.7: Chemical composition (%) of essential oil of rose-scented geranium as affected by irrigation frequencies and a one-week stress period in the tunnel. IR1, 2, 3 are twice a day, once a day and every second day irrigation frequency, respectively

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

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

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

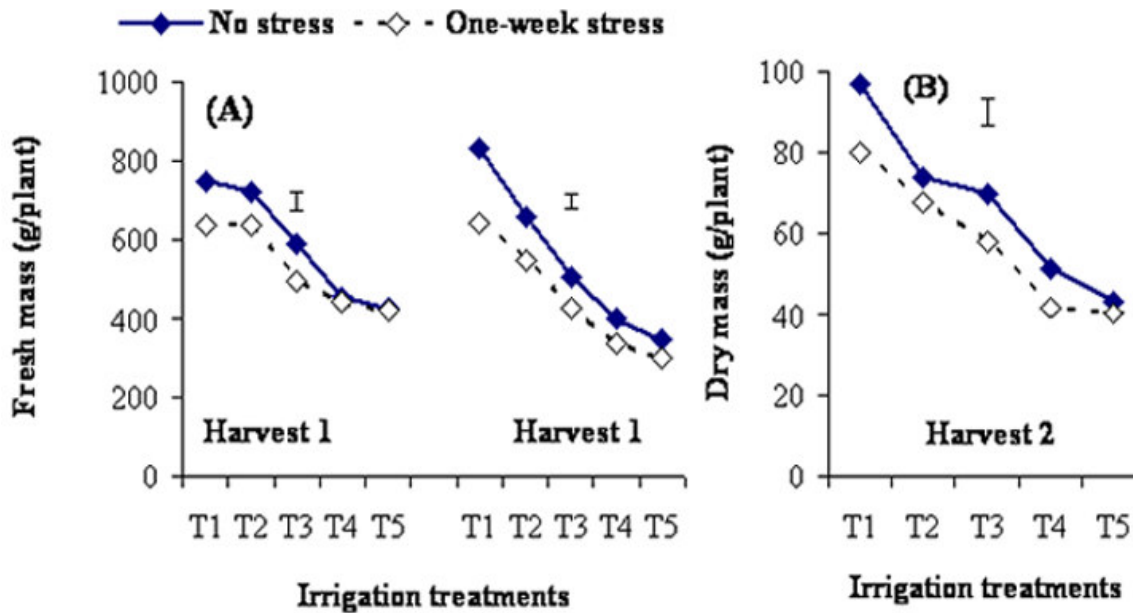


Figure 5.8: Fresh (A) and dry (B) herbage mass of rose-scented geranium as affected by irrigation frequency and a one-week irrigation-withholding period in the glasshouse. The vertical bars represent LSD (at $\alpha = 0.05$); Harvests 1 and 2 were conducted in June and October 2006, respectively; T 1,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

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

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

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

Essential oil yield

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

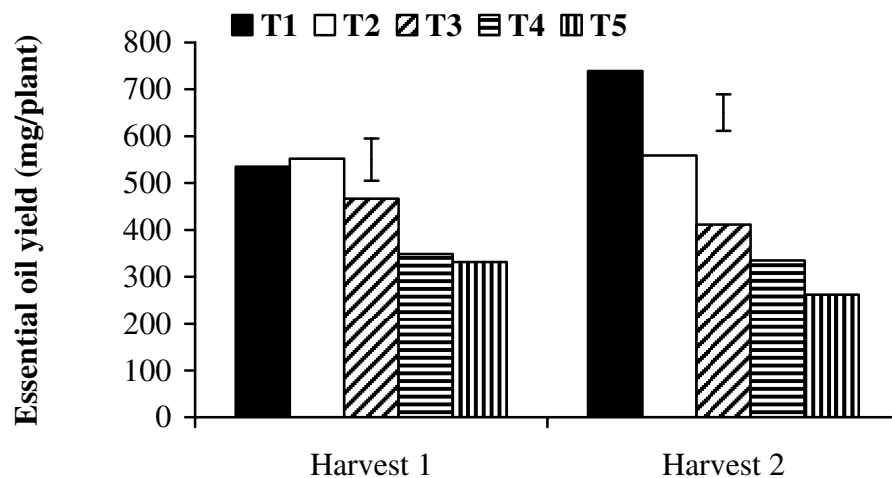


Figure 5.9: Effect of irrigation frequency on essential oil yield of rose-scented geranium in the glasshouse. The vertical bars represent LSD at $\alpha = 0.05$; Harvests 1 and 2 were conducted in June and October 2006, respectively; T 1, 2, 3, 4 and 5 represent daily, and every second, third, fourth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

Essential oil content

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

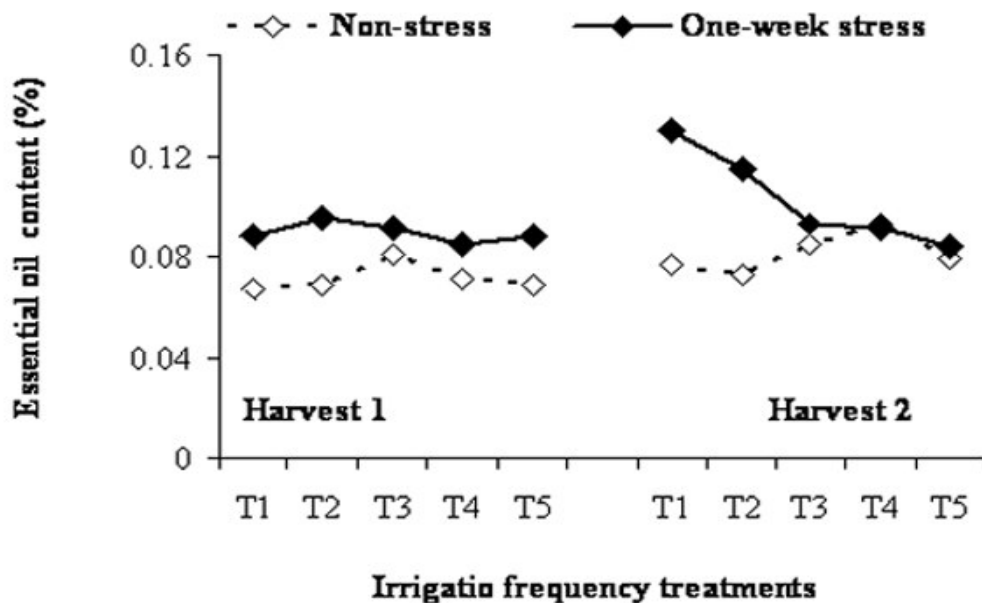


Figure 5.10: Essential oil content (percentage oil on fresh herbage mass basis) of rose-scented geranium as affected by irrigation frequency and one-week irrigation-withholding treatments. Harvests 1 and 2 were conducted in June and October 2006 in the glasshouse; T1, 2, 3, 4 and 4 represent daily, and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

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

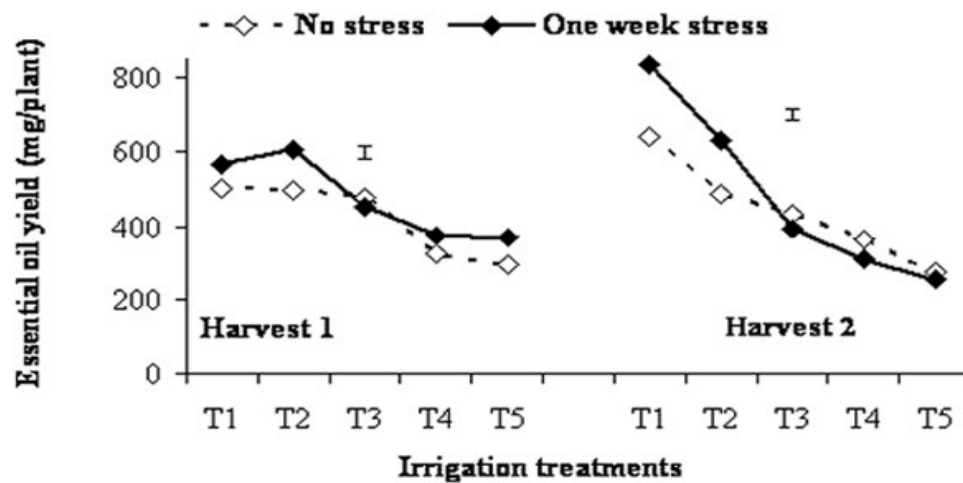


Figure 5.11: Essential oil yield of rose-scented geranium grown in different irrigation frequencies and a one-week water stress period. The vertical bars are LSD (at $\alpha = 0.05$); Harvests 1 and 2 in the glasshouse were conducted in June and October 2006, respectively; T 1, 2, 3, 4 and 5 represent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

Essential oil composition

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

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

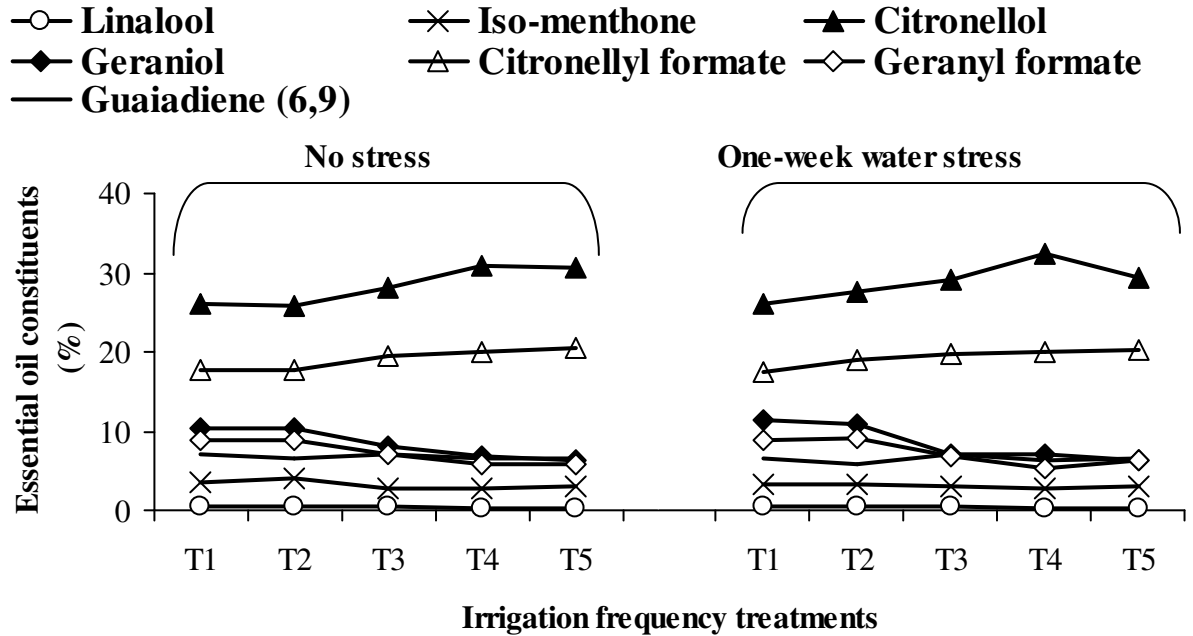


Figure 5.12: Composition (percentage of essential oil) of rose-scented geranium as affected by irrigation frequency and irrigation withholding for the week prior to harvesting treatments in the glasshouse. T1, T2, T3, T4 and T5 represent daily and every second, third, fourth and fifth day irrigation,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

5.4.7 Water use and water-use efficiency (WUE)

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

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

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

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

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

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

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

5.5 CONCLUSIONS

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