High Irrigation Frequency and Brief Water Stress Before Harvest Enhances Essential Oil Yield of Rose-scented Geranium (*Pelargonium capitatum* × *P. radens*)

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Abstract. Pot experiments were conducted to investigate the effects of irrigation frequency and withholding irrigation during the week before harvesting on rose-scented geranium herbage yield 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) were conducted in a tunnel. One week before harvest, irrigation was withheld for half of the pots in each treatment combination. In a glasshouse, sandy clay soil was used as growing medium, and five irrigation frequencies (everyday, everyday with 1-week irrigation withholding period, every second day, every third day, and every fourth day irrigation to pot capacity) were applied as treatments. Results showed that essential oil content (percent oil on fresh herbage weight basis) increased as the irrigation frequency decreased. Both herbage yield and essential oil yield positively responded to frequent irrigation. Thus, higher herbage and essential oil yields were obtained from the highest irrigation frequency. A 1-week stress period significantly increased total essential oil yield as well as oil content per fresh herbage weight. The results highlighted that conditions of high soil water availability followed by brief water stress just before harvesting would maximize rose-scented geranium essential oil yield. Citronellol and citronellyl formate tended to increase with an increase in the stress level, but the reverse was true for geraniol and geranyl formate. Other major essential oil components were unaffected by water stress.

Soil water supply is one of the major abiotic factors that determine the biosynthetic processes in plants (Letchamo et al., 1995). Response of essential oil yield and composition to water stress varies with duration and severity of stress. According to the literature, production of primary metabolites and essential oil yield may decline when plants are exposed to sustained water stress. Letchamo et al. (1995), for instance, reported that optimum conditions for photosynthesis indirectly enhanced essential oil yield of thyme (*Thymus vulgaris* L.). The authors found positive correlations among photosynthesis, herbage yield, and essential oil yield in thyme plants. Putievsky et al. (1990) also reported that as irrigation intervals became more extended, herbage yield and essential oil yield were reduced in *Pelargonium grave-*

olens. Similarly, Rajeswara Rao et al. (1996) reported that a wet season encouraged vegetative growth of rose-scented geranium and resulted in higher essential oil yield.

Based on results he obtained from his previous studies on rose-scented geranium, Weiss (1997) suggested that climatic factors (wet season for instance) that encourage herbage growth would have a negative effect on essential oil yield. Similarly, Simon et al. (1992) reported that 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 previously mentioned report, short-term stress (withholding irrigation for 8 d) did not change essential oil yield and oil composition of *Melaleuca alternifolia* (List et al., 1999).

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. Thus, we investigated 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* × *P. radens*) grown in South Africa.

Materials and Methods

Plant establishment. Pot trials were conducted in a tunnel and in a glasshouse (from Jan. 2005 to Dec. 2006) at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa. Geranium seedlings (*Pelargonium capitatum* × *P. radens*) (raised in seedling trays from stem cuttings under a mist bed) obtained from a commercial nursery were transplanted into 10-L plastic pots in a tunnel on 26 Jan. 2005. Typical oil characteristics from this species are presented by Araya et al. (2006) and Motsa et al. (2006). The pots were filled with either silica sand or sandy clay soil (52:8:38 coarse sand, silt, and clay, respectively). Volumetric water-holding capacity of the silica sand was 9.7% at field capacity and 3.8% at permanent wilting point; and that of the sandy clay soil was 29% and 17% at field capacity and permanent wilting point, respectively. Plants were allowed to grow for 6 months until uniform growth was attained. Thus, irrigation treatments were started on 22 June 2005. Data of four harvests (Sept. 2005, April, August, and Dec. 2006) were collected for evaluation.

One hundred and twenty plants that were grown in 10-L plastic pots in the tunnel, but had not been included in the water stress trials, were transferred into 12-L ceramic pots with their soil (sandy clay) and moved into a glasshouse. After the plants started to grow well and uniformly, treatments were started on 13 Nov. 2005 and harvesting was done on 12 Feb. 2006 (after 3 months of regrowth).

Treatments. Irrigation treatments in the tunnel were twice a day (IRR1), once a day (IRR2), and every other day (IRR3) in either silica sand or a sandy clay soil. The irrigation frequency by soil type treatment combinations were arranged in a randomized complete block design with four replications. Each plot consisted of two adjacent rows (75 cm apart) of 21 pots each. A 1-week irrigation withholding period before harvesting was imposed on 50% of the plants in each plot (as a split). A computer-regulated drip irrigation system (spaghetti water emitters with an average discharging rate of 2175 mL·h⁻¹) was installed and used to monitor the irrigation intervals and amount of water given to each treatment to refill to pot capacity. To minimize drainage, the amount of water applied was estimated by measuring water collected in drainage-collecting containers put in a hole near representative pots with gutters at the bottom.

In the glasshouse experiment, only the sandy clay soil was used. Five irrigation intervals of once a day with no brief stress (T1), once a day with a 1-week stress just before harvesting (T2), once every second day (T3), once every third day (T4), and once every fourth day (T5) were applied as treatments. At each irrigation event, plants were watered to pot capacity. The treatments

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were arranged in a randomized complete block design with four replications.

In both experiments, the regrowth durations were 3 months $\pm$ 1 week depending on the weather conditions during the brief stress treatment (on noncloudy days). The plants appeared to be sensitive to water stress during the first month after cutting. Hence, in the first month of regrowth, no water stress was applied. Also, cultural practices (fertilizer application and some pest control measures) were done within that period. Irrigation treatments were applied during the next 2 months of regrowth.

Fertilizer application. During each regrowth period, each plant received 3 g N, 4.5 g P, and 3 g K as a split in Week 1 and Week 7 [in the form of 2:3:2 (22) N-P-K fertilizer granules], and 1 g N and 1 g K (in the form of ammonium nitrate and potassium nitrate) on Week 9. To avoid any salt accumulation, plants were overirrigated on the first and second days of each regrowth period.

Data recorded. During harvesting, plant shoots were cut to $\approx$ 15 to 20 cm above the surface of the pots. Herbage fresh weight was measured immediately after cutting. Fresh herbage samples ($\approx$ 9 kg each) were sent for oil content determination and oil composition analysis. From the oil content, the respective treatment oil yields were calculated. Oil samples were pooled per treatment and analyzed by gas chromatography (GC). For GC analysis, an Agilent GC (FID) (Agilent Technologies, Santa Clara, CA) model 6890N, fitted to 30 m $\times$ 0.25 mm fused silica capillary column and a film thickness of 0.25 μ m, was used. Helium gas was used as a carrier. The temperature program was 50 to 200 $^{\circ}$ C with a ramp amount of 5 $^{\circ}$ C/min $^{-1}$ and a detector and an injector temperature of 220 $^{\circ}$ C. Constituents were identified by comparing their retention time and retention indices to standard values (Adams, 2004). The recorded data were subjected to analysis of variance (ANOVA) using MSTATC, a data-analyzing microcomputer program (MSTATC, 1991). Where there were significant differences in ANOVA, means were compared using the least significant difference test. Because oil samples were pooled for oil composition analysis, statistical analysis could not be performed on these data.

Results and Discussion

Irrigation frequency experiment in the tunnel. Herbage yield was sensitive to irrigation frequency. Statistical analysis showed that every reduction in irrigation frequency resulted in a significant reduction in herbage yield (Table 1). The herbage yield reduction rate was consistently higher between IRR2 and IRR3 (ranged from 42% to 58%) than between IRR1 and IRR2, where it ranged from 16% to 37%. These results agree with results reported by Rajeswara Rao et al. (1996), who found that an increase in soil water availability encouraged vegetative growth of rose-scented geranium. Similarly, Singh (1999) found significant lower herbage

Table 1. Fresh herbage mass of four harvests of rose-scented geranium grown under different irrigation frequencies in a tunnel.

Irrigation frequency	Fresh herbage mass (g/plant)			
	September	April	August	December
IRR1 ²	644.75 a ³	895.47 a	1006.00 a	833.4 a
IRR2	508.16 b	627.71 b	844.60 b	521.3 b
IRR3	275.79 c	323.98 c	356.70 c	299.3 c
Grand mean	476.23	615.72	735.70	551.31
Coefficient of variation (%)	8.49	9.22	6.96	7.07
Least significant difference ($\alpha = 0.05$)	30.46	43.2	41.80	41.52

²IRR1, 2, and 3 are twice a day, once a day, and every second day irrigation frequency, respectively.

³Numbers followed by the same letters within a column are not significantly different at 5% level of probability.

Table 2. Average minimum and maximum temperatures and radiant energy inside and outside of the tunnel and glasshouse during each regrowth period.

Harvesting month	Growth system	Minimum ($^{\circ}$ C)		Maximum ($^{\circ}$ C)		Light energy (MJ-m $^{-2}$ -d $^{-1}$)	
		Inside	Outside	Inside	Outside	Inside	Outside
Sept. 2005	Tunnel	9.1	8.1	20.2	24.8	10.2	15.7
Apr. 2006	Tunnel	18.2	15.1	33.4	26.4	10.3	15.9
Aug. 2006	Tunnel	10.4	4.6	26.4	21.4	8.3	12.8
Dec. 2006	Tunnel	19.3	14.6	34.2	29.1	11.6	17.9
Feb. 2006	Glasshouse	20.2	16.3	35.6	28.3	12.8	20.7

Table 3. Essential oil yield of rose-scented geranium under different irrigation frequencies in a tunnel.

Irrigation frequency	Essential oil yield (mg/plant)		
	September	April	August
IRR1 ²	197.92 b ³	761.63 a	408.60 a
IRR2	238.70 a	764.47 a	409.70 b
IRR3	145.31 c	390.42 b	207.30 c
Grand mean	193.98	654.35	341.88
Coefficient of variation (%)	8.49	8.04	8.15
Least significant difference ($\alpha = 0.05$)	23.1	49.51	22.64

²IRR1, 2, and 3 are twice a day, once a day, and every second day irrigation frequency, respectively.

³Numbers followed by the same letters within a column are not significantly different at 5% level of probability.

Fig. 1. Essential oil contents of rose-scented geranium under different irrigation frequencies in a tunnel. Vertical bars are least significant difference $\alpha = 0.05$; IRR1, 2, and 3 represent twice a day, once a day, and every second day irrigation frequency, respectively.

yield of *Pelargonium graveolens* grown in 0.3 irrigation water:cumulative pan evaporation (IW:CPE ratio) than in an 0.6 IW:CPE ratio soil water regime.

In addition, the data showed that there was a clear impact of season on herbage yield. When plants experienced cold weather (Sept. 2005 harvest), the herbage yield was reduced

(Table 1). In that particular regrowth period, the average minimum and maximum temperatures inside the tunnel were 9 and 20 °C, respectively (Table 2). Although the growth period of the third harvest (Aug. 2006) was also a winter season, the temperature-controlling system was switched off because of malfunctioning and the maximum temperature (during the day) inside the tunnel was, therefore, higher (average maximum, 26 °C) than the temperature outside. As a result, the herbage yield was as high as or even higher than the growth during Spring 2005 (April and December harvest). Motsa et al. (2006) also reported that herbage yield of rose-scented geranium was higher in seasons with higher temperatures (spring/summer).

Compared with herbage yield, essential oil yield was less sensitive to the differences in irrigation frequencies (Table 3). 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 the result of a tendency of essential oil content (percent oil on herbage fresh weight basis) to increase with a decrease in irrigation frequency (Fig. 1). In all three harvests, essential oil yield was significantly reduced when plants were subjected to relatively severe water stress in the every second day irrigation schedule. Several research reports also highlighted that secondary metabolites such as essential oils are positively related to primary metabolites (Letchamo et al., 1995; Sangwan et al., 2001; Srivastava and Luthra, 1993). Rajeswara Rao (2002) also reported that total essential oil yield of rose-scented geranium was positively related to fresh herbage yield.

Essential oil yield performance among harvests was also affected by season. Despite the higher herbage yield obtained from the August harvest, essential oil yield per plant was lower than that of the April harvest (Table 3). This result could be attributed to the lower night temperatures or the wider range between day and night temperatures in the winter season during the regrowth of the August harvest (Table 2). Similarly, Motsa et al. (2006) reported that rose-scented geranium essential oil content tended to decline with decrease in night temperatures.

Irrigation frequency and brief water stress in the tunnel experiment. Figure 2 illustrates the data for herbage yield response to long-term water stress (irrigation frequency) and 1-week irrigation withholding treatments in the two growth media (silica sand and sandy clay soil). The data show that withholding irrigation for 1 week decreased fresh herbage mass significantly in IRR1 (irrigated more often), but not in IRR2 and IRR3 (irrigated less often). This could be an indication that the plants in the lowest irrigation frequency had developed a water-conserving mechanism or had limited stored water that could be lost as evapotranspiration. The data also showed that herbage yield was lower in the silica sand than in the sandy clay soil, presumably as a result of the lower water-retaining capacity of the silica sand.

Fig. 2. Herbage yield of rose-scented geranium grown in a tunnel under different irrigation frequencies and a 1-week stress period before harvest. Vertical bars are least significant difference $\alpha = 0.05$; IRR1, 2, and 3 represent twice a day, once a day, and every second day irrigation frequency, respectively; S and N represent a 1-week stress and a stress-free period, respectively.

Fig. 3. Essential oil content (percent fresh herbage mass basis) of rose-scented geranium grown in a tunnel under different irrigation frequencies and a 1-week stress period before harvest. Vertical bars are least significant difference $\alpha = 0.05$; IRR1, 2, and 3 are twice a day, once a day, and every second day irrigation frequency, respectively; S and N represent a 1-week stress and a stress-free period, respectively.

Thus, the overall result implies that high soil water results in high vegetative growth in rose-scented geranium as also reported by Weiss (1997) and Rajeswara Rao (2002).

Withholding irrigation for the last 1 week before harvesting in most cases significantly improved essential oil content (percent oil on herbage fresh weight basis) (Fig. 3). The increase in essential oil content resulted in an increase in essential oil yield per plant (Fig. 4) despite the general decline in fresh herbage yield reported in Figure 2. In general, the highest essential oil yield was obtained from a combination of high irrigation frequency (IRR1 and IRR2) and 1-week stress in the sandy clay soil. Similar to our results, De Abreu and Mazzafera (2005) reported that several plant secondary metabolites in *Hypericum brasiliense* Choisy showed an increasing trend under water stress condi-

tions. 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 observed that when plants were subjected to mild or moderate water stress, the oil content per dry weight increased by almost 100%. In addition, Weiss (1997) mentioned that rose-scented geranium essential oil yield tended to increase in water-stressed conditions. List et al. (1999), however, did not observe any increase in essential oil yield of *Melaleuca alternifolia* after the plants were stressed for nearly 1 week. These contradictory results may imply that different plant species respond differently to duration and degree of water stress.

In most cases, the contribution of irrigation frequency and brief water stress before harvest to changes in oil composition was limited (Fig. 5). To some extent, the highest

irrigation frequency (IRR1) favored 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). The overall results showed that geraniol and geranyl formate were negatively related to 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 stress levels. The relationship between geraniol and citronellol observed in the current experiment agrees with work of Rajeswara Rao et al. (1996) who reported that water and thermal stress conditions 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*.

Glasshouse experiment. The overall results of the glasshouse experiment were similar to the results in the tunnel experiment. Fresh herbage yield progressively decreased with a decrease in irrigation frequency (Fig. 6). A significant reduction was observed when irrigation was scheduled for every second day (T3) or a longer interval (T4 and T5). Although statistically not significant, the 1-week irrigation withholding event applied on T2 reduced herbage fresh weight.

In agreement with the results from the tunnel experiment and reports of Rajeswara Rao (2002) and Singh (1999), essential oil yield positively responded to irrigation frequency (Fig. 7). Thus, the results proved that essential oil is a function of primary metabolites or herbage growth (Letchamo et al., 1995; Sangwan et al., 2001; Srivastava and Luthra, 1993). Similar to the tunnel experiment, the highest essential oil yield obtained from T2 (a treatment irrigated everyday as T1 but subjected to 1-week water stress before harvesting) confirmed that imposing brief water stress before harvesting enhances essential oil yield of rose-scented geranium.

Results in Figure 8 show that essential oil content (percent oil on herbage fresh weight basis) was much higher in treatments irrigated less often (T4 and T5) and in the treatment in which a brief stress was imposed (T2). The apparent increase in essential oil content, but lower total oil yield in T4 and T5, to some extent, showed that water stress negatively affected total essential oil yield by lowering vegetative growth.

Figure 9 shows that both irrigation frequency and a 1-week irrigation withholding event did not significantly affect the composition of the seven major essential oil constituents. Geraniol and geranyl formate remained low in all treatments. In contrast, citronellol and citronellyl formate were increased and comprised more than 50% of the total recovered essential oil. High temperatures (maximum and minimum temperatures were 35 and 20 °C, respectively) during most of the regrowth period could be

Fig. 4. Essential oil yield of rose-scented geranium grown in a tunnel under different irrigation frequencies and a 1-week stress period before harvest. Vertical bars are least significant difference $\alpha = 0.05$; IRR1, 2, and 3 represent twice a day, once a day, and every second day irrigation frequency, respectively; S and N represent 1-week stress and a stress-free period, respectively.

Fig. 5. Pooled mean chemical composition (%) of essential oil of rose-scented geranium grown in a tunnel under different irrigation frequencies and a 1-week stress period. IRR1, 2, and 3 are twice a day, once a day, and every second day irrigation frequency, respectively; S and N represent a 1-week stressed and a stress-free period, respectively.

Fig. 6. Fresh herbage mass of rose-scented geranium grown in a glasshouse. Vertical bars represent least significant difference $\alpha = 0.05$; and T1, T2, T3, T4, and T5 are control (stress-free), stress-free followed by a 1-week stress (just before harvesting), every second day, third day and fourth day irrigation frequency, respectively.

the reason for the extremely high C:G ratio, which ranged between 7.5 and 10, whereas the desirable ratio is unity as reported by Motsa et al. (2006). Contrary to the present results, Doimo et al. (1999) reported that low

minimum temperatures (lower than 5.5 °C) increased C:G ratio. Motsa et al. (2006) also reported that, unlike geraniol, citronellol content showed an increasing tendency with a decrease in night temperatures.

Fig. 7. Essential oil yield of rose-scented geranium grown under different irrigation frequencies in a glasshouse. Vertical bars represent least significant difference $\alpha = 0.05$; and T1, T2, T3, T4, and T5 are control (stress-free), stress-free followed by a 1-week stress (just before harvesting), every second day, third day and fourth day irrigation frequency, respectively.

Fig. 8. Essential oil content (per fresh herbage mass) in a glasshouse. Vertical bars represent least significant difference $\alpha = 0.05$; and T1, T2, T3, T4, and T5 are control (stress-free), stress-free followed by a 1-week stress (just before harvesting), every second day, third day and fourth day irrigation frequency, respectively.

Fig. 9. Chemical composition (percent of essential oil) of rose-scented geranium grown in a glasshouse under different irrigation frequencies. T1, T2, T3, T4, and T5 represent control (stress-free), stress-free followed by a 1-week stress before harvest, and every second day, third day, and fourth day irrigation frequency, respectively.

The current study indicates that long-term water stress brings about parallel reduction in primary and secondary metabolites. Herbage yield seems to be an indicator of essential oil yield, i.e., essential oil yield is a function of primary metabolites. A brief period of water stress, on the other hand, enhanced essential oil yield. This could be an indication of reallocation of primary metabolites to secondary metabolites at certain water stress levels or duration.

At field level, applying a 1-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 authors suggest that, for the 1-week withholding period to be effective in improving geranium oil yield, certain deficit irrigation techniques (FAO, 2000) might have to be adopted to keep the subsoil as dry as possible by applying shallower but more frequent irrigation during the regrowth period.

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