ESSENTIAL OIL YIELD AND COMPOSITION OF ROSE-SCENTED GERANIUM (*PELARGONIUM* SP.) AS INFLUENCED BY HARVESTING FREQUENCY AND PLANT SHOOT AGE

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Essential Oil Yield and Composition of Rose-scented Geranium (*Pelargonium* SP.) as Influenced by Harvesting Frequency and Plant Shoot Age

by

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DECLARATION

I, Nozipho Mgcibelo Motsa, hereby declare that the dissertation, which I hereby submit for the degree MSc. (Agric) Agronomy at the University of Pretoria is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution

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N. M. Motsa

Dated this ____________________ day of ________________________2006

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Abstract

Rose-scented geranium is a world known essential oil plant priced for its high grade essential oil mostly utilized in the perfumery industry. However, South Africa has just joined the geranium oil production industry and still battling with the variations in oil yield and oil composition. It is not clear when and at what growth stage the herbage should be harvested or the stage at which it can produce the greatest essential oil. Another controversy that surrounds the production of geranium essential oil is the variation in oil composition which is speculated to be influenced by environmental factors. This study was carried out to investigate the effect of plant shoot age, temperature, seasonal changes and harvesting frequencies on herbage yield, oil yield and essential oil composition of rose-scented geranium (*Pelargonium* species, cultivar Bourbon).

The response of herbage yield, oil yield and essential oil composition to plant shoot age and temperature effects was conducted in the field. Rose-scented geranium was harvested every week for eighteen weeks per experimental cycle. Irrigation, fertilizer application and general crop management was conducted according to the crop's requirements. Measurements were made on fresh mass yield which was later computed into herbage yield, oil yield and oil composition. Oil content was generally higher (0.088%) in the spring/summer harvested plants than those harvested in autumn/winter (0.064%) cycle. Decreases in oil content in the autumn/winter cycle were negatively related to decreases in night temperatures. Greater herbage yields resulted to higher oil yields. Geraniol concentration decreased with plant shoot age in both experimental cycles and with decreases in night (minimum) temperatures in the autumn/winter experiment. The double decrease effect on geraniol concentrations in the autumn/winter experiment resulted into very high C: G ratios. Citronellol concentration was not affected by plant shoot age but was affected by decreasing night temperatures in the autumn/winter harvesting cycle.

Similarly, the response of rose-scented geranium's herbage yield, oil yield and oil composition to harvesting frequency was studied in the field. The treatments were harvesting after every two, three and four months. Standard crop management was also conducted to suit the crop requirement. Herbage yield, oil yield and oil composition were measured at each harvest. Seasonal effects on the measured variables were recorded. Harvesting frequency had a highly significant effect ($P>0.01$) on herbage yield and oil yield. Closer harvesting intervals (after two months) produced less herbage yield and oil yield while longer harvesting intervals produced more herbage yield and oil yield. Harvesting at two month intervals improved the oil composition of rose-scented geranium to lower C: G ratios but did not improve oil yield. The season of harvest also showed highly significant effects on herbage yield, oil yield and oil composition. Herbage yield, oil yield and oil composition improved when harvested in summer, compared to autumn and winter. The results suggested that high oil yield of rose-scented geranium can be obtained by harvesting after three or four months in summer. However, low C: G ratio oil, is obtainable from two month harvesting frequency, irrespective of the harvesting season.

Leaf samples were collected from the harvesting frequency experiment to study the variations of essential oil glands with leaf age. The leaf samples were treated and fixed under laboratory environments and then viewed under a scanning electron microscope for the appearance of oil glands. Two types of oil glands were identified on the leaf surfaces of rose-scented geranium and both appeared on both leaf surfaces. The adaxial leaf surface recorded higher densities of oil glands and trichomes than the abaxial leaf surfaces. Leaf position on the plant showed a significant effect in the density of oil glands. The top younger leaves contained more oil glands (all types) than the bottom older leaves.

Keywords: Rose-scented geranium, harvesting frequency, seasonal effect, herbage yield, essential oil yield, oil percentage, oil composition, citronellol, geraniol, citronellyl formate, geranyl formate, guaia-6,9-diene

INTRODUCTION

Rose geranium (*Pelargonium radens* x *Pelargonium capitatum*) is an essential oil plant that belongs to the Geraniaceae family. *Pelargonium* species originated around the southern tip of Africa and were introduced to Britain and Holland through spice trade and medicinal plant collection by sailors (Miller, 2002). Weiss (1997) also indicated that the first *Pelargonium* (*P. cucullatum*) was collected from Table Mountain in 1672, which makes South Africa to be the centre of the genus. Out of 25 *Pelargonium* species, only four species are important in the production of essential oil.

The four essential oil species are *P. graveolense*, *P. odoratissium*, *P. capitatum* and *P. radens*. The prolific essential oil producer is cultivar Rosé, a cross between *P. capitatum* and *P. radens*, which originated in Réunion and was introduced to other countries as 'Bourbon type' (Weiss, 1997).

The 'Bourbon type' cultivar is an erect, branching perennial shrub that grows up to 1.3 m in height, forming compact clumps to about 1 m in diameter. The root system grows up to 30 cm deep and can spread extensively. It has multiple soft green or green to grey stems becoming woody with age. The stems are covered with two kinds of bristles, some are long and fine while others are short and scarcely visible. The number, length and degree of branching vary (Swamy, Sreshta & Kalyanasundaram, 1960; Weiss, 1997; Demarne, 2002; Miller, 2002).

The leaves are sharply denticulated with 5 to 7 lobes which are more or less divided, green and opposite on the stem. They are soft, hairsute, fragrant, rose-scented. An essential oil is obtained from the leaves and tender shoots by distillation (Weiss, 1997; Demarne, 2002). Hay & Waterman (1993) defined essential oil as the volatile oil stored in extracellular spaces in the epidermis or mesophyll cells of plants, has a low boiling point and can be recovered from the plant tissues by steam distillation. The ability of these plant parts to produce the essential oil necessitates maximum production of herbage and preservation of the subsequent ability of the shoot to develop (Demarne & van de Walt, 1989).

The essential oil of the 'Bourbon type' of geranium possesses a tenacious rose-like odour with various nuances, such as citrus and minty undertones. The main constituents of the essential oil are citronellol, geraniol, iso-menthone, citronellyl formate, geraniol formate (Swamy *et al*., 1960; Weiss, 1997; Miller, 2002; Peterson *et al*., 2005) and guaia-6,9-diene (Rajeswara Rao, 2000). Rose-scented geranium oil resembles that of rose oil because of similar constituents, geraniol, citronellol and linalool (Anon, 2006a)

The essential oil is largely utilized in the perfumery, cosmetic and aromatherapy industries all over the world. It has since become indispensable aromatherapy oil. It is one of the best skincare oils because it is good in opening skin pores and cleaning oily complexions (Swamy *et al*., 1960; Laurence, 1996; Weiss, 1997; Miller, 2002; Peterson, Goto, Machmuah, Roy, Sasaki, & Hirose, 2005; Anon, 2006a).

Other uses of geranium essential oil that are becoming more and more popular include the treatment of dysentery, hemorrhoids, inflammation, heavy menstrual flows, and even cancer. The French medicinal community is currently treating diabetes, diarrhea, gallbladder problems, gastric ulcers, jaundice, liver problems, sterility and urinary stones with this oil. The leaves are used as a form of herbal tea to de-stress, fight anxiety, ease tension, improve circulation and to cure tonsillitis (Peterson *et al*., 2005; Anon, 2006b).

The Chinese homeopathies, on the other hand, believe that it (essential oil) opens up the liver charka and promote the expulsion of toxins that prohibit the achievement of balance within the body (Higley & Higley, 2001). In a recent study between the essential oil of geranium and tropical capsaicin, a commonly prescribed conventional remedy for shingles pain, it was discovered that geranium essential oil was extremely useful in reducing pain due to post-herpetic neuralgia followed by shingles (Greenman, Fromw, Engles, & McLellan, 2003).

The commercial variety of rose-scented geranium has undergone a lot of breeding such that it is no longer the same geranium that originated in South Africa. The right time, age and frequency of harvesting rose-scented geranium plants has always been a controversy. It is not clear when and at what growth stage the herbage should be harvested or the stage at which it can produce the greatest essential oil. Another controversy that surrounds the production of geranium essential oil is the variation in oil composition which is speculated to be influenced by environmental factors.

A study was designed to investigate the problems that surround the production of geranium essential oil with the following objectives:
Determine the seasonal changes in herbage yield, oil yield and the variation in the concentration of the major components of rose-scented geranium with plant shoot age;
determine the influence of harvesting frequency and seasonal changes on herbage yield, oil yield and oil composition;

- study the variations of essential oil glands with leaf age.

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

A large number of plant species are of commercial interest because they have the ability to produce essential oils. Essential oil is defined as the volatile oil stored in extracellular spaces in the epidermis or mesophyll cells of plants. It has a low boiling point (Hay & Waterman, 1993). Many of the essential oil plants are used as medicinal plants, mostly in folk medicine. Several of these plants are sometimes used in large amounts in the pharmaceutical, the food and the cosmetic industries. This has led to an increase in the number of essential oil plant species that are studied for their volatile components (Scheffer, 1993).

Essential oil is contained in specialized structures in all or some plant parts; cavities or ducts in the epidermis of eucalyptus leaves or citrus fruit peels; and glands or hairs originating from epidermal cells of the modified leaf hairs of geranium. Why plants secrete oils or waxes has yet to be fully explained, although certain cavities can reasonably be attributed to their presence. The oil might be produced to act as an insect repellent, to deter browsing animals or to increase disease resistance. Some terpenes from eucalyptus leaves are known to contribute to allelopathic effects on the forest floor, thus inhibiting germination and growth of competitors. Highly scented oil contained in flowers of essential oil crops is generally accepted as an aid to attract pollinators for the reproduction process (Swamy *et al.*, 1960; Weiss, 1997; Rajeswara Rao, 2000).

The most common method of essential oil extraction from plant materials is by distillation with water or steam (Bauer, Garbe & Surburg, 1990; Hay & Waterman, 1993; Wilson, 1995; Coleman, 2003). Other methods of oil extraction that exist in the perfumery and food industries include cold pressing or scarification, which is used to release the volatile substances from peels of fruits, especially citrus peels, effleurage which is used to extract oil from delicate plant materials such as flowers, solvent extraction and the latest being supercritical carbon dioxide extraction (Bauer *et al*., 1990; Wilson, 1995; Anitescu, Doneanu, & Radulescu, 1997; Coleman, 2003).

The aroma of each oil is a result of the combination of the aromas of all components. The trace components of a particular oil are more important because they give the odour a particular natural characteristic. This makes it very important that any essential oil extraction procedure maintains the natural proportion of these components. Extraction by distillation technique has been the best so far and has survived unchanged for centuries, but some improvements have been made to date since essential oil industrialization begun (Bauer *et al*., 1990; Anitescu *et al*., 1997).

Plant species that are already known to produce essential oils are often studied in order to investigate whether different chemotypes within a species do exist which possess interesting new oil composition (Scheffer, 1993). Weiss (1997) indicated that oil composition, especially the terpene component, is now important in plant classification as chemotaxonomy, since physical characters may be virtually impossible to differentiate. Biotechnology can influence the breeding or development of essential oil plants, and could theoretically produce plants containing oil of a required composition or odour. It is, however, possible agronomically to modify particular oil by varying the time of harvest and thus regulating the abundance of a specific constant.

1.2 HARVESTING TIME AND HERBAGE YIELD

The ultimate profitability of an essential oil production enterprise depends on the interrelationship of three aspects of ontogeny: (1) the time course of biomass (dry-matter) production, (2) the time course of oil content per unit of biomass and (3) the time course of oil composition. Factors 1 and 2 jointly determine the quantity of oil which can be recovered from the crop, whereas 3 determines the quality (in relation to national or international standards). Each of these aspects of ontogeny can be influenced independently by changes in management which includes harvesting the crop at maximum production or by environmental factors (Hay & Waterman, 1993).

1.2.1 Seasonal effects

Temperature

Temperature plays an important role in plant growth and yield as a whole. Plants respond to temperature changes in most of their metabolic activities such as photosynthesis, respiration and transpiration (Murtagh, 1996). For example, Weiss (1997) described geranium as a plant that produces maximum leaf growth with high oil content under warm sunny conditions. These findings were in agreement with that of Kumar, Bahl, Bansal, & Naqvi (2001) who also reported that warm environments of the Lucknow plains (India) favoured vegetative growth of geranium (per unit time).

Contrary to the results of Kumar *et al*. (2001), other researchers such as Rasejwara Rao, Kaul, Mallavarapu & Ramesh (1996) reported that during summer, geranium subjected to thermal (atmospheric as well as soil) and moisture stresses ended up producing low biomass yield. It was then suggested that this was caused by the reduction in levels of photosynthesis and damaging effects of solarization.

Other essential oil crops such as peppermint were strikingly influenced by small changes in temperature. Plant dry matter, frequency of oil glands on leaves, morphological development and oil yield responded positively to higher temperatures. Maximum leaf, stem and root matter was produced under 30^0C day temperature and the leaf mass ratio showed an increase with increasing day temperature. The combination of high day and low night temperatures (Figure 1.1) produced the greatest leaf mass ratio (Duriyaprapan, Britten & Basford, 1986).

Tea tree is another aromatic crop adapted to warmer climates. It was reported that leaf yield was more depressed (59%) in cool temperatures indicating the added effect of lower temperatures on the proportion of leaves and twigs in tea leaf (Murtagh, 1996).

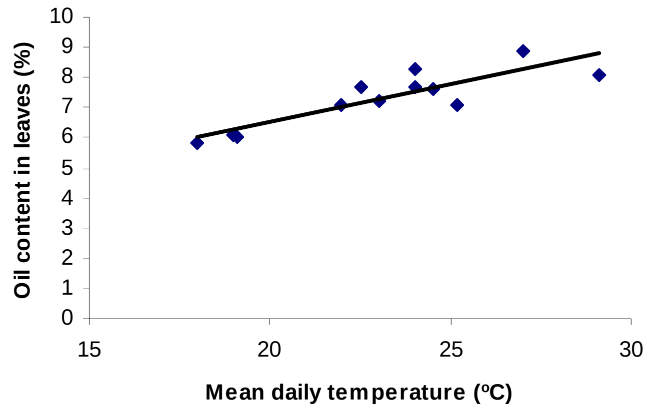


Figure 1.1: Relation between mean daily temperature and percentage oil content in the leaves of Japanese mint under four day and night temperature treatment combinations (after Duriyaprapan *et al*., 1986)

Moisture

Water is one of the most important environmental factors required for plant growth and development. The water regime plays an important role in the growth and development of the entire plant. Usually, a limited supply of water or water stress has a negative effect on the development of the plant. The negative effects are expressed in the rate of vegetative growth, reproduction, flowering, fruit set and yield. However, when focusing on the biosynthesis of secondary metabolites, water stress is not always completely detrimental (Yaniv & Palevitch, 1982; Murtagh, 1996).

Photosynthetic activity is known to be reduced in crops subjected to low light levels and water deficit conditions. Letchamo & Xu (1995) hypothesized that dry matter formation and accumulation of essential oil in thyme was closely related to photosynthesis, and limitations in the net CO_2 assimilation rates had a direct or indirect effect on shoot growth and production of the volatile oil.

The growth of geranium was reported to require an annual rainfall of 1000 to 1500 mm evenly distributed over the main growing period. Regions with sharply defined wet and dry seasons were able to produce good crop yield, provided there were no extended periods of water logging. For example, low herbage and high oil yield were recorded in geranium after three dry months as compared to three wet months (Table 1.1) in Kenya (Weiss, 1997).

Table 1.1: Effect of dry and wet periods on oil and herbage yield of geranium in Kenya (adopted from Weiss, 1997)

Time	Herbage t/ha	Oil yield	
		kg/t	kg/ha
After 3 wet months	28.2	0.73	20.6
After 3 dry months	21.8	1.07	23.3

The observation in Table 1.1 has often been reported over time, even the oldest literature indicated that geranium plants grew well in subtropical climate and could tolerate drought conditions to a certain extent, but dislike incessant heavy rains (Swamy *et al*., 1960). Weiss (1997) then added that geranium, though drought tolerant, can be severely retarded in growth, oil content, and change in oil characteristics by long dry spells.

Photoperiodic regimes

Photoperiod has a strong effect on plant performance, with respect to vegetative growth and reproductive behaviour (Farooqi, Sangwan & Swangwan, 1999). Runkle (2002) proposed that photoperiod may also influence plant height, branching and other plant growth characteristics. It may also exert a direct influence through modulation of relevant metabolic pathways, from photosynthetic carbon production and its partitioning to the Rohmer route (non-mevalonate pyruvate-glyceraldehyde-3-phosphate driven isopentenyl pyrophosphate synthesis), further leading to generation of essential oil terpenoids (Farooqi, *et al*., 1999).
These metabolic processes have a direct relevance to essential oils obtained from the foliage of certain plants such as mints. This was evident in the three different *Mentha* species (*M.*

arvensis, M. citrate, M. cardiaca) subjected to different photoperiodic treatments where it was discovered that the three species were long-day plants, exhibiting substantially higher vegetative proliferation under long day conditions. Shorter-day conditions resulted in slower growth and reduced herbage yield.

Leaf area and leaf greenness increased under long-day photoperiods in geranium. Plant dry mass was also reported to increase as a consequence of increased chlorophyll content (Langton, Adams & Cockshull, 2003). Adams & Langton (2006) took the studies of photoperiod further by observing the effect of long-day lighting on plant growth in winter. The authors reported that geranium plants increased in fresh shoot mass and the marketing stage was reached earlier than normal. These findings indicated that geranium grows better under long-day photoperiods, which was contrary to the studies of Runkle (2002) who suggested that geranium was a day neutral plant.

Letchamo & Xu (1996) measured the photosynthetic light responses of thyme and related it to physiological traits, dry matter, shoot formation and essential oil accumulation. The authors found that variability in shoot yield and essential oil was indeed associated with photosynthetic activities. All plants grown under supplemental light had an upright growth with relatively deep green coloured leaves and were aesthetically more appealing, while those grown under natural light showed a prostrate, open type of growth, an adaptation that would improve light penetration to interior leaves. Shorter, stouter and thicker leaves with more tillers and branches were developed by plants grown under supplemental light. However, the leaf length and width of natural light grown plants were significantly higher than variants grown under supplemental light.

Non-availability or restricted availability of photosynthates adversely affects essential oil synthesis and accumulation since it is biosynthesized as a secondary plant metabolite, i.e. product of photosynthesis (Rajewara Rao *et al*., 1996). The environment under which these plants are produced should allow high photosynthetic rates since the oil yield is a product of leaf yield and oil content. Both parameters are affected by the foliage density within a plot (Badi, Yazadani, Mohamed Ali & Nazari, 2004).

1.2.2 Growth stage

The main objective in essential oil crop production is to maximize or optimize biomass production, and to harvest the crop before any deterioration in oil content or quality occurs. Studies conducted on a number of aromatic crops clearly indicated that the chemical composition of the essential oil was related to the age of the leaves, thus emphasizing the importance of the growth stage at which harvesting should take place (Hay & Waterman, 1993).

It was found from some Lamiaceae species (*Mentha pipperita* and *Salvia officinalis*) that synthesis and secretion of secretory materials into the subcuticular space in the peltate glandular hairs start in very young leaves. Some authors have suggested that the number of glandular hairs was established early during leaf differentiation, while others have concluded that it increases throughout all stages of leaf development (Croteau, Felton, Karp & Kjonaas, 1981; Latchamo & Xu, 1995).

The question has often arisen whether the number of glandular hairs is fixed during the life span of the leaf or if it increases with age. Oosthuizen & Coetzee (1984) reported that the spiny hairs are initiated before glandular hairs (oil glands) in the leaf primordial of *Pelargonium*. The authors further suggested that the spiny hairs are either initiated at a lower rate than glandular hairs or are initiated only during the primordial stages of leaf development with no further spiny hairs development during leaf expansion. Knowing the stage of high glandular hair initiation is of utmost importance in geranium oil production.

Oosthuizen & Coetzee (1983) also studied the morphogenesis of trichomes of *Pelargonium scabrum* and they reported that essential oil secretion occurs from the young stage of the glandular hairs to the young and old five-celled glandular hairs. The only disadvantage is that the cuticle ruptures to release the essential oil since there are no pores in the cuticle. The glandular hair is then forced to produce another cuticle under the ruptured one so as to preserve the secretory products.

1.2.3 Method and frequency of harvesting

Most perennial aromatic crops experience reduction in biomass yield due to repeated harvesting, thus affecting essential oil yield. Repeated harvests can either be beneficial or detrimental to oil production, depending on other environmental factors. For example, herbage yield is usually high at first harvest, becomes constant and declines with repeated harvests (Murtagh, 1996; Weiss, 1997).

Kothari, Bhattacharya & Ramesh (2004) found that biomass yield was greater in the first harvest and gradually declined in the subsequent harvests of *Ocimum tenuiflorum*. The method of harvesting had no effect on biomass yield. Contrary to the decrease in biomass yield, essential oil content in general was lower in the first harvest and increased gradually in subsequent harvests to reach maximum in the fourth harvest.

Rose geranium (*Pelargonium* sp.) has a life span of six to eight years under commercial production and the first harvest is carried out at 6 - 8 months after planting. Subsequent harvests are then conducted at 3 – 4 month intervals. Considerable time is taken before harvesting the first crop so that it could establish itself. It is, thereafter, harvested at close intervals (3 – 4 months) to avoid losses in oil yield due to leaf senescence (Weiss, 1997; Rajeswara Rao, 2000). Harvesting of secondary branches of *O. tenuiflorum* led to maximum plant height, plant spread and number of secondary branches during second and subsequent harvests (Kothari *et al*., 2004).

Rose-scented geranium plants are normally cut at 15 to 20 cm above ground to allow re-establishment of new leaves for the process of photosythesis (Weiss, 1997; Demarne, 2002). The same height is maintained when harvesting tea tree and European pennyroyal. This cutting height may have a negative effect on the amount of herbage sent for oil distillation and analysis because the basal parts of the foliage remain on the field (Stengele & Stahl-Biskup, 1993).

Rajeswara Rao, Singh & Bhattachary (1990) investigated two harvesting methods on rose-scented geranium. In one experiment, all the herbage was harvested (leaving 2-3 nodes from ground level) leaving one branch for rejuvenation. In another experiment, the top 15 cm herb consisting of green leaves and tender stems were harvested. The results obtained showed that the plants cut at ground level produced more herbage but less essential oil. They then proposed that harvesting the top 15 cm produces higher essential oil yield due to higher leaf/stem ratio.

1.3 HARVESTING TIME ON OIL YIELD AND OIL COMPOSITION

The essential oil in geranium is mostly contained in the leaves, therefore, the greater the proportion of leaves in the harvested produce the better the recovery and yield of oil (Rajeswara Rao *et al*., 1990). Murtagh & Smith (1996) described oil yield as the mathematical product of leaf yield and oil concentration/content. The oil concentration has the same weighting as leaf yield in determining the economic return of a crop. Determination of the correct harvesting time is extremely important both for maximum yield and for highest oil quality. Kumar *et al*. (2001) also found out that essential oil yield of geranium was predominantly affected by herbage yield and only to a negligible extent by the oil content in the herb. These (herbage yield and oil concentration), according to Murtagh (1996), appears to accumulate and fluctuate independently.

1.3.1 Seasonal and environmental conditions

The season or month of harvesting was observed to exert remarkable influences on the oil yield of essential oil crops. Weiss (1997) reported that the highest oil content of geranium was observed in July (rainy/monsoon) and lowest in February (spring) in southern India. Doimo, Mackay, Rintoul, Aray & Fletcher (1999) reported that not only the seasons and months of harvest affected oil yield, but that the geographic area where these crops were grown also influenced yield. Eighteen world types of geranium were studied in Australia where it was found that they did not produce the amount of oil and oil composition they would produce in their native countries. For example Chinese oil was higher in both yield and oil costituents than Réunion oil, yet the world’s geranium oil is rated using Réunion oil as standard. The chemical composition of the essential oil was also observed to be influenced by environmental changes i.e. seasonal changes with different soil water content, temperature and photoperiod. These environmental conditions may increase or decrease different terpenoids in the crop.

Moisture

In an irrigation experiment of fifteen medicinal plants (*Officinal* sp.) rich in essential oil, it was found that the formation and accumulation of the essential oil tended to increase under dry growing condition. In xerophyte species, such as *Artemisia absinthium*, *Matricaria chamomilla* and *Lavendula spica*, the irrigated plants contained more essential oil as expressed in volumetric units. However, the relative values were largest in non-irrigated plants. The levels of the substances (composition) present depended on the developmental stage at which the irrigation had been applied (Yaniv & Palevitch, 1982).

Langenheim, Stubblebine & Foster (1979) found no significant variations in oil composition and oil yield of *Hymenaea courbaril* due to water stress. Although some changes in oil composition occurred under dry conditions, leaf development was retarded and the variation was consistent with the progressive change in composition with leaf development.

Photoperiodic regimes

Manipulation of the photoperiod may benefit essential oil yield and composition at the expense of the crop biomass. In a *Mentha* experiment, the short-day condition in all three species (*M. arvensis, M. citrate, M. cardiaca*) yielded the greatest essential oil content and best composition while the long-day plants produced high oil yield (Farooqi *et al*., 1999).

Contray to the *Metha* experiment, *Thymus vulgaris* was observed to increase the accumulation of oil in the leaves under supplemental light as compared to natural light grown plants. It was concluded that the photosynthetic input of CO_2 increased the number or the density of essential oil glands per given leaf area, or it increased both the number and the size at the same time (Letchamo *et al*., 1995).

Temperature

High or low temperatures may favour the development of oil glands on leaf surfaces. An experiment on Japanese mint revealed that the number of oil glands per unit leaf area on the adaxial leaf surface responded differently to high day temperature treatment than those on the abaxial leaf surface. The oil glands on the adaxial surfaces were greater in number at 35^0C day temperature and it was observed that they increased in number with increases in night temperature while those on the abaxial surface remained costant at all temperatures (Duriyaprapan *et al*., 1986).

Studies conducted on two chemotypes of rose-scented geranium showed that weather parameters such as temperature and rainfall influenced the content and chemical constituents of the oil. Hot months were observed to favor the accumulation of citronellol while cool months favoured geraniol (Prakasa Rao & Ganesha Rao 1995; Rajeswara Rao, 2002). Rajeswara Rao *et al*. (1996) studied the response of citronellol concentration on geranium essential oil and observed that it peaked in summer rather than in winter-spring. The proposed mechanism involved adjustments to moisture and thermal stress and direct effect of weather parameters (temperature and photoperiod). Other studies on rose-scented geranium indicated that citronellol concentration peaked during the late winter-spring and was minimal in autumn (Doimo *et al*., 1999).

When comparing the citronellol: geraniol ratio, it was found that cold periods (where the minimum dry bulb temperature was reduced to 2 ^{o}C) resulted in rapid decrease in geraniol as compared to citronellol. The levels of geraniol were reduced in winter but no distinct peak was determined, although it tended to be higher in spring-summer (Doimo *et al*., 1999). Increases in day temperatures also increased menthone concentration in Japanese mint but menthanole was not affected much (Duriyaprapan *et al*., 1986).

1.3.2 Growth stage

Oil composition and oil yield may decrease or increase with age. In an experiment on sage (*Salvia officinalis* L.), Croteau *et al*. (1981) noted an increase in total number of oil glands (all types included) during development of leaves and demonstrated that immature sage leaves synthesized and accumulated camphor most rapidly. The camphor content increased as the leaves expanded. The same pattern of growth was observed in *Ocimum gratissimum*, but the oil content and oil composition decreased in mature senescing leaves (Charles & Simon, 1993).

White & Iskandar (1987) reported similar findings in peppermint *(Mentha piperita*) where menthol occurred in older leaves and menthone in small immature leaves but declined as they matured. This means that the mixture of old and new leaves provided a blend of menthol and menthone in the crop, which changed as the crop aged. On the other hand, Charles & Simon (1993) reported that leaf oil content decreased with maturity in most *Mentha* species. Harvesting time was determined by the oil composition desired by the farmer.

Rajeswara Rao *et al*. (1993) studied the changes in the profile of essential oil of rose-scented geranium leaves during their ontogeny. The results showed that essential oil concentration decreased with leaf age inspite of an increase in leaf area. The authors suggested that accumulation of essential oil was controlled by the balance between synthesis and catabolism of essential oil. In rose-scented geranium, synthesis of oil appears to exceed catabolism only in the first leaf. According to Rajeswara Rao *et al*. (2002) and Weiss (1997), the oil was high from the onset of flowering, highest at full bloom, and rapidly decreased thereafter. The authors noted that the plant usually did not flower at all. This was in agreement with Kumar *et al*. (2001) who reported an increase in the expression of secondary metabolites involved in the accumulation and synthesis of essential oil in the shoot of geranium, thus the high density of oil glands on young leaves. Rajeswara Rao, *et al*. (1996) also found that the moment of sampling/harvesting (time of the day) of plant material was another important factor that affected oil composition. It (moment of sampling) influenced the terpenoid composition such as geraniol which was reported to decrease with leaf age.

Stengele & Stahl-Biskup (1993) studied oil content in different plant parts of *Mentha pulegium* by separating the leaves into three sections; Part A (whorls 1-6 from the ground), part B (middle part, whorls 7-12) and part C (top of the plant, whorl 13). Part A contributed 0.07% oil, part B 0.13% and part C 0.45%. They concluded that the lower part A made only one tenth of the total oil of a plant because of its low oil content. Thus emphasizing the fact that lower leaves contribute a negligible amount of oil to the total oil yield per plant.

In the same experiment of *M. pulegium*, these researchers harvested the plants at different growth stages (young shoots, vegetative stage and flowering stage). Oil composition that was produced at full bloom (the common harvesting stage) recorded the lowest pulegon concentration and highest concentrations of menthone. Pulegon is the main compound of *M. pulegium*.

1.4 EFFECTS OF DISTILLATION METHODS ON OIL COMPOSITION

Kaul, Rajeswara Rao, Bhattacharya, Sigh & Sigh (1995) distilled geranium plants by two methods of hydro-distillation, i.e. water distillation and steam distillation, and compared the composition of volatile oil recovered. Desirable results were obtained from steam distilled oil since there was little hydrolysis as compared to water distilled oil.

Kaul & Babu (2005) explored the distillation methods even further. They compared the two distillation methods with and without cohobation and recycling treatment. The results showed that steam distilled geranium oil was rich in ester content and was the most preferred since esters comprise the larger portion of the important compounds used in flavours and fragrances. Oil produced from recycling enabled the extraction of an alcohol rich oil dissolved in the hydrosols.

Guerere *et al*. (1985) as quoted by Gomes, Mata & Rodrigues (2004) compared different oil extraction methods (hydrodistillation both in copper still and in laboratory glass apparatus and hexane extraction) and found that the geraniol concentration was lower in both hydrodistillation methods. To explain this, they proposed a reaction mechanism where geraniol was transformed into linalool through water vapour action.

Chiasson, Belanger, Bostanian, Vincent & Poliquin (2001) distilled the essential oils of *Artemisia absinthium* and *Tenacetum vulgare* to be used as an insecticide for controlling twospotted spider mite. The authors also used steam distillation and water distillation to extract the essential oil. All extracts were lethal to the spider mite but with variable results. Steam distilled extracts were more effective in controlling the spider mite than the water distilled extracts. They concluded that extraction techniques quantitatively and qualitatively affected the constituents of the natural oil.

The results of Chiasson *et al*. (2001) agree with those of Charles & Simon (1990), who compared water and steam distillation on basil. The authors reported that steam distillation produced higher yield than water distillation. No significant differences were found in the oil constituents of the two methods.

CHAPTER 2

PLANT SHOOT AGE AND TEMPERATURE EFFECTS ON HERBAGE YIELD, OIL YIELD AND OIL COMPOSITION OF ROSE-SCENTED GERANIUM

2.1 INTRODUCTION

Oil yield is the mathematical product of leaf yield and oil concentration. The oil concentration has the same weighting as leaf yield in determining the economic return from a crop (Murtagh & Smith, 1996). In a general study of essential oil crops, Hay & Waterman (1993) indicated that the ultimate profitability of any essential oil production enterprise depends on the interrelationship of three aspects of ontogeny: (1) the time course of biomass (dry-matter) production, (2) the time course of oil content per unit of biomass and (3) the time course of oil composition. One and 2 jointly determine the quantity of oil which can be recovered from the crop, whereas 3 determines the quality (in relation to national or international standards). Each of these aspects of ontogeny can be influenced independently by changes in management (which includes harvesting the crop at maximum production) or environmental factors.

Like all essential oil crops, geranium oil yield is a product of the three components of which the main ones are leaf yield and oil concentration within the above ground plant material (leaves and branches), and each appears to accumulate and fluctuate independently (Murtagh, 1996). The fluctuations in the biomass yield and oil concentration is due to seasonal changes and locality effect, and in addition to that, oil concentration can change in the short term (Murtagh & Smith, 1996). Rajeswara Rao *et al*. (1996) identified temperature changes, soil water regimes, photoperiod and season of harvest as the main factors that exert a profound influence on the variability of biomass and essential oil yield.

The moment of sampling/harvesting was also identified as an important factor that may strongly influence the composition of the essential oil isolated. Rajeswara Rao *et al*. (1996) suggested that the terpenoid composition of rose-scented geranium was strongly influenced by the age of the leaves. Some terpenoids were found in higher concentration in young leaves than in the older leaves. The same observation was recorded on sage (*Salvia offinalis*), where the camphor content was synthesized and accumulated rapidly from the young immature leaves. The only difference from rose-scented geranium was that the camphor content increased as the leaves expanded (Croteau *et al*., 1981).

Studies by Ram & Kumar (1996) on the effect of plant age of geranium (harvesting geranium shoots at 4, 6, and 8 fully expanded leaves) revealed that plants harvested at 4 fully expanded leaves produced high essential oil yield with high citronellol and geraniol content, and low iso-menthone content. The 6 and 8 fully expanded leaf treatments produced higher plant biomass, with an accompanying increase in iso-menthone, and decreased citronellol and geraniol content.

The effect of temperature on biomass has been investigated, but little has been done to investigate the role of temperature on oil composition. Doimo *et al*. (1999) studied the effect of seasonal changes in the major alcohols (citronellol and geraniol) of geranium and their relationship with temperature over an extended period of time. The authors discovered that citronellol concentration peaked during late winter-spring and was minimal in autumn. Geraniol level, on the other hand, was reduced in winter but no distinct peak period was identified. Its concentration tended to be higher in spring-summer. These findings were not in line with the findings of Rajeswara Rao *et al*. (1996) who indicated that citronellol production was highest in summer. They further proposed that the increase in citronellol concentration was a mechanism to fight/adjust to moisture and thermal stress.

This experiment was aimed at investigating the seasonal changes in herbage yield, oil yield and the variation in the concentration of the major components of rose-scented geranium oil with plant shoot age.

2.2 MATERIALS AND METHODS

2.2.1 General site and climate description

A field experiment was conducted at the University of Pretoria's Experimental Farm (Hatfield) located at latitude 25^{o} 45'S, longitude 28^{o} 16'E and at an altitude of 1372 m above sea level. The region is a summer rainfall area with an average rainfall of about 650 mm per annum (October – March). Climatic data for the period of experimentation is presented in Table A1 of the Appendix. The first harvesting cycle was carried out in the autumn/winter season and the second was carried out in the spring/summer season of 2005 to 2006. The experimental layout was a randomized complete block design (RCBD) with three blocks. Figure 2.1 shows the over view of the weekly harvesting field experiment.

2.2.2 Planting and general crop management

Geranium seedlings were planted on a sandy clay loam soil (22.2% clay) in October 2004. The soil was cultivated and harrowed to a fine tilth and seedlings were planted at 0.625 m intra-row and 1.0 m inter-row spacings. The plants were given six months to establish, and then cut back to approximately 15 to 20 cm height before applying treatments. Plants were allowed to grow uniformly for six weeks before commencement of weekly harvests. Harvesting was conducted every Thursday at the same time of the day for eighteen weeks.

The first cycle began on 31 March 2005 and ended on 28 July 2005 (autumn/winter experiment). The plants were cut back for the second cycle of data collection, which began on 27 October 2005 and ended on 23 February 2006 (spring/summer experiment). Weekly irrigation was scheduled using a neutron probe (Neutron water meter, model 503 DR, CPN Corporation, Martinez, California) where plants were irrigated to field capacity. Standard plant management and plant protection practices were followed. Nitrogen, P and K were applied in three splits at a rate of 60, 90 and 60 kg ha^{-1} year^{-1}, respectively.

Figure 2.1: View of the weekly harvesting field experiment

2.2.3 Plant growth recording

The vegetative data collected and analyzed in this experiment were leaf area, plant height, plant fresh and dry mass and moisture content. These growth parameters were recorded at each harvest, i. e. every week for the duration of the experiment.

Leaf area index

Two plants were randomly harvested from each plot every week (Thursday) to determine the leaf area index. Leaf area (LA) of each plot was measured using a belt driven leaf area meter LI 3100 (LiCor, Lincoln, Nebraska, USA). Leaf area index (LAI) was determined using the relationship between leaf area (LA) and harvested land area (HA) as expressed in Equation 2.1.

$$\mathbf{LAI = LA/HA} \qquad \mathbf{(2.1)}$$

Canopy height, fresh and dry mass

Plant height was recorded using a tape measure before plants were cut for leaf area measurement. Immediately after the determination of leaf area, the sampled canopy was dried in an oven at 68 ^{o}C for 72 hours to determine the oven-dried mass. The oven-dried samples were weighed and subtracted from the fresh mass so to determine the moisture content.

2.2.4 Total yield recording, essential oil distillation and analysis

Fresh mass was measured at each harvesting date and later computed into total herbage yield per month. Approximately 5 - 8 kg of fresh biomass was collected for estimation of oil concentration. Oil concentration was determined by steam distillation for one hour using a custom built unit. Oil analysis was determined by gas chromatography (GC). An Agilent GC (FID) model 6890N fitted with 30 m x 0.25 mm fused silica capillary column, and film thickness of 0.25 µm, was used. Temperature was programmed from 50 ^{o}C – 200 ^{o}C at 5 ^{o}C/min and with detector and injector temperatures at 220 ^{o}C. Component identification was done by comparing retention times and Kovat indices to standard values (Adams, 2004). Experimental data was analyzed using the Statistical Analysis System (SAS, 1999), and ANOVA was conducted to a significance level of $P \leq 0.05$. Regression analysis of the data was run using MS Excel.

2.3 RESULTS AND DISCUSSION

2.3.1 Impact of shoot age

Leaf area index (LAI)

Leaf area index values from the two harvesting experiments are presented in Table 2.1. The results show that LAI positively responded to plant shoot age. LAI gradually increased from week 1 to week 14 of the autumn/winter experiment while in the spring/summer experiment, it increased up to week12 and thereafter, declined until the last harvesting week (Figure 2.2).

The highest values were recoded in weeks 14 and 12 in the autumn/winter and spring/summer experiments, respectively. This observation is an indication that LAI of geranium plants increases with plant shoot age up to a certain point, i.e. up to 14 weeks in autumn/winter and up to 12 weeks in spring/summer (Figure 2.2). Plant growth was generally accelerated in the spring/summer experiment compared to the autumn/winter experiment. The decrease in LAI after weeks 14 and 12 of the two experiments might have been be due to leaf senescence since plants had already reached the highest point of leaf production and expansion.

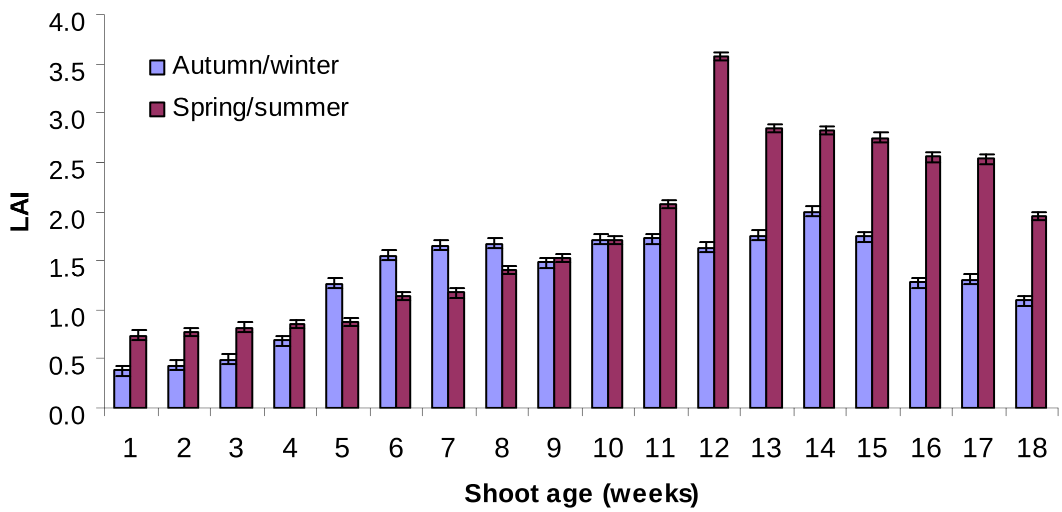


Figure 2.2: Leaf area index of rose-scented geranium as affected by plant shoot age in the autumn/winter and spring/summer harvesting experiments

It was also observed that the average LAI was higher in the spring/summer experiment (average of 1.78) than in the autumn/winter experiment, where it was 1.32. LAI was favoured by the spring/summer conditions than the autumn/winter conditions. A similar observation was also recorded for plant height. The spring/summer experiment recorded higher average plant height (38.11 cm) than the autumn/winter experiment (37.00 cm) (Table 2.1).

Table 2.1: Impact of plant shoot age on plant height, LAI and oil content in different growing seasons

	Autumn/winter experiment			Spring/summer experiment		
Shoot age (weeks)	Plant height[z] (cm)	LAI	Oil content (%)	Plant height (cm)	LAI	Oil content (%)
1	-	0.38 k	0.070	18.00 i	0.74 m	.
2	-	0.43 kj	0.040	21.00 h	0.77 lm	0.051
3	24.00 k	0.49 j	0.102	24.33 g	0.82 lkm	0.065
4	26.33 j	0.69 i	0.082	26.67 feg	0.85 lk	0.062
5	30.00 i	1.26 g	0.062	26.33 fg	0.87 k	0.065
6	34.00 h	1.55 fe	0.070	27.33 fe	1.14 j	0.073
7	37.33 g	1.65 ced	0.091	29.33 e	1.17 j	0.132
8	39.00 f	1.67 cbd	0.074	34.00 d	1.40 i	0.098
9	42.33 d	1.48 f	0.064	39.00 c	1.52 h	0.101
10	48.00 a	1.71 cbd	0.056	39.33 c	1.70 g	0.094
11	40.67 e	1.72 cbd	0.075	46.67 b	2.07 e	0.071
12	45.67 b	1.63 ed	0.070	50.00 a	3.57 a	0.072
13	40.67 e	1.75 b	0.045	51.00 a	2.84 b	0.103
14	42.67 d	2.00 a	0.038	52.00 a	2.82 cb	0.114
15	45.33 b	1.74 cb	0.050	51.00 a	2.75 c	0.119
16	43.00 cd	1.27 g	0.041	52.67 a	2.55 d	0.070
17	41.67 ed	1.30 g	0.062	51.33 a	2.53 d	0.101
18	44.33 cb	1.09 h	0.060	46.00 b	1.95 f	0.097
Grand mean	37.00	1.32	0.064	38.11	1.78	0.088
$LSD_{(\alpha = 0.05)}$	1.54	0.101		2.97	0.087	
C V (%)	2.37	4.57		45.10	2.95	

[z] **Means with the same letter within a column are not significantly different at 5% level of probability**

The similarities in the response of LAI and plant height to plant shoot age led to further investigations. A correlation between the two variables showed a positive relationship (r^2 = 0.88 and r^2 = 0.70) in the spring /summer and autumn/winter experiments respectively. This relationship means that LAI will keep on increasing as long as plants continue to grow (increase in height).

Rose-scented geranium plants are valued for their essential oils, extracted from the plant canopy. Kumar *et al*. (2001) indicated that the leaves are the most important plant part that produces geranium oil since they contain most of the oil glands and trichomes. Lower LAI values were recorded in the weeks of higher oil content (week 3 of the autumn/winter experiment and week 7 of the spring/summer experiment) (Table 2.1).

As the LAI increased, the oil content showed a tendency to decrease in the autumn/winter experiment, while it fluctuated in the spring/summer experiment (Figure 2.3). Both experiments recorded low oil content at higher LAI. An oil content of 0.038% (the lowest recording in the experiment) was recorded in week 14 of the autumn/winter experiment, coinciding with the highest LAI recording. The same was true for the spring/summer experiment, where an oil content of 0.072 % was recorded in week 12.

Based on this observation, a correlation between LAI and oil content was made to observe whether there was a relationship between the two variables. No relationship was obtained found between oil content and LAI in both experiments. This means that an increase in LAI does not benefit essential oil content in rose-scented geranium plants. Unlike in camphor production (Croteau *et al*., 1981) where camphor increased with leaf expansion. However, this observation is in line with that of Rajeswara Rao *et al*, (1993) who reported that essential oil concentration decreased with leaf age in spite of an increase in leaf area.

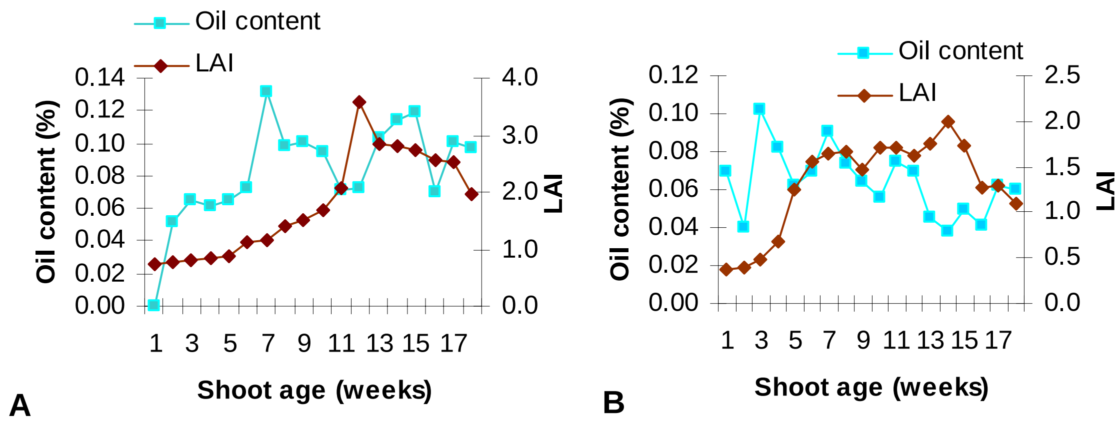


Figure 2.3: Changes in oil content and leaf area index during the two harvesting experiments. A) Autumn/winter harvesting experiment, B) Spring/summer harvesting experiment

Herbage yield and essential oil yield

The greatest fresh mass was produced by plants harvested from week 12 up to the last week (week 18) in the spring/summer experiment (Table 2.2), while plants that recorded the greatest fresh mass in the autumn/winter experiment were harvested from week 6 to week 16 (Table 2.3). Plants fresh mass and herbage yield steadily increased in the spring/summer experiment up to the last experimental week as compared to the autumn/winter experiment. The results showed a clear correlation ($r^2 = 0.83$) between fresh mass per plant and total herbage yield in the spring/summer experiment (Figure 2.4) as compared to the variations observed in the autumn/winter experiment (Figure 2.4).

These results illustrated that geranium plants reached maximum herbage production after 12 weeks and maintained that for six weeks without significant decreases in the spring/summer experiment. In the autumn/winter experiment, plants reached maximum herbage production after 6 weeks, maintained that for ten weeks and thereafter, showed a steady decrease.

Table 2.2: Impact of plant shoot age on herbage yield and essential oil yield in the spring/summer harvesting experiment

Shoot age (weeks)	Herbage yield[z] (t/ha)	Fresh mass (kg/plant)	Dry mass (kg/plant)	Moisture content (%)	Oil yield (kg/ha)	Oil content (%)
1	6.51 [f]	0.31 [h]	0.11 [e]	59.06[bac]	.	.
2	6.98 [f]	0.53 [hg]	0.14 [e]	68.72[bac]	3.58 [h]	0.051
3	10.97 [ef]	0.60 [hfg]	0.12 [e]	79.81[ba]	7.15 [hg]	0.065
4	7.86 [f]	0.51 [hg]	0.13 [e]	73.78[ba]	4.87 [h]	0.062
5	11.29 [ef]	0.51 [hg]	0.15 [e]	69.59[bac]	7.39 [hg]	0.065
6	11.09 [ef]	0.78 [hfg]	0.22 [de]	72.17[ba]	8.11 [hfg]	0.073
7	13.47 [edf]	0.95 [efg]	0.20 [de]	78.58[ba]	17.77 [efg]	0.132
8	19.43 [ed]	1.23 [efd]	0.22 [de]	81.68[a]	19.08 [ef]	0.098
9	21.76 [ed]	1.12 [efg]	0.287 [de]	74.45[ba]	21.94 [ed]	0.101
10	39.02 [c]	1.23 [efd]	0.44 [de]	60.82[bac]	36.56 [bc]	0.094
11	44.88 [bc]	1.97 [bc]	0.38 [de]	80.45[a]	32.04 [dc]	0.071
12	59.75 [a]	2.84 [a]	1.52 [a]	46.64[c]	42.81 [bac]	0.072
13	52.19 [ba]	1.79 [bcd]	0.38 [de]	78.84[ba]	53.71 [a]	0.103
14	45.09 [bc]	2.33 [ba]	1.07 [bc]	57.97[bc]	51.35 [a]	0.114
15	38.24 [c]	2.03 [bc]	0.47 [de]	76.96[ba]	45.64 [ba]	0.119
16	56.94 [a]	2.89 [a]	1.30 [ba]	58.69[bac]	39.75 [bc]	0.070
17	23.45 [d]	2.06 [bc]	0.65 [dc]	70.05[ba]	23.68 [ed]	0.101
18	40.53 [c]	1.53 [ecd]	0.49 [de]	66.65[bac]	39.32 [bc]	0.097
Grand mean	28.30	1.40	0.46	69.71	26.75	0.088
$LSD_{(\alpha = 0.05)}$	11.26	0.63	0.48	23.37	11.26	
C V (%)	23.97	27.24	62.84	20.20	25.30	

[z] Means with the same letter within a column are not significantly different at 5% level of probability

Table 2.3: Impact of plant shoot age on herbage yield and essential oil yield in the autumn/winter harvesting experiment

Shoot age (weeks)	Herbage yield[z] (t/ha)	Fresh mass (kg/plant)	Dry mass (kg/plant)	Moisture content (%)	Oil yield (kg/ha)	Oil content (%)
1	3.36 k	0.31 g	0.06 d	81.24 bdac	2.35 l	0.07
2	6.60 j	0.38 g	0.06 d	82.84 bac	2.64 l	0.04
3	9.31 i	0.66 f	0.07 d	89.33 a	9.49 gh	0.102
4	11.93 h	0.73 f	0.09 d	87.36 ba	9.78 g	0.082
5	15.12 g	1.107 e	0.41 bc	64.81 edc	9.37 h	0.062
6	19.01 b	1.59 c	0.60 ba	62.13 edc	13.31 d	0.070
7	17.31 cd	1.73 b	0.56 ba	68.10 ebdc	15.75 a	0.091
8	18.76 b	1.70 b	0.48 bac	71.72 ebdac	13.88 b	0.074
9	21.46 a	1.95 a	0.75 a	61.65 ed	13.75 cb	0.064
10	16.86 de	1.98 a	0.69 ba	65.31 edc	9.50 gh	0.056
11	14.76 g	1.96 a	0.60 ba	69.03 ebdac	11.07 e	0.075
12	19.34 b	1.44 d	0.44 bac	68.20 ebdc	13.47 cd	0.070
13	16.36 fe	1.35 d	0.50 bac	63.28 edc	7.41 j	0.045
14	17.65 c	1.65 cb	0.65 ba	60.39 e	6.64 k	0.038
15	16.03 f	1.64 cb	0.61 ba	62.83 edc	8.00 i	0.050
16	19.35 b	1.39 d	0.45 bac	67.72 ebdc	7.95 i	0.041
17	16.48 fe	1.46 d	0.41 bc	71.37 ebdac	10.16 f	0.062
18	16.33 fe	1.04 e	0.23 dc	77.73 ebdac	9.80 gf	0.060
Grand mean	15.33	1.34	0.43	70.84	9.68	0.064
$LSD_{(\alpha = 0.05)}$	0.596	0.11	0.32	20.84	0.38	
C V (%)	2.34	4.99	44.62	17.73	2.36	

[z] Means with the same letter in within a column are not significantly different at 5% level of probability

The decrease in herbage yield might have been caused by plant age and early leaf senescence as a result of cold unfavourable weather conditions in winter. This indicated that plant aging is probably more accelerated in autumn/winter conditions than spring/summer conditions.

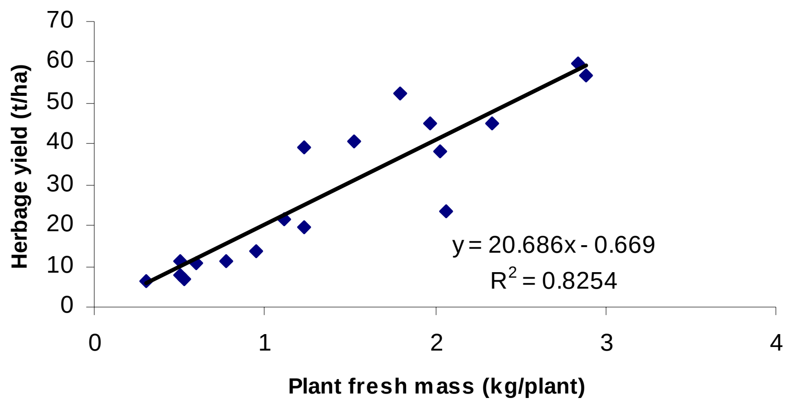


Figure 2.4: Relationship between herbage yield and plant fresh mass in the spring/summer experiment

Rose-scented geranium plants harvested in the spring/summer experiment had greater average fresh mass (1.40 kg) when compared to plants harvested in the autumn/winter experiment (1.34 kg) (Tables 2.2 and 2.3). The greater plant fresh mass resulted in a higher average herbage yield (28.3 t/ha) in the spring/summer experiment. The autumn/winter experiment on the other hand, resulted in an average herbage yield of 15.3 t/ha.

Oil content (percentage) was higher in the spring/summer experiment (0.088%) than in the autumn/winter experiment (0.064 %) (Tables 2.2 and 2.3). The greatest oil content was recovered in week 3 (0.102%) and week 7 (0.132%) in autumn/winter and spring/summer experiments, respectively. Greater plant fresh mass did not result into greater oil content in both experiments. The highest oil yield in the autumn/winter experiment was recorded from week 7 (15.75 kg/ha) to week 12. It then decreased from week 13 up to the end of the experimental period. The oil yield decreased even in the weeks that recorded higher herbage yield (weeks 14 – 16). This observation was rather strange for geranium oil yield since it was reported to be entirely dependant on herbage yield (Kumar *et al*., 2001; Rajeswara Rao, 2002). The decrease in oil yield can hardly be blamed on lower oil contents because the herbage yield was expected to compansate for that. The oil content on the other hand was relatively constant throughout the eighteen harvesting weeks.

The greatest oil yields in the spring/summer experiment (53.71 and 53.35 kg/ha) corresponded to the weeks that produced more plant mass and consequently higher herbage yield. The oil content was also relatively high in the plants distilled during these weeks. The essential oil yield was generally increased from week 10 to 18 similar to herbage yield. The increase in plant fresh mass and herbage yield (in spring/summer) may be related to a better response of plants to higher/suitable temperatures and increased metabolic activities such as photosynthesis, respiration and transpiration.

The increase in oil content towards the end of the spring/summer experiment led to further investigations. Oil content was correlated to plant shoot age and no relationship was observed between the two variables in both experiments. Another correlation was made between oil content and moisture content to find out if increases in moisture content had an influence on oil content. No relationship was observed between moisture content and oil content in both experiments. This might mean that geranium oil is produced independently of the plant sap.

The general observation from the two experiments indicated that herbage yield was the dominant factor influencing total oil yield rather than oil content. This result agrees with studies by Kumar *et al*. (2001), which indicated that geranium essential oil yield was predominantly affected by herbage yield and only to a negligible extent by the oil content in the leaves.

Oil composition

Geranium essential oil consists of a number of compounds that are responsible for its odour. Only seven of these compounds were analyzed for in the current experiment namely linalool, iso-menthone, citronellol, geraniol, geranyl formate, citronellyl formate and guaia-6,9-diene. All seven compounds were present in the essential oil at different concentrations (Tables 2.4 and 2.5). There was no oil to analyze on week 1 of the spring/summer experiment due to inadequate plant material sent for oil distillation.

Statistically significant variation in concentration of all compounds analyzed for occurred in both harvesting experiments. There was a negative linear relationship between geraniol content and plant shoot age for both experiments (r^2 = 0.87 in autumn/winter and r^2 = 0.81 in spring/summer). Geraniol content, therefore, decreased with plant shoot age. Citronellol content, on the other hand, was not significantly affected by plant shoot age in either harvesting experiments. Consequently, the citronellol: geraniol ratios increased with plant shoot age (Figure 2.4). This data supports earlier observations by Rajeswara Rao *et al*. (1993) and Gomes *et al*. (2004), which indicated that geraniol is affected by leaf age. Geraniol and linalool contents are decreased by 40 – 70% in the yellow leaves (old leaves) while citronellol and its esters maintain their levels. Rajeswara Rao *et al*. (1993) proposed that geraniol is biosynthesized early in oil formation and accumulation, and is subsequently metabolized by the leaves.

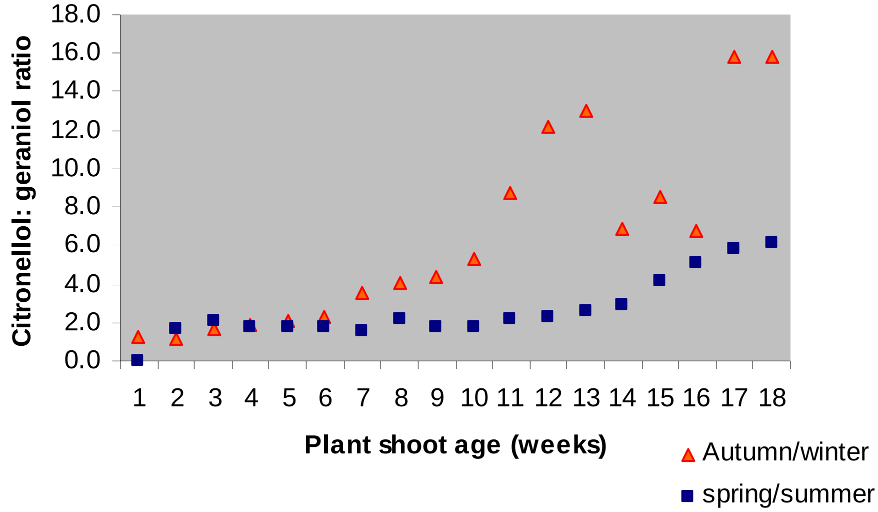


Figure 2.5: Citronellol: geraniol ratios as affected by plant shoot age in the autumn/winter and spring/summer experiments

Table 2.4: Effect of plant shoot age on the composition (percentage) of rose-scented geranium oil (autumn/winter experiment)

Shoot age (weeks)	Linalool	Iso-menthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Guaia-6,9-diene
1	0.23	1.60	20.62	16.25	17.58	12.19	8.28
2	0.97	3.73	24.08	20.41	16.51	12.07	4.73
3	0.85	3.92	27.75	17.02	17.73	11.33	4.04
4	0.92	4.16	27.87	14.91	18.58	11.66	5.25
5	1.40	3.14	28.80	13.61	19.94	11.45	4.83
6	1.06	5.20	27.75	12.34	19.61	11.72	4.91
7	0.74	3.97	33.18	9.32	23.61	11.12	5.47
8	0.67	3.69	32.98	8.11	21.62	10.12	4.72
9	0.91	3.33	31.13	7.04	21.13	8.64	4.96
10	0.80	5.06	33.44	6.27	21.94	8.36	4.67
11	0.46	3.04	35.98	4.14	25.61	7.74	4.80
12	0.35	4.95	35.59	2.93	25.10	6.50	4.44
13	0.37	4.01	33.18	2.56	23.96	6.41	4.70
14	0.37	2.35	31.34	4.54	22.93	7.17	4.94
15	0.51	6.43	34.10	3.99	24.72	6.44	4.18
16	0.53	4.51	28.01	4.14	23.67	8.24	5.42
17	0.35	3.99	31.78	2.01	25.25	5.02	4.82
18	0.26	5.90	31.65	2.00	25.76	4.91	4.22
LSD(α= 0.05)	0.64	0.35	2.92	0.78	1.70	1.07	1.70
C V (%)	5.90	5.23	4.34	5.59	4.66	7.32	20.59

Table 2.5: Effect of plant shoot age on the composition (percentage) of rose-scented geranium oil (spring/summer experiment)

Shoot age (weeks)	Linalool	Iso-menthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Guaia-6,9-diene
1	-	-	-	-	-	-	-
2	0.99	4.31	27.43	16.80	18.36	8.08	5.94
3	0.55	2.38	29.28	14.09	20.67	7.89	4.84
4	0.92	6.50	30.59	17.29	20.12	7.83	4.33
5	1.06	4.26	29.70	16.59	18.45	7.62	4.18
6	1.68	7.51	28.90	16.30	18.79	8.18	3.70
7	0.85	4.82	25.24	16.61	16.41	7.64	5.63
8	1.01	0.38	30.86	13.82	17.99	7.33	5.18
9	1.04	5.62	26.94	15.26	16.13	7.18	4.97
10	0.64	4.25	25.31	13.91	15.85	6.38	6.54
11	0.99	2.72	30.79	13.86	17.13	6.73	4.78
12	0.98	4.39	30.96	13.77	17.29	7.11	4.90
13	0.87	4.33	32.82	12.75	18.93	7.23	5.76
14	0.61	5.85	29.19	10.02	16.24	6.27	5.64
15	0.57	0.36	34.01	8.12	19.73	5.56	6.89
16	0.56	2.61	35.45	6.92	18.70	5.25	5.82
17	0.35	1.44	31.79	5.48	18.91	5.07	7.23
18	0.33	4.62	29.65	4.83	17.51	4.57	7.12
LSD(α= 0.05)	0.07	1.38	0.23	0.81	0.22	1.10	0.81
C V (%)	5.15	21.27	0.47	3.81	0.07	9.74	8.91

2.3.2 Impact of temperature changes

Herbage yield and essential oil yield

Statistical analysis of the variation in average weekly maximum and minimum temperatures showed the only significant variation to be for the minimum (night) temperatures in the autumn/winter period (Table A1 of the Appendix). The oil content was then correlated to the night temperatures recorded during the autumn/winter experiment. The night temperatures in autumn/winter had a negative relationship to oil content (r^2 = 0.52). The same was true when correlating herbage yield and LAI with the minimum temperatures. Both correlations showed a negative relationship (r^2 = 0.58).

Oil composition

Since the variation in minimum temperatures was significant during the autumn/winter experiment, it was therefore, imperative to correlate the changes in oil composition relative to minimum temperature for this period. Geraniol (r^2 = 0.84) and geranyl formate (r^2 = 0.67) decreased with decreasing night temperatures, while citronellol (r^2 = 0.53) and citronellyl formate (r^2 = 0.73) increased with decreasing night temperatures in the autumn/winter experiment. This resulted in a drastic increase in the citronellol: geraniol ratio during the autumn/winter experiment (Figure 2.4). The observations from this experiment were, however, contrary to those of Diomo *et al*. (1999) who reported that the citronellol: geraniol ratio decreased rapidly during cold periods. Guaia-6,9-diene, linalool and iso-menthone were not influenced by either temperature or plant shoot age in both harvesting experiments.

Prakasa Rao *et al*. (1995) had earlier suggested that weather parameters such as temperature and rainfall influences the content and the chemical constituents of rose-scented geranium oil. The compounds that are mostly affected are geraniol, citronellol and citronellyl formate. The current results further proposes that geranyl formate was also affected by temperature changes. The oil extraction method also plays a role in the composition of oil collected at the end of the day. Guerere *et al.* (1985) as quoted by Gomes *et al* (2004) reported that the geraniol concentration was lower in hydro-distilled oils than in oil extracted by other methods (supercritical carbon dioxide extraction and hexane). The authors justified their findings by proposing a reaction mechanism of transformation of geraniol in linalool through water vapour action.

2.4 CONCLUSIONS

The data collected during the two harvesting experiments indicated that rose-scented geranium herbage yield, oil yield and oil content was high in the spring/summer compared to autumn/winter. Plant growth rate and leaf area index were the main determining factors of herbage yield although oil content was not affected by LAI in both experiments.

Moisture content did not influence oil content and it was found that it played no role in geranium essential oil production. Most importantly, essential oil content appeared not to be affected by shoot age in both experiments. It was, however, influenced by night temperatures in autumn/winter, although the same relationship was not observed in the spring/summer period because there were no changes in night temperatures with time.

Geraniol concentration was influenced by plant shoot age in both harvesting periods and by decreasing night temperatures in autumn/winter. Citronellol, citronellyl formate and geranyl formate concentrations were not influenced by shoot age, but were influenced by night temperatures in autumn/winter. The citronellol: geraniol ratio, therefore, appeared to be influenced by both seasonal changes and plant shoot age and was affected by both factors in autumn/winter, thus the high C: G ratio. The spring/summer harvesting season, therefore, appears superior to the autumn/winter harvesting season in all aspects of rose-scented geranium essential oil production.

2.5 SUMMARY

Geranium shoots are the most important plant parts because they produce most of the essential oil. Knowing the plant shoot age that can produce high oil content and oil yield is very important. Therefore, this study was to investigate the seasonal changes in herbage yield, oil yield and the variation in the concentration of the major components of rose-scented geranium oil with plant shoot age. The experiment was carried out in two seasons (autumn/winter and spring/summer harvesting cycles). The main variable was harvesting every week (Thursdays) for eighteen weeks. The second variable was changes in temperatures during the experimental periods.

Oil content was generally higher (0.088%) in the spring/summer harvested plants than those harvested in autumn/winter (0.064%) cycle. Decreases in oil content in the autumn/winter cycle were negatively related to decreases in night temperatures. Greater herbage yields resulted to higher oil yields. Geraniol concentration decreased with plant shoot age in both experimental cycles and with decreases in night (minimum) temperatures in the autumn/winter experiment. The double decrease effect on geraniol concentrations in the autumn/winter experiment resulted into very high C: G ratios. Citronellol concentration was not affected by plant shoot age but was affected by decreasing night temperatures in the autumn/winter harvesting cycle.

CHAPTER 3

HERBAGE YIELD, ESSENTIAL OIL YIELD AND OIL COMPOSITION OF ROSE-SCENTED GERANIUM AS INFLUENCED BY HARVESTING FREQUENCY

3.1 INTRODUCTION

Geranium oil is mainly produced by the leaves and young stems and these (plant materials) should be cut in a way that preserves their perennial characteristics. Harvesting of these plants is done in such a way that there will be maximum production of biomass and preservation of subsequent shoot development (Dermane, 1992; Weiss, 1997).

The ability and speed of plant shoot development governs the frequency and yield of the subsequent harvest. This will depend on the compatibility of the plant to the environmental conditions. Cutting of plants may be determined by weather conditions and other farming operations, but to obtain maximum oil yield, plants should be routinely sampled to determine oil content. The moment of harvesting/sampling may also influence the oil composition (Lawrence, 1996; Rajeswara Rao *et al*., 1996; Weiss 1997).

The moment of harvesting is not only related to time of the day but also to the growth period/stage and growing season. Weiss (1997) described geranium as a plant that produces maximum leaf growth with high oil content under warm, sunny conditions and oil content normally increases from the onset of flowering and reduces after full bloom. This is in agreement with the observations of Kumar *et al*. (2001), who suggested that the highest oil yield from geranium was related to better vegetative growth per unit time in the hotter environments of the Lucknow plains (India).

Oil composition may also change over time and these changes corresponds to the growth stages of the plant, and that is why harvesting should be conducted at the optimum time for oil content and good oil composition (Weiss, 1997). Rajeswara Rao (2002) indicated that changes in temperature, humidity and rainfall have a greater effect on the essential oil production, especially in the oil composition. Hot months will thus, favour some compounds at the expense of others.

Harvesting of rose-scented geranium is usually conducted at 3 – 5 month intervals using sickles. The main determining factors considered before harvesting are plant developmental stage, weather conditions, crop management and labour availability. Labor availability is an important aspect mainly because rose-scented geranium is largely harvested by hand (Dermane, 2001; Rajeswara Rao, 2002). Mechanical harvesting results in low herbage yield sent for distillation. Mechanical harvesters only solve the problem of re-growth since they cut the utmost top of the plants (Dermane, 2002).

Other studies of perennial essential oil crops indicated that these crops experience reduction in biomass yield due to repeated harvesting. This may affect the essential oil yield recovered from these crops, but the effect is very subjective because the environmental factors also play a greater role (Murtagh, 1996). Konthari *et al*. (2004) differed a bit from other authors. In a study on *Ocimum tenuiflorum* it was found that even though there was a decrease in biomass yield on the subsequent harvests, essential oil in general, was lower in the first harvest and increased gradually in the subsequent harvests, and reached maximum in the fourth harvest.

The present experiment was aimed at investigating the influence of harvesting frequency and seasonal changes on herbage yield, oil yield and oil composition of rose-scented geranium under local conditions.

3.2 MATERIALS AND METHODS

3.2.1 Site and experimental design

The experiment was conducted at the Hatfield Experimental Farm of the University of Pretoria, and the location of the field is as described in Chapter 2, Section 2.2. The experimental layout was a randomized complete block design (RCBD) with four blocks

(Figure 3.1). Each block was 62 m^2. Data collection began in spring and ended in winter (September 2005 to July 2006).

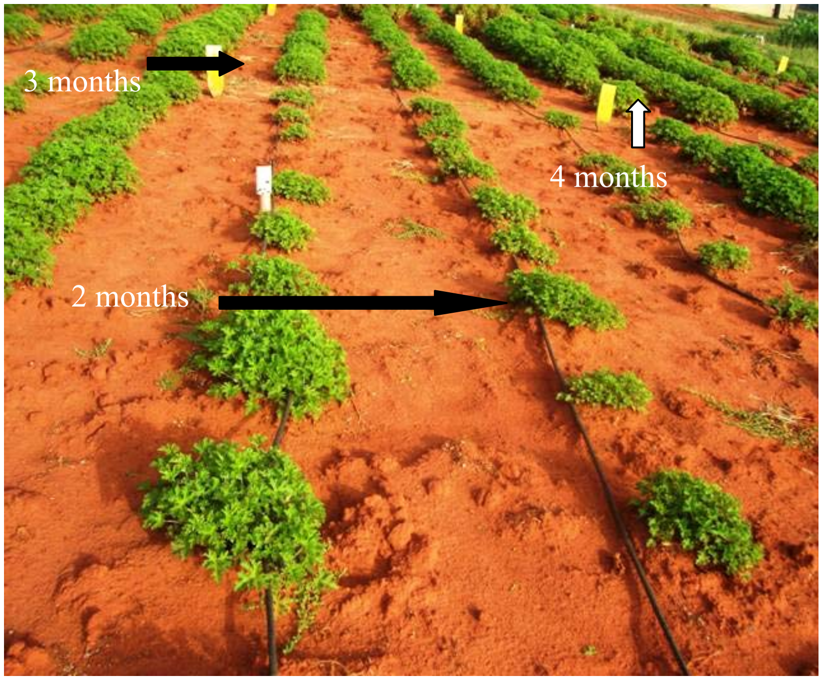


Figure 3.1: General view of the harvesting frequency field experiment

3.2.2 Harvesting, oil distillation and oil analysis

Rose-scented geraniums were planted in 2004 at a population of 16 000 plants/ha. Plants were allowed to grow for a year to establish in the field, and then cut back in September 2005 so as to begin data collection in spring and thus, imitate a typical commercial rose-scented geranium farm. Harvesting was conducted after every two, three or four months of growth. Weekly irrigation was scheduled using a neutron probe (Neutron water meter, model 503 DR, CPN Corporation, Martinez, California), and standard plant management and plant protection practices were followed. Nitrogen, P and K were applied in three splits at a rate of 60, 90 and 60 kg ha^{-1} $year^{-1}$, respectively, after each harvest.

Fresh mass and leaf area was measured at each harvesting date and later computed into total herbage yield and LAI, respectively. Approximately 5 - 8 kg of fresh biomass was collected for estimation of oil concentration. Oil concentration was determined by steam distillation for one hour using a custom built unit. Oil analysis was determined by gas chromatography (GC). An Agilent GC (FID) model 6890N fitted with 30 m x 0.25 mm fused silica capillary column, and film thickness of 0.25 µm, was used. Temperature was programmed from 50°C – 200 °C at 5 °C/min and with detector and injector temperatures at 220 °C. Component identification was done by comparing retention times and Kovat indices to standard values (Adams, 2004).

3.3 RESULTS AND DISCUSSION

3.3.1 Effect of harvesting frequency

Harvesting rose-scented geranium plants every two months resulted in a total of four harvests, while harvesting every three and four months resulted into totals of three and two harvests, respectively. The four harvests from the two months harvesting frequency represents all four seasons of the year. Harvesting plants at three months interval represents three seasons and harvesting plants at four months interval represents only two seasons of the year.

Leaf area index (LAI)

The response of leaf area index to harvesting frequency was not consistent in the two months harvesting treatment. LAI increased from the first harvest to the second harvest and, thereafter, decreased. However, only the second harvest recorded significantly higher LAI, while the rest of the harvests were the same (Figure 3.2). The three and four months harvesting frequencies showed a steady decrease in LAI in the subsequent harvests. The first and the second harvests recorded the same LAI in the three months harvesting frequency (Figure 3.2).

It was observed that LAI was higher in plants harvested at towering heights in all three treatments. A positive relationship (r^2 = 0.78 and r^2 = 0.87) between LAI and plant height was observed in the two and three months harvesting frequencies. The increase in LAI was related to an increase in plant height (Table 3.1). It was not possible to make any correlations between LAI and plant height in the four months treatment because only two harvests were made.

These results were contrary to the findings of Konthari *et al*. (2004) who reported that repeated harvesting (done at three months harvesting frequency) of *Ocimum tenuflorum* plants resulted into a significant increase in plant height and plant spread. Rose-scented geranium plants harvested at two months interval followed the same pattern as *O. tenuflorum* only up to the second harvest and, thereafter, followed the same pattern as the other two treatments (i.e. decreased in plant height and plant spread).

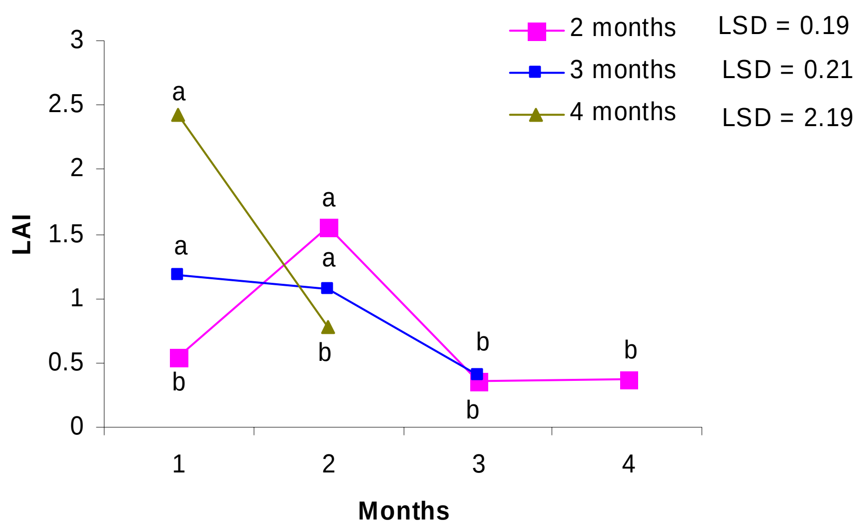


Figure 3.2: Effect of harvesting frequency on leaf area index (LAI)

An overall comparison of the three treatments indicated that plants harvested after four months produced a significantly higher LAI (1.6) while the other treatments recorded LAI of 0.71 (two months) and 0.89 (three months), respectively. Plants were also taller in the four months harvesting frequency followed by the three months treatment then the two months treatment. The positive response of LAI and plant height to the four months harvesting frequency was similar to the findings of Badi *et al*. (2004) who investigated harvesting time and spacing on *Thymus vulgaris*. The authors reported that harvesting thyme at fruit set (after four months) increased plant height and plant diameter.

Table 3.1: Response of plant height and LAI to three harvesting frequencies

Harvests	Plant height (cm)	LAI
	Two months harvesting frequency	
1	30.13b[z]	0.55b
2	39.75a	1.55a
3	15.25c	0.35b
4	14.00c	0.38b
Grand mean	24.81	0.71
CV (%)	4.15	18.32
LSD (α = 0.05)	1.64	0.21
	Three months harvesting frequency	
1	40.00a	1.18a
2	32.38b	1.08a
3	23.50c	0.40b
Grand mean	31.96	0.89
CV (%)	4.16	12.37
LSD (α = 0.05)	2.30	0.19
	Four months harvesting frequency	
1	52.50a	2.43a
2	30.75b	0.78a
Grand mean	41.63	1.60
CV (%)	8.13	60.86
LSD (α = 0.05)	7.62	2.19

[z] **Means with the same letter within a column are not significantly different at 5% level of probability**

Herbage yield and oil yield

The two months harvesting frequency

There was a significant increase in herbage yield (15.78 t/ha) on the second harvest of the two months harvesting frequency. The first and third harvest recorded statistically similar amounts of herbage (5.3 and 5.18 t/ha, respectively) and the lowest herbage yield was recorded on the fourth harvest (Table 3.2). The increase in herbage yield on the second harvest was due to higher fresh mass per plant (1.23 kg/plant) as compared to the other three harvests. Plants sampled on the second harvest also recorded the highest LAI, plant height and dry matter content.

The increase in herbage yield consequently led to a higher oil yield on the second harvest. This result concurs with findings in Chapter 2 which indicated that the total oil yield of rose-scented geranium was mainly dependent on herbage yield and less dependant on oil content. The oil content (0.084%) in the second harvest was lower than the fourth harvest (0.122%) but since the second harvest recorded a greater herbage yield, the total oil yield was subsequently higher.

The generally low herbage yield in the two months harvesting frequency was caused by repeated cutting of the plant material. The plants were, therefore, not given enough time to fully recover from the previous cutting. Harvesting was also conducted while the plants were still recovering and battling to adapt to the environmental conditions, and just before reaching peak growth rates. Weiss (1997) indicated that cutting geranium plants too early often kills many plants and re-growth becomes very slow. A similar response was observed in the two months harvesting frequency, where the number of experimental plants was reduced to almost half at the end of the experiment. Another reason for the reduction in herbage yield and oil yield could be related to the findings of Murtagh (1996) who reported that repeated harvesting reduced the biomass yield and oil yield of tea tree.

Table 3.2: Response of herbage yield and oil yield of rose-scented geranium harvested at two months interval

Harvest	Fresh shoot mass (kg/plant)	Dry shoot mass (kg/plant)	Herbage yield (t/ha)	Oil yield (kg/ha)	Oil content (%)
1	0.48b[z]	0.07b	5.30b	3.50b	0.066
2	1.23a	0.22a	15.78a	13.25a	0.084
3	0.18c	0.05cb	5.18b	2.75b	0.053
4	0.10c	0.03c	2.55c	3.13b	0.122
Grand mean	0.50	0.09	7.20	5.66	0.081
CV (%)	22.45	25.41	16.91	16.82	
LSD (α = 0.05)	0.18	0.04	1.95	1.52	
Totals per year	1.99	0.37	28.81	22.63	

[z] Means with the same letter within a column are not significantly different at 5% level of probability

The cutting height (15 – 20 cm) for rose-scented geranium might also have contributed to the decrease in herbage yield over time. All the leaves and tender stems were harvested and sent for oil distillation leaving few basal leaves on the plant stump. These leaves were either too old or had already reached the senescence stage, thus they had low photosynthetic rates, which retarded the rejuvenation process. This observation partially agrees with the results of Rajeswara Rao *et al*. (1990) who reported that plants cut at ground level produced higher herbage yield but less oil yield. The current experiment produced generally low yield in terms of both herbage and oil yield.

The three months harvesting frequency

There was a steady decrease in herbage yield and oil yield from the first harvest to the last harvest in the three months harvesting frequency (Table 3.3). The decrease in herbage yield occurred irrespective of the plant mass recorded throughout the experimental period. The first harvest recorded the highest herbage yield (25.90 t/ha) followed by the second and then the last harvest.

Table 3.3: Response of herbage yield and oil yield of rose-scented geranium harvested at three months interval

Harvest	Fresh shoot mass (kg/plant)	Dry shoot mass (kg/plant)	Herbage yield (t/ha)	Oil yield (kg/ha)	Oil content (%)
1	1.08a	0.30a	25.90a	21.42a	0.083
2	1.10a	0.18b	14.70b	14.12b	0.096
3	0.85a	0.10c	7.53c	4.08c	0.058
Grand mean	1.01	0.19	16.04	11.95	0.079
CV	14.50	15.06	11.51	12.22	
LSD (α =0.05)	0.25	0.05	3.19	2.72	

[z] **Means with the same letter within a column are not significantly different at 5% level of probability**

The first harvest also recorded the highest LAI, plant height and dry shoot mass. There was a significant decrease in dry shoot mass due to repeated harvesting, despite the similar fresh shoot mass recorded throughout the experimentation period. The total oil yield followed the same pattern as the herbage yield. The first harvest recorded highest oil yield followed by the second then the last harvest. These results are not different from previous results (Chapter 2), where the total oil yield increased with increases in herbage yield. The oil content in the first two harvests was higher than in the last harvest. The difference in total oil yield between the first and second harvests was mainly caused by less herbage yield in the second harvest. The last harvest recorded the lowest herbage yield, oil content and subsequently low oil yield.

The four months harvesting frequency

The herbage yield from the four months harvesting frequency followed the same trend as the three months harvesting frequency (Table 3.4). The first harvest recorded a significantly higher herbage yield (39.68 t/ha) than the second harvest. The higher herbage yield in the first harvest was a result of a significantly greater shoot mass (3.20 kg/plant). The first harvest was generally superior to the second harvest in all aspects of plant production.

The highest oil yield (43.73 kg/ha) was recorded in the first harvest. This result also confirms the results in Chapter 2 which indicated that oil yield was mainly dependant on herbage yield than oil content. This was clearly illustrated in this treatment because it recorded the same oil content in both harvests.

When comparing all three harvesting frequencies (Table 3.5), it was observed that harvesting rose-scented geranium plants after three and four months produced higher herbage yield and automatically higher oil yield compared to the two month harvesting intervals. The four months harvesting frequency also recorded higher oil content in the plant material compared to the other harvesting frequencies. It was probably increased by the amount of plant material taken for oil distillation, which comprised of a combination of young and older leaves. This combination allowed for extensive extraction of oil, even the little oil contained in the basal leaves. A similar combination was reported to increase oil content in European pennyroral and *Ocimum tenuiflorum* (Stengele *et al*., 1993; Kontari *at al*., 2004). The high oil content in the two months harvesting frequency (especially in the last 4th harvest) did not contribute to the total oil yield since there was little herbage sent for distillation.

Table 3.4: Response of herbage yield and oil yield of rose-scented geranium harvested at four months interval

Harvest	Fresh shoot mass (kg/plant)	Dry shoot mass (kg/plant)	Herbage yield (t/ha)	Oil yield (kg/ha)	Oil content (%)
1	3.20a	0.55a	39.68a	43.73a	0.110
2	0.48b	0.20b	9.45b	10.50b	0.111
Grand mean	1.84	0.38	24.56	27.11	0.110
CV	23.80	24.34	5.11	5.19	
LSD (α =0.05)	0.98	0.21	2.82	3.17	

[z] **Means with the same letter within a column are not significantly different at 5% level of probability**

Table 3.5: Summary table of averages per year for plant growth, herbage yield and oil yield for the different harvesting frequencies

Harvesting frequency (months)	Plant height (cm)	LAI	Dry plant mass (kg)	Fresh plant mass (kg)	Herbage yield (t/ha)	Oil yield (kg/ha)	Oil content (%)
2	24.81c	0.71b	0.09c	0.49c	7.20c	5.66c	0.081
3	31.96b	0.89b	0.19b	1.01b	16.04b	10.16b	0.079
4	41.63a	1.60a	0.38a	1.84a	24.56a	27.11a	0.110
CV	11.79	54.35	34.32	56.31	26.93	47.41	
LSD (α=0.05)	3.18	0.46	0.06	0.47	3.29	4.93	

[z] **Means with the same letter within a column are not significantly different at 5% level of probability**

When comparing the cumulative herbage yield and oil yield that could be recovered per annum, it was found that the three and four months harvesting frequencies produced the highest herbage yield (Figure 3.3). The four months harvesting frequency recorded highest herbage yield and oil yield, even though it had fewer harvests than the other treatments. The oil yield recorded from the three months harvesting frequency did not correspond to the herbage yield. This was as a result of the higher oil content (0.11%) recorded for the four months harvesting frequency treatment.

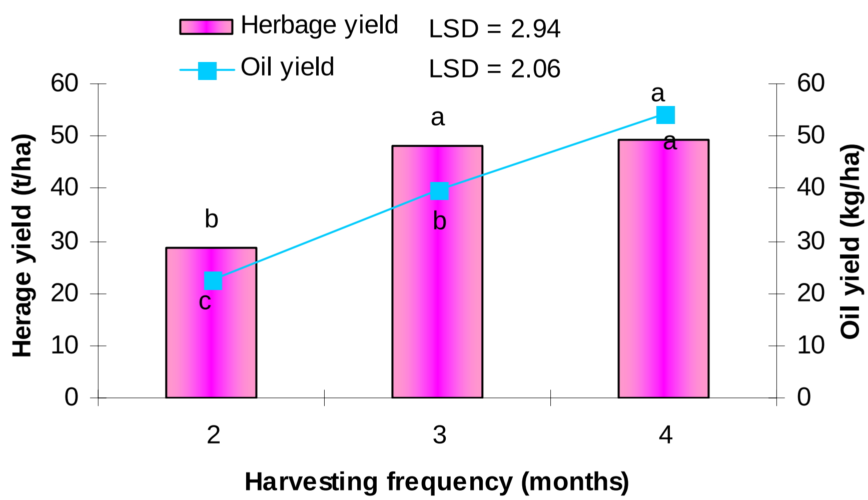


Figure 3.3: Cumulative herbage yield and oil yield of rose-scented geranium from three different harvesting frequencies

3.3.2 Seasonal effect

General plant growth

Herbage yield of rose-scented geranium showed a significant increase during the summer season (Table 3.6) irrespective of the harvesting frequency. LAI (Figure 3.4), fresh shoot mass and plant height recorded higher value during this season. Since the harvesting seasons showed a significant effect on LAI, the two variables were correlated to determine if any relationships existed. A polynomial relationship (r^2 = 0.54) between LAI and seasons was found. This meant that LAI increased from spring to summer and thereafter, decreased steadily in autumn and reached its minimal value in winter. These results indicated that the summer season was the most favourable growing season for the growth of rose-scented geranium. Table A1 of the Appendix indicates that the average temperature and rainfall was high during summer months. This result concurred with findings of Weiss (1997) who indicated that geranium produces maximum leaf growth under warm, sunny conditions.

Table 3.6: Response of rose-scented geranium plants to seasonal changes and different harvesting frequencies

Harvesting season	Plant height (cm)	Herbage yield (t/ha)	Oil yield (kg/ha)	Fresh plant mass (kg)	Dry plant mass (kg)	Oil content (%)
			Two months harvesting frequency			
Spring	30.13c[z]	5.30e	3.50e	0.475d	0.07e	0.066
Summer	39.75b	15.78c	13.25c	1.225b	0.22c	0.084
Autumn	15.25e	5.18e	2.75e	0.175e	0.05e	0.053
Winter	14.00e	2.55f	3.13e	0.100e	0.03e	0.122
			Three months harvesting frequency			
Summer	40.00b	25.90b	21.43b	1.08cb	0.30b	0.083
Autumn	32.38c	14.70c	14.12c	1.10cb	0.18dc	0.096
Winter	23.50d	7.53ed	4.08e	0.85c	0.10de	0.058
			Four months harvesting frequency			
Summer	52.50a	39.68a	43.73a	3.20a	0.55a	0.110
Winter	30.75c	9.45d	10.50d	0.48d	0.20c	0.111
CV (%)	5.83	12.85	13.35	20.43	29.88	
LSD (α = 0.05)	2.61	2.61	2.51	0.29	0.08	

[z] **Means with the same letter within a column are not significantly different at 5% level of probability**

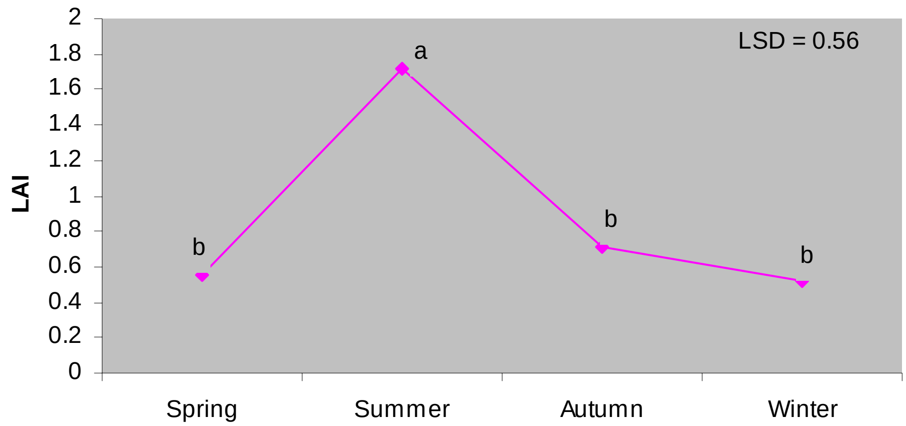


Figure 3.4: Effect of season on LAI of rose-scented geranium plants

A similar finding was reported by Duriyaprapan *et al*. (1986) on peppermint. It was found that leaf mass ratio increased with increasing day temperatures and the maximum peppermint leaf, stem and root matter were produced under 30 ^{o}C day temperatures. The good response of rose-scented geranium plants to the summer season is similar to the findings of Letchamo *et al*. (1995) who hypothesized that dry matter formation in thyme was closely related to photosynthesis. Limitations in water and net carbon dioxide assimilation were reported to have a negative effect on shoot growth and production of volatile oils. Since the summer season recorded high rainfall figures and water is one of the major inputs required for the process of photosynthesis, the availability of water increased the rate of photosynthesis during the summer period thus, plants produced higher oil yield.

The other advantage of the summer season over the other seasons was the longer photoperiod. This characteristic is beneficial to some major metabolic activities in plant growth such as photosynthesis, transpiration and respiration. Increases in leaf area and leaf greenness under long-day photoperiod were reported in geranium. Plant dry mass was also reported to increase as a consequence of increased chlorophyll (Langton *et al*., 2003). Other essential oil plants (*Mentha* sp and thyme) were tested under different photoperiodic regimes where they responded by increases in the vegetative growth and chlorophyll concentration under long-day photoperiod (Letchamo *et al*., 1995; Farooqi *et al*., 1999).

Herbage yield and oil yield

The highest herbage yield and oil yield were recorded in the summer season across all the harvesting frequencies. The four months harvesting frequency recorded the highest herbage and oil yield out of the three treatments (Table 3.6). This response was in agreement with the findings of Kumar *et al*. (2001) and Weiss (1997) who reported that the highest herbage yield and oil yield was recorded on plants harvested in summer.

The autumn harvest in the three month harvesting frequency recorded the same yield (herbage and oil yield) as the two month harvesting frequency in summer (Table 3.6). There was no significant difference in the yield recorded in spring and autumn from the two month harvesting frequency. This might have been caused by the low oil content in the herbage and simultaneously less herbage yield in both seasons. Weiss (1997) also indicated that spring harvested geranium (in southern India) produced lower oil yield as compared to summer harvested geranium.

All treatments were also harvested in winter, and this once again provided an opportunity to compare their performance during that season (Table 3.6). The herbage yield from the three months harvesting frequency (7.53 t/ha) was not significantly different from that harvested from the four months harvesting frequency (9.45 t/ha). The oil yield recovered from the three months harvesting frequency (4.08 kg/ha), however, was much lower than that recovered from the four months harvesting frequency (10.50 kg/ha).

In winter, oil yield recovered from the three month harvesting frequency was statisically equal to that recovered from the two months harvesting frequency (3.13kg/ha and 4.08kg/ha) yet the herbage yield was much lower for the latter (two months treatment). It was expected that the three months harvesting frequency will record the same amount of oil as the four months harvesting frequency since both treatments recorded equivalent amounts of herbage. The result of the three months harvesting frequency totally disagreed with reports from other geranium researchers who found that rose geranium produced high oil yield when harvested after 3 – 4 months. This finding was, however, true for the four months harvesting frequency (Weiss, 1997; Demarne, 2002; Rajeswara Rao, 2002).

Most interesting result was the herbage yield from the two months harvesting frequency in winter. It was the lowest herbage yield in the whole experiment yet its oil yield was equal to that recorded from the three months harvesting frequency in the same season. This finding continued to discredit the three months harvesting frequency. The increase in oil yield might have been a result of the high oil content (0.122%) during the winter season in the two months harvesting frequency. This was the highest oil content recorded in the whole experiment. In contrast, the three months harvesting frequency recorded the lowest oil content (0.058%) during the same season. As expected, it took advantage of the greater herbage yield and surpassed the two months harvesting frequency.

To confirm whether the three months harvesting frequency was indeed an inferior harvesting frequency or not, it was compared with other results obtained in the 2005 growing season. These results only represents three seasons (summer, autumn and winter). Plants harvested in summer (both growing seasons) produced higher yields in the three months harvesting treatment (Table 3.7). The herbage yield and oil yield were within the same range as those recorded in this experiment (2005/2006). Herbage yield and oil content, therefore, play a big role in total oil yield.

The three months harvesting frequency produced higher herbage yield (24.32 t/ha) in autumn 2005 than in autumn 2006 (14.70 t/ha). However, the oil yield was almost equal to that recovered in autumn 2006. This might have been caused by the low oil content in plants harvested during the 2005 growing season. The oil content became the dominant factor that influenced total oil yield. This result, however, contradicts the findings of Kumar *et al*., (2001) who reported that oil yield was predominantly affected by herbage yield and only to a negligible extent by oil content.

Herbage yield and oil yield recorded in autumn 2005 was higher than that recorded in autumn 2006 in the two months harvesting frequency (Table 3.7). The oil content could not be a possible reason for the differences in oil yield but rather the herbage yield because both growing seasons recorded approximately the same oil content (0.05% in 2005 and 0.053% in 2006). The autumn season of 2005 was, seemingly, more productive when compared to autumn of 2006. This is supported by the higher herbage yield and consequently higher oil yield from the four months harvesting frequency in that season.

Table 3.7: Herbage yield and oil yield of rose-scented geranium recorded in three seasons of the 2005 production year compared to 2006

Season	Herbage yield (t/ha)		Oil yield(kg/ha)		Oil content (%)	
	2005	2006	2005	2006	2005	2006
			Two months harvesting frequency			
Spring	-	5.30e^{z}	-	3.50e	-	0.066
Summer	-	15.78c	-	13.25c	-	0.084
Autumn	9.81c^{z}	5.18e	4.91c	2.75e	0.050	0.053
Winter	2.46d	2.55f	1.65d	3.13e	0.067	0.122
			Three months harvesting frequency			
Summer	26.58b	25.90b	23.88a	21.43b	0.090	0.083
Autumn	24.32b	14.70c	13.38b	14.13c	0.055	0.096
Winter	4.98d	7.53ed	4.43dc	4.08e	0.089	0.058
			Four months harvesting frequency			
Summer	-	39.68a	-	43.73a	-	0.110
Autumn	40.81a	-	24.49a	-	0.060	-
Winter	10.56c	9.45d	4.65dc	10.50d	0.044	0.111
CV (%)	12.85	16.88	13.35	18.57		
LSD(α = 0.05)	2.61	4.24	2.51	3.02		

^{z} **Means with the same letter within a column are not significantly different at 5% level of probability**

A lot of inconsistencies in herbage yield and oil yield were observed in the winter season (of 2005 and 2006) in all three harvesting frequencies (Table 3.7). The three months harvesting frequency recorded similar oil yield in both winters yet the herbage yield in winter 2006 was higher than that recorded in winter 2005. The reason was, yet again, related to the low oil content in the 2006 harvest. The two months harvesting frequency produced equivalent herbage yield in both winters. The oil yield though, was very low in 2005. This was another scenario where the oil content decreased the overall oil yield.

Similar results as the two months harvesting frequency was true for the four months harvesting frequency (Table 3.7). The oil yield in the 2006 winter season was higher than the oil yield recovered in 2005. This was also associated with low oil content in the 2005 growing season. The climatic conditions e.g. temperature/rainfall might have had an influence on the oil yield from these treatments since they were harvested in the same season. The month of harvest was also reported to increase or decrease the oil yield recovered from geranium plants (Doimo *et al*., 1999).

3.3.3 Effect of harvesting frequency on oil composition

Plants from the two months harvesting frequency produced an essential oil high in linalool, iso-menthone, geraniol and geranyl formate concentrations (Table 3.8). This was the only treatment that produced the highest geraniol concentration at all harvests. Geraniol is one of the most important compounds that constitute geranium essential oil. Weiss (1997) and Peterson *et al*. (2005) described geraniol as the compound responsible for the flowery rose-like odor of geranium oil. The previous chapter (Chapter 2) indicated that this compound decreased with plant shoot age hence, harvesting after every two months assured that plant shoots were very young at harvest. These results certainly concur with the findings in the previous chapter and with other geranium oil researchers (Rajeswara Rao *et al*., 1993).

Table 3.8: Oil composition of rose-scented geranium recovered from three different harvesting frequencies

Season	Linalool	Iso-menthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Guaia-6,9-diene
			Two months harvesting frequency				
Spring	1.29b[z]	3.87b	29.00b	16.93c	20.25b	8.00e	4.26f
Summer	1.10c	5.68a	27.64c	18.39b	17.27f	9.05d	4.89e
Autumn	1.78a	3.61e	18.38g	23.06a	14.59h	11.85b	5.40d
Winter	0.75d	3.24g	21.13f	16.99c	15.89g	13.63a	5.85b
			Three months harvesting frequency				
Summer	1.70a	3.67d	27.19c	15.11d	17.35ef	7.57fe	5.58c
Autumn	0.26f	3.76c	25.72d	9.01g	17.62ed	5.53g	7.65a
Winter	0.00g	1.037i	23.48e	5.13h	21.98a	7.70e	7.60a
			Four months harvesting frequency				
Summer	0.68d	3.37f	32.27a	12.70e	18.86c	7.10f	5.85b
Winter	0.55e	2.34h	28.14cb	10.95f	17.73d	10.22c	5.42d
CV (%)	5.31	0.28	2.91	2.35	1.06	3.74	0.59
LSD (α = 0.05	0.08	0.02	1.29	0.57	0.33	0.57	0.06

[z] **Means with the same letter within a column are not significantly different at 5% level of probability**

Citronellol is another important compound and its concentration is as important as that of geraniol (Table 3.8). It is reported to be associated with the sweet, soapy rose-like odor of geranium oil (Weiss, 1997; Peterson *et al*., 2005). This compound is always in high concentrations in geranium oil, and as a consequence, it is mostly responsible for the increases in citronellol: geraniol (C: G) ratio. The C: G ratio is commonly used to rate geranium oil in the commercial market, and it is considered best within the range of 1.0 – 3.0. Plants from the two months harvesting frequency were capable of providing such ratios due to their high geraniol concentration. The other compounds analysed for in the essential oil are also important but in low amounts. It was reported that these are responsible for the natural characteristics of the oil. If absent or in high concentrations, the oil loses its natural odour (Bauer *et al*., 1990; Anistescu *et al*., 1997).

The geraniol concentration in the four months harvesting frequency was higher than the last two harvests of the three months harvesting frequency. The three months treatment recorded an acceptable geraniol concentration in the very first harvest only, otherwise it decreased drastically in subsequent harvests. This result was rather strange because plants were harvested at a younger age than the four months harvesting frequency. It was expected that its geraniol concentration would be higher than the four months harvesting frequency as found in Chapter 2.

Guaia-6,9-diene is the third most important compound out of the seven compounds analyzed for in the geranium oil. It is reported to be responsible for the powerful tenacious odour of geranium oil (Weiss, 1997; Peterson *et al*., 2005). Its concentrations (concentrations greater than 5%) (Table 3.8) in the three months harvesting frequency indicated that the oil could be fixed for a long time. The other harvesting treatments also recorded acceptable concentrations of this compound.

Oil composition from the 2005 growing season was not different from that recorded in the current experiment (Table 3.9). Plants harvested after two months recorded a relatively high concentration of geraniol compared to those harvested after three and four months. The three months harvesting frequency always recorded low geraniol concentrations throughout the 2005 growing season, but it was not lower than the four months harvesting frequency. The two months harvesting frequency recorded low C: G ratios even in the 2005 season due to the high geraniol concentrations. The citronellol concentration was very high in the 2005 season and this resulted in generally high C: G ratios, especially in the four months harvesting frequency. Guaia-6,9-diene concentration was, yet again, high in the three months harvesting frequency, although not as high as in the 2006 experiment.

Table 3.9: Oil composition of rose-scented geranium from the 2005 harvesting season

Season	Linalool	Iso-menthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Guaia-6,9-diene
			Two months harvesting frequency				
Autumn	0.58dc[z]	2.45c	27.36d	15.63a	18.19c	11.71a	4.53c
Winter	1.29a	4.39b	32.61ba	10.18b	22.03b	10.82a	5.02bc
			Three months harvesting frequency				
Summer	0.69c	4.99ba	26.80d	9.00cb	20.82b	11.48a	5.84bac
Autumn	0.45d	3.62bc	29.50c	8.20c	17.36c	5.00c	6.51ba
Winter	0.94b	4.37b	33.70a	10.55b	22.53b	7.30b	4.70c
			Four months harvesting frequency				
Autumn	0.27e	0.40c	31.27b	5.46d	18.69c	5.00c	6.67a
Winter	0.52d	6.51a	33.63a	5.12d	24.92a	8.82b	4.78bc
CV	13.68	24.28	3.25	10.91	5.00	11.64	15.99
LSD($\alpha = 0.05$)	0.16	1.62	1.75	1.75	1.81	1.75	1.52

[z] Means with the same letter within a column are not significantly different at 5% level of probability

3.3.4 Seasonal effects on oil composition

The two months harvesting frequency was harvested in all four seasons of the year, while the three months harvesting frequency was harvested in three seasons and the four months harvesting frequency in two seasons (Table 3.8). The two months treatment recorded relatively higher geraniol concentration in all four seasons and the highest concentration was in autumn (23.06%). The three months treatment recorded higher geraniol concentration in summer.

Since all treatments were harvested in winter and summer, this gave an opportunity to compare the treatments' performance during both seasons. The lowest C: G ratio (Figure 3.5) in the summer season was obtained from the two months and three months treatments. The high C: G ratio in the four months treatment was probably due to old plant material which is reported to produce low geraniol concentrations (Rajeswara Rao *et al*., 1993; Kontari *at al*., 2004). The highest C: G ratio was obtained from the three months treatment in winter while the two months treatment continued to record lower C: G ratio during this season.

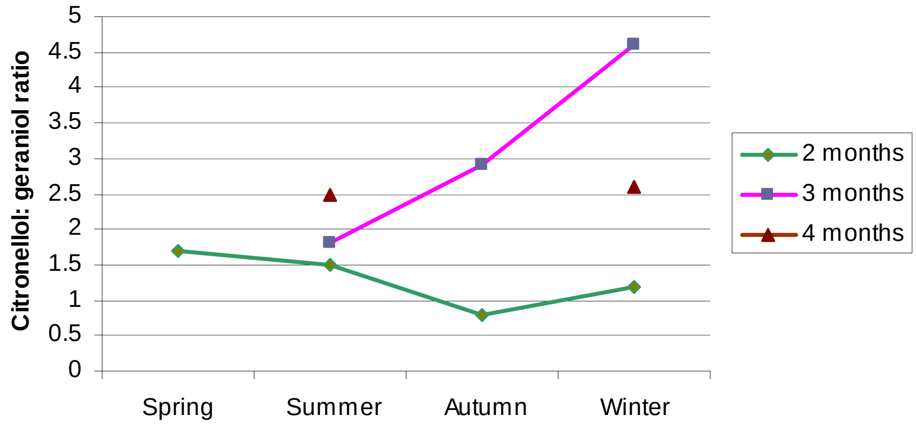


Figure 3.5: Citronellol: geraniol ratio from the three different harvesting frequencies and their respective harvesting season

Scheffer (1993) indicated that oil composition may vary to a large extent depending on the time of harvesting (in relation to season and month of harvest). Doimo *et al*. (1999) found that geraniol concentration increased in spring and summer season, concurring with the findings of the current experiment, irrespective of the harvesting frequency. Guaia-6,9-diene concentrations were not favoured by the summer season but were above 5% in the three and four months harvesting treatment and comparable to values recorded in the 2005 summer season (Table 3.9). Iso-menthone concentration was generally high in the two months harvesting frequency, and very high in summer.

Only two treatments were harvested in autumn (Table 3.8). The two months harvesting frequency recorded the highest geraniol and lowest citronellol concentrations in this season. This resulted in a very low C: G ratio. The citronellol concentration was not very high in the three months harvesting frequency, but it recorded a high C: G ratio due to the low geraniol concentration. The citronellol behavior followed the same pattern as reported by Doimo *et al*. (1999) that citronellol concentrations were minimal in autumn. This cannot be validated because observations from the 2005 growing season recorded higher concentrations of this compound in autumn (Table 3.9). Guaia-6,9-diene concentration was higher in the three months harvesting frequency, and it seemed to be favoured by the autumn season because it recorded the same concentrations even in the 2005 growing season.

The geraniol concentration was very low in winter (Table 3.8) except for the two months treatment. The three months harvesting frequency recorded the lowest concentration thus, resulted into the highest C: G ratio in the experiment (Figure 3.5). The two months harvesting frequency maintained its low C: G ratio even during the winter season due to high geraniol concentration. When comparing these results to those recorded in the 2005 growing season (Table 3.9), it was found that geraniol concentration decreased in winter in all harvesting frequencies, unlike in the 2006 experiment (Table 3.8), where the lowest concentration was recorded in the three months harvesting frequency. Results from the three and four months harvesting frequencies in the 2006 experiment and those from the 2005 experiment concur with the findings of Doimo *et al*. (1999).

Findings by Rajeswara Rao *et al*. (1999) indicated that citronellol concentrations increased in summer as a mechanism to adjust to thermal stress. This experiment partly disagrees with this result because citronellol was very high in winter (2005) as per the observations of Doimo *et al*. (1999). Maybe the increase or decrease might be caused by changes in the climatic conditions at different months of harvest as indicated by Doimo *et al*. (1999).

3.4 CONCLUSIONS

Herbage yield was affected by both harvesting frequency and seasonal changes. Harvesting at close intervals (two months) resulted in low herbage yield and consequently low oil yield irrespective of the oil content. Plants that were given enough time to grow and recover from previous cutting (three and four months harvesting frequencies) produced higher herbage yield and higher oil yield. Oil content increased in summer and it coincided with an increase in herbage yield, thus rendering the summer season as the best season for geranium oil production. It was also found that the oil content can influence the total oil yield to a greater extent depending on harvesting frequency. This was evident mostly in the winter harvests of the two growing seasons (2005 and 2006).

The oil composition was mainly influenced by the harvesting frequency. Plants harvested at a younger stage produced a relatively low C: G ratio than plants harvested at an older stage. Furthermore, the summer season produced a relatively low C: G ratio than the winter season.

The three months harvesting frequency produced the worst oil yield and oil composition. This result renders it the most undesirable harvesting frequency. The four months harvesting frequency, on the other hand, was desirable because of its high oil yield. Oil yield is an important characteristic in geranium producer because the oil is paid according to the mass of oil produced. The two months harvesting frequency was desirable for its consistent low C: G ratio recorded even in the winter season where it is reported to increase. The oil composition from this treatment (two months harvesting frequency) was considered of good quality although very subjective because geranium oil quality is mainly dependant on the buyer. One buyer may favor oil with low C: G ratio while another may prefer one with a higher C: G ratio. The harvesting interval that can be the best compromise between oil yield and oil composition is after every four months.

3.5 SUMMARY

The objective of this experiment was to study the effects of harvesting frequency and seasonal effects on herbage yield, oil yield and oil composition of rose-scented geranium. The experiment was carried out in four seasons (2005 – 2006). The main variables were harvesting after every two, three and four months of growth. Season (spring, summer, autumn and winter) was the second variable studied.

Harvesting frequency had a highly significant effect ($P>0.01$) on herbage yield and oil yield. Closer harvesting intervals (after two months) produced less herbage yield and oil yield, while longer harvesting intervals produced more herbage yield and oil yield. Harvesting at two month intervals improved the oil composition of rose-scented geranium to lower C: G ratios but did not improve oil yield. The season of harvest also showed highly significant effects on herbage yield, oil yield and oil composition. Herbage yield, oil yield and oil composition improved when harvested in summer compared to autumn and winter. The cumulative results of rose-scented geranium yield suggested that higher oil yield can be obtained by harvesting after three or four months. However, low C: G ratio oil, is obtainable from two months harvesting frequency irrespective of the season of harvest

CHAPTER 4
SIZE AND DENSITY OF OIL GLANDS ON LEAVES OF ROSE-SCENTED GERANIUM AS INFLUENCED BY HARVESTING FREQUENCY

4.1 INTRODUCTION

Leaves of geranium are the most important plant part because they produce higher essential oil yield than other plant parts (Mallavarapu *et al*., 1997). Essential oil is contained in glandular trichomes located on both surfaces of the leaf, the tender stem, buds and the inflorescences. More than half of the essential oil is produced by the leaves (Mallavarapu *et al*., 1997; Demarne, 2002). The ability of these plant parts to produce essential oil necessitates maximum production of herbage and preservation of subsequent shoot development (Demarne & van de Walt, 1989).

Oosthuizen & Coetzee (1984) reported that spiny hairs were initiated before glandular hairs and this process either ended at an early age of leaf development or continued at a very slow rate. Glandular hair initiation continued during leaf development at a higher rate than spiny hairs but the rate was slower than that of leaf development. It was also reported that the initiation (of glandular hairs) occurred at varying rates during the different stages of leaf development. Van de Walt & Demarne (1988) identified two types of glandular hairs differing in size and structure on the leaf surfaces of geranium plants whose concentration might affect the total oil yield. The position of the leaf on the plant shoot was also reported to have a significant effect on geranium oil concentration and composition (Konthari *et al*., 2004).

Variations in oil content and oil yield in the previous chapters brought about another element of investigation into the experiment. Since the leaves were reported to be the highest contributor of essential oil compared to other plant parts, it was imperative to focus the main objective on the leaves. The objective of this experiment was to study the variations of essential oil glands at different harvesting frequencies.

4.2 MATERIALS AND METHODS

Leaf materials used for studying oil glands were collected from the harvesting frequency experiment (Chapter 3). Three leaves were collected from different positions on the plant, comprising of top, middle and bottom sections. A leaf sample (1.0 X 1.5 cm) was cut from each leaf sapmles and fixed in gluteraldehyde (3% w/v), washed thoroughly in phosphate buffer (0.1 M, pH 7.0) and post fixed for 1.5 hours in osmium tetroxide (1% w/v). After several washing with distilled water, the leaf samples were dehydrated using ethanol series (30 – 100% w/v) in a critical point drying apparatus (Bio-Rad E300, Watford, England). The dried samples were mounted on copper stubs and coated with gold in a vacuum coating unit (Polaron E5200C, Watford, England). The gold coated samples were observed under a JSM – 840 scanning electron microscope (JEOL, Tokyo, Japan) at 65X magnification. Clearly visible oil glands were counted on the abaxial and adaxial surfaces of the leaves (Figures 4.1 and 4.2).

4.3 RESULTS AND DISCUSSION

The two types of oil glands (differing in size and structure) found by van de Walt & Dermane (1988) on *P. graveolens* and *P. radens* were also present on the leaf surfaces of the geranium (Bourbon type) used for this experiment. This might be due to the fact that *P. radens* is one of the 'parents' (P. *capitatum* X *P. radens*) of this geranium variety.

4.3.1 Oil glands as influenced by harvesting frequency

Harvesting frequency showed a highly significant ($P < 0.01$) effect on density of oil glands per unit leaf area (Table 4.1). All harvesting frequencies recorded lower densities of large oil glands compared to smaller glands. The total number of oil glands per harvesting frequency decreased as the harvesting frequency lessened. A similar trend was also recorded from the small oil glands. The densities of smaller oil glands decreased from the two months harvesting frequency to the three months harvesting frequency then four months harvesting frequency. Larger oil gland densities on the other hand, increased in the four months harvesting frequency while the densities were consistent in the other harvesting frequencies.

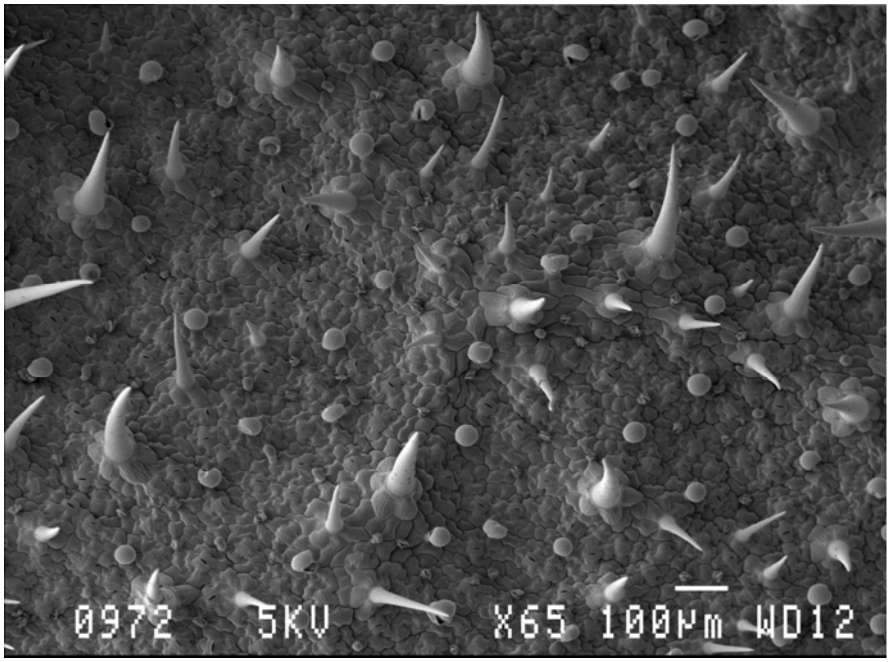


Figure 4.1: An adaxial leaf surface of rose-scented geranium viewed under a scanning electron microscope

The oil gland density of rose scented geranium was also affected by whether the leaf surfaces were abaxial or adaxial. The adaxial leaf surface recorded higher densities of both types of glands with an exception of the smaller glands. The appearance of large oil glands on the abaxial leaf surfaces was more pronounced in the four months harvesting frequency.

An interaction between harvesting frequency and leaf surface was only recorded in the two months harvesting frequency (Figure 4.3). The abaxial leaf surface showed a highly significant ($P < 0.01$) effect on the density of small oil glands of the two months harvesting frequency. Lower densities of oil glands (small oil glands) were found in the two moths harvesting frequency as compared to three and four months harvesting frequency. It is not known whether the decrease in oil gland densities in this harvesting frequency (two months) is related to the findings of Oosthuizen & Coetzee (1984) who reported that glandular hair initiation continues at a low rate during leaf development.

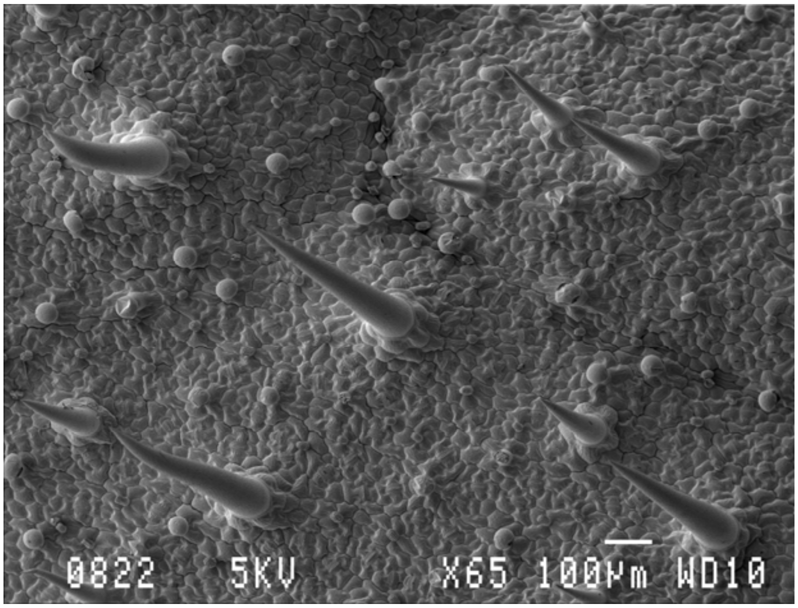


Figure 4.2: An abaxial leaf surface of rose-scented geranium viewed under a scanning electron microscope

The density of trichomes was also very high on the adaxial surface of leaves (Figure 4.1). It was observed that trichomes appearing on the leaves of the two months harvesting frequency consisted of circular warts extending from the top to the base of the trichome (Figure 4.4). The same observation was reported by Oosthuizen & Coetzee (1983) on *P. scabrum*. The appearance of warts was described by the authors as a signal of maturity of the trichomes. The other harvesting frequencies had smooth trichomes on both leaf surfaces.

Table 4.1: Summary table for the density of oil glands on the abaxial and adaxial leaf surfaces of rose-scented geranium harvested at different harvesting frequencies

Leaf position on plant	Oil glands				Total no. of oil glands	
	Bigger		Smaller			
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
	Two months harvesting frequency					
Top	40.50b[z]	63.00a	60.75cb	122.50a	101.25b	185.50a
Middle	22.50cb	35.75b	46.50cbd	65.75b	69.00c	101.50b
Bottom	13.00c	26.00cb	28.00d	42.00cd	41.00c	68.00c
CV (%)		41.98		23.76		22.67
LSD (α = 0.05)		20.87		21.50		31.79
	Three months harvesting frequency					
Top	44.00a[z]	44.50a	70.50ba	74.50a	114.50a	119.00a
Middle	18.50b	46.00a	60.50ba	65.25ba	79.00b	111.25a
Bottom	15.75b	27.75b	30.50c	55.00b	46.25c	82.75b
CV (%)		30.56		21.76		19.00
LSD (α = 0.05)		14.87		19.19		26.00
	Four months harvesting frequency					
Top	35.25bac	53.25a	55.75a	66.75a	91.00ba	120.00a
Middle	26.25dc	50.50ba	50.00ba	45.25ba	76.25bc	95.75ba
Bottom	21.75d	38.00bac	32.25b	46.00ba	54.00c	84.00bc
CV (%)		28.51		30.10		24.93
LSD (α = 0.05)		15.89		22.06		32.16

[z] **Values followed with the same letter in their respective columns are not significantly different form each other at α = 0.05 according to the t-test**

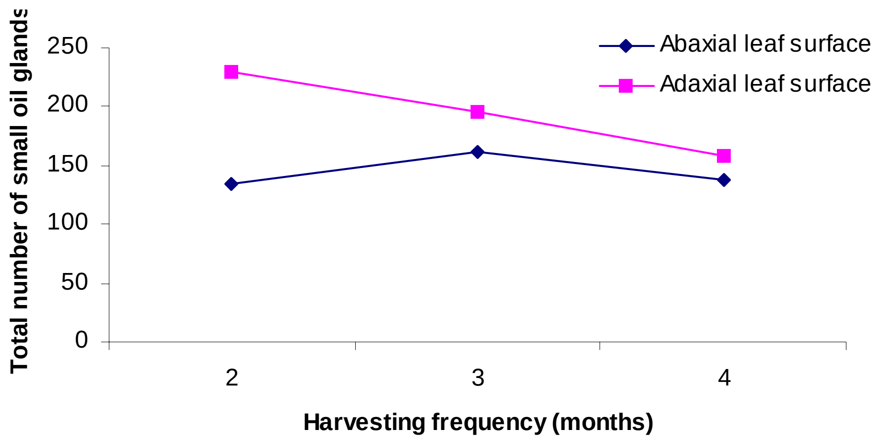


Figure 4.3: Interaction between leaf surface and the two months harvesting frequency

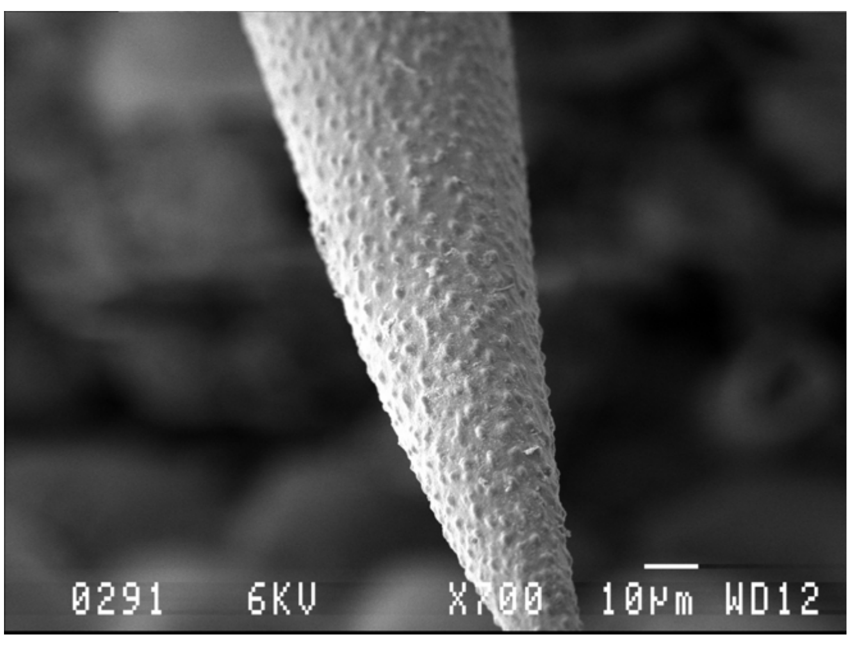


Figure 4.4: A warty trichome of rose-scented geranium viewed under a scanning electron microscope

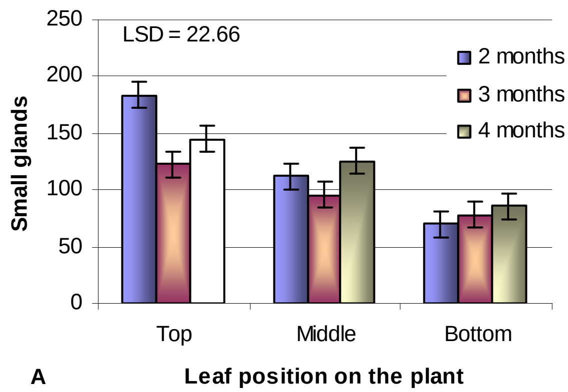
It has not yet been determined whether the composition of the essential oil produced by the two types of oil glands differs (van de Walt & Dermane, 1988). Mallavarapu *et al*. (1997) indicated that the leaves of geranium contributed maximum essential oil content making them (leaves) the most important part of geranium. These authors further indicated that leaf oil contains the highest percentage of oxygenated monoterpenes than oil of the petioles and stems.

4.3.2 Oil glands as influenced by leaf position on the plant

The density of oil glands was significantly affected by leaf position on the plant (Table 4.1). The top leaf recorded highest density of oil glands, followed by the middle leaf then the bottom leaf. The top leaf recorded more oil glands irrespective of the leaf surface or harvesting frequency. The two months harvesting frequency tended to show higher oil gland density in the top leaf as compared to the other harvesting frequencies, but the oil gland density decreased more than the other frequencies as the leaf position moved down the plant.

The density of small oil glands in the bottom leaf of the two months harvesting frequency was not significantly different from the other harvesting frequencies (Figure 4.5A). The small oil glands were generally low in the three months harvesting frequency in all the leaf positions (Figure 4.5A). The large oil glands showed a different response to the harvesting frequencies, as they tended to be in higher densities in the three months harvesting frequency compared to the two months harvesting frequency (Figure 4.5B). The bottom leaves of the three months harvesting frequency recorded higher densities of the large oil glands than the other two (two and four months) harvesting frequencies. These results were in line with the findings of Rajeswara Rao *et al*. (1993) who reported that the density of oil glands was maximum in the first (top) leaf and decreased with leaf age, resulting in reduction of essential oil concentration as the leaves aged.

The reason for the decrease in oil glands per surface area with plant age might be following the findings of Oosthuizen & Coetzee (1984) who reported that glandular hair initiation continued during leaf development at a higher rate than spiny hairs but the rate was slower than that of leaf development. It was also reported that the initiation (of glandular hairs) occurred at varying rates during the different stages of leaf development.



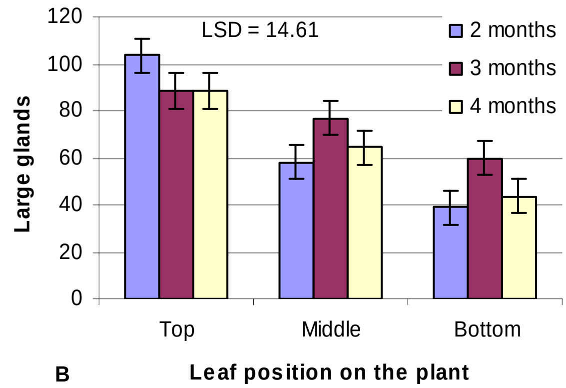


Figure 4.5: Density of small (A) and large (B) oil glands from different leaf positions of rose-scented geranium harvested at different harvesting frequencies

Studies by Oosthuizen & Coetzee (1984) clearly indicated that the rate of glandular hair (oil glands) initiation varied relative to leaf expansion. As leaf area increased, glandular hair initiation decreased or stopped completely. Glandular hair initiation might, however, continue after leaf expansion has stopped depending on the metabolic reaction of the plant.

Essential oil from the different leaf positions was not analyzed to determine if there were differences in oil composition in the current study. Rajeswara Rao *et al*. (1993) distilled and analyzed oil from different positions on the plant, and found that geraniol and linalool concentration was higher in the top younger leaves and it progressively decreased with leaf age. Perhaps this result provides a better explanation to the increases in geraniol concentration in the two months harvesting frequency. The concentration of citronellol, citronellyl formate and geranyl formate was not affected by leaf age.

4.4 CONCLUSIONS

The density of oil glands in the leaves is mainly affected by the position of the leaf on the plant. The uppermost (younger) leaves contain more oil glands (both types on both leaf surfaces) than the bottom (older) leaves. This means that the age of the herbage has an important role in oil yield. For farmers to obtain younger herbage, they will have to harvest at a very young stage (close intervals) which will subsequently lead to lower oil yield since fewer leaves would have been formed at harvesting time. Harvesting at three and four months intervals will produce higher herbage yield and oil yield because even the little oil contained in the fewer oil glands in the older leaves will be extracted and will contribute to the total oil yield. It can be safely concluded that many leaves with fewer oil glands results into higher oil yield than fewer leaves with many oil glands.

The two months harvesting frequency can still be referred to as the most desirable harvesting frequency as far as oil composition is concerned. This is based on the observations from the previous chapter where it was the only treatment that continuously produced oil with low C: G ratio. The oil gland density experiment did not include seasonal effects on the oil glands and essential oil composition, which makes it very difficult to draw conclusions regarding seasonal changes. It is therefore, recommended that an experiment that will study the effects of harvesting frequencies, leaf position and seasonal changes on the oil yield and composition of rose-scented geranium be taken into consideration as a follow up from this experiment.

4.5 SUMMARY

Most of the oil glands in rose-scented geranium are located on the leaves. The objective of this experiment was to study the variations of essential oil glands at different harvesting frequencies. Leaf samples were collected from the harvesting frequency experiment and oil glands identification was achieved through the use of a scanning electron microscope.

Two types of oil glands (smaller, slightly elongated type and larger, spherical type) were identified on both leaf surfaces of rose-scented geranium. The adaxial leaf surface recorded higher densities of oil glands (both types) and trichomes than the abaxial leaf surfaces. An interaction between harvesting frequency and leaf surface was only significant in the two months harvesting frequency. Total number of oil glands (all types) was higher on the abaxial leaf surface as compared to the adaxial leaf surface of rose-scented geranium. The density of oil glands was also affected significantly, by the leaf position on the plant. The top younger leaves contained more oil glands (all types) than the bottom older leaves.

GENERAL DISCUSSION AND CONCLUSIONS

Rose-scented geranium is a world known essential oil plant priced for its high grade essential oil mostly utilized in the perfumery industry. South Africa has just joined the geranium oil production industry and is still battling with the variations in oil yield and oil composition. It is not clear when and at what growth stage the herbage should be harvested or the stage at which it can produce the greatest essential oil. Another problem that surrounds the production of geranium essential oil is the variation in oil composition which is speculated to be influenced by environmental factors.

Researchers in rose-scented geranium (Weiss, 1997; Dermane, 2002; Rajeswara Rao, 2002) reported that the best harvesting time for higher oil yield was at intervals of 3 – 4 months. The problem of oil composition though, was not solved. Rajeswara Rao *et al*. (1993) tried to address the problem of oil composition by harvesting and distilling leaves of different ages and it was found that oil composition changes with leaf age. These investigations did not solve the yield variation problems in South Africa though, since the investigations were not conducted under South African conditions. Therefore, the study was carried out in a South African environment to determine:

- Seasonal changes in herbage yield, oil yield and the variation in the concentration of the major components of rose-scented geranium with plant shoot age
- The influence of harvesting frequency and seasonal changes on herbage yield, oil yield and oil composition
- The harvesting frequency that can produce plants leaves with high density of oil glands

Oil yield and oil composition was significantly affected by plant shoot age in both seasons of experimentation and by temperature changes during the autumn/winter harvesting cycle. Greater herbage yield produced higher oil yield. These results demonstrated the importance of the plant growth stage at which rose-scented geranium should be harvested for higher oil yield. Oil content/percentage is also important in essential oil production but, the total herbage yield contributes massively to the overall oil yield. The oil composition though, did not change with herbage yield. The commonly acceptable oil composition was only recovered in younger plant shoots. These young plant shoots, however, produced lower herbage and oil yield. Higher concentrations of geraniol were recorded in young plant shoots in both experiments. This shows how crucial the harvesting time of rose-scented geranium is, since it either determines the desirable yield and composition of essential oil or the opposite. Environmental conditions also play a vital role in the oil yield and composition. Geraniol and citronellol concentrations were mostly affected by temperature changes. This was more prevalent during the autumn/winter experimental cycle. It resulted into an extremely high C: G ratio since citronellol increased with decreasing temperatures, while geraniol decreased. The fact that geraniol was decreased by both age and decreasing night temperatures in the autumn/winter experimental cycle gives enough explanation why the C: G ratio was very high. Other compounds affected were geranyl formate and citronellyl formate; they increased and decreased respectively, under lower temperatures.

The harvesting frequency experiment indicated that greater herbage/biomass yield produces higher oil yield irrespective of the oil content/percentage. The less frequently harvested treatments (three and four months) produced greater herbage which consequently led to higher oil yield. The oil percentage was mainly to complement herbage yield. These results confirm findings from the literature and from the first experiment (plant shoot age experiment) that biomass yield of essential oil crops (in general) determines the amount of oil yield that can be produced.

Different results were recorded in the oil composition from the harvesting frequencies. The more frequently harvested plants (two month intervals) produced a low C: G ratio oil. As the harvesting frequency decreased (longer intervals), the C: G ratio increased to commercially less desirable oil. This means that harvesting rose-scented geranium plants at a younger age produces a highly recommended oil composition. However, constant production of younger plants could be very challenging under commercial geranium oil production conditions. It would mean increases in the cost of production through frequent oil distillation, which would increase the running costs

The variation trend in the composition of the essential oil with further development of the plant as a whole and the stage of the leaves in particular still needs to be elucidated. Some essential oil investigators blame it on a *de novo* synthesis of oil compounds or re-metabolization (Stengele *et al*., 1993). A commercial harvesting strategy would consider timing that allows the maximum amount of vegetative material to be present, bearing maximal quantities and good composition of oil, but it seems that the composition of oil is maximal at low quantities.

Most if not all essential oil crops are subjected to different environmental changes that pose detrimental conditions to the plant and affect the desired yield and composition. The seasons in which harvesting is conducted play a vital role in the oil yield and oil composition of rose-scented geranium. Seasons affect plant growth by changes in temperature, soil water, photoperiod, humidity and management practises and all these factors jointly affect oil yield. Dry conditions and/or decrease in water supply and optimum temperature is believed to cause an increase in the content and yield of secondary metabolites. Since essential oil is a secondary metabolite that the plant produces as a response to environmental stress or as a defence mechanism against its predators, it is believed to increase under stressful environments. Results from the harvesting frequency experiment indicated that the summer season was more favourable to geranium oil production. Oil yield increased despite increases in temperatures, soil water and photoperiod. It only decreased in autumn and winter when environmental factors were harsher. According to Rajeswara Rao *et al*. (1996) oil yield was expected to increase during this period (autumn and winter) following reports that oil yield of geranium increases in winter because of reductions in thermal stress.

Changes in the oil composition at different seasons cannot be concluded yet because most of the results found in this study, as well as in the literature were inconsistent. Besides the seasons' influence on oil composition, there is also the influence of geographic location where the crop is grown. The crop may perform better in other places than it does in Pretoria (South Africa). Depending on what the essential oil producers want, they can manipulate the harvesting time to suit their desired oil composition considering the differences in oil composition collected at different times of the year. It is always important to mention the collection period of the essential oil under study.

Knowing the density of oil glands on the leaf surfaces does not help much in the production of rose-scented geranium oil. The oil glands are present in all developmental stages with an exception of very young leaves, i.e. oil can be recovered from all the leaves of the plant. The only problem though, is decreases in oil gland density as the leaves age. The senescing leaves contain less oil than the young ones, but can still contribute to the overall yield if included in the oil extraction process. Most interestingly, is the decrease in oil gland density from leaves of the same plant, irrespective of the harvesting frequency. Persistent harvesting of younger shoots will result into lower oil yield because of lower herbage. For high oil yield a high leaf number is more important than a high leaf area because the number of oil glands do not vary with leaf size. It is likely that the highest oil yield coincide with maximum number of mature leaves because of senescence. It is important to know the location and stage of leaf where the majority of the oil is accumulated. This will help determine the growth stage on which harvesting should take place since the percentage of terpenoids concentration changes with leaf development. Another solution would be to breed a variety that can prolong leaf aging so that maximum herbage yield is reached while most of the leaves are still young with higher densities of oil glands.

The spring/summer harvesting season appeared superior to the autumn/winter harvesting season in all commercial aspects of rose-scented geranium essential oil production. Harvesting rose-scented geranium at four months interval was the ideal harvesting frequency for higher oil yield although lower C: G ratio oil was achieved at two months harvesting interval. The density of oil glands does not contribute to the total oil yield of rose-scented geranium instead the herbage yield is the main factor that determines total oil yield.

GENERAL SUMMARY

Field trials were undertaken at the Hatfield Experimental Farm to determine the response of oil yield and oil composition to plant shoot age, harvesting frequency and seasonal changes. The treatments were: harvesting of rose-scented geranium plants every week (Thursday) for eighteen weeks, in two harvesting cycles (autumn/winter and spring/summer) and harvesting rose-scented geranium plants at three frequencies (harvesting after 2, 3 and 4 months). Weekly irrigation was scheduled using neutron probe and standard plant management and plant protection practices were followed. Nitrogen, P and K were applied at a rate of 60, 90 and 60 kg ha^{-1}, respectively, in equal splits after each harvest. Measurements made included leaf area, LAI, plant height, fresh plant mass (later computed to herbage yield), dry mass, oil content, oil composition and a count of the number of oil glands.

LAI values increased with plant height in all treatments but oil content did not increase with LAI. Herbage yield increased, from week 12 and was maintained to the last harvesting week during the spring/summer cycle, while it increased from week 6 to week 16 in the autumn/winter cycle. The herbage yield recorded in weeks 17 and 18 began to decrease due to leaf senescence. Aging (of plant shoot) was more accelerated in the autumn/winter cycle than in the spring/summer experimental cycle. Oil content was generally higher (0.088%) in the spring/summer harvested plants than those harvested in the autumn/winter (0.064%) cycle. The decreases in oil content in the autumn/winter cycle were negatively related to night temperatures. Oil content decreased with decreasing night temperatures. Oil yield increased from week 10 up to the last (18^{th}) week in the spring/summer harvesting cycle, while it increased from week 7 up to week 12 in the autumn/winter cycle. Geraniol concentration decreased with plant shoot age in both experimental cycles and with decreases in night (minimum) temperatures in the autumn/winter experiment. The double decrease effect on geraniol concentrations in the autumn/winter experiment resulted into very high C: G ratios. Citronellol concentration was not affected by plant shoot age but was affected by decreasing night temperatures in the autumn/winter harvesting cycle. It increased with decreasing temperatures, thus causing the C: G ratio to increase even more. Other compounds affected by temperature changes in the autumn/winter experiment were geranyl formate and citronellyl formate. Geranyl formate decreased while citronellyl formate increased with decreasing night temperatures.

Plant height and LAI decreased from the second harvest to the last harvest in all the harvesting frequencies. A positive relationship between LAI values and plant height was also recorded in the harvesting frequency experiment. The highest LAI values were recorded during the summer season in all treatments. Harvesting at four month intervals produced the highest yield with regards to herbage and oil yield. The yields were escalated during the summer season. The sudden increases in herbage yield and oil yield in the second harvest of the two month harvesting frequency might have been a response to favourable summer conditions. The subsequent harvests (in all treatments) recorded lower herbage yield, and most probably because they were harvested during the autumn and winter seasons.

Harvesting frequency itself, had a significant effect on the herbage yield and oil yield. Herbage yield increased as harvesting frequency lessened, thus greater oil yield was recorded in the three and four month harvesting intervals. Lower average oil content was recorded from the three month harvesting interval but since the herbage yield was higher than the two month harvesting interval, it recorded greater oil yield. The highest average oil content and cumulative oil yield was recorded in the four month harvesting interval. Oil content (other than herbage yield) had a slight influence in the total oil yield recorded from the two and three months harvesting frequencies during the winter season. The results recorded in this (harvesting frequency) experiment were relatively similar to those recorded in the 2005 harvesting seasons. In instances of variations in the oil yield and oil content, it was probably due to differences in the months of harvest since plants were not persistently harvested on the same months.

Oil composition from the two months harvesting frequency contained higher concentrations of geraniol and citronellol, irrespective of the harvesting season. This resulted into a relatively low C: G ratio as compared to the other harvesting intervals. The three months harvesting frequency recorded the lowest geraniol concentrations in the last two harvests, thus resulting into higher C: G ratios. Seasonal effects on the C: G ratio was more evident in the three months harvesting frequency yet in 2005 harvesting season, it affected all treatments, mostly the four months harvesting interval. Citronellol showed a sharp increase during this harvesting season (2005) than it did during the experimental period. This also indicated that citronellol may increase or decrease depending on the year of production.

Two types of oil glands were identified on the leaf surfaces of rose-scented geranium. One type was smaller and slightly elongated while the other one was larger and spherically shaped. The adaxial leaf surface recorded higher densities of oil glands and trichomes than the abaxial leaf surfaces. Larger oil glands tended to be in higher densities in the four months harvesting frequency while the smaller oil glands decreased as the harvesting frequency lessened. An interaction between harvesting frequency and leaf surface was only significant in the two months harvesting frequency. Total numbers of oil glands on the abaxial leaf surface were lower as compared to the adaxial leaf surface of rose-scented geranium. The density of oil glands was also affected significantly, by the leaf position on the plant. The top younger leaves contained more oil glands (all types) than the bottom older leaves.

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APPENDIX

Table A1: Weather parameters during the autumn/winter and spring/summer harvesting cycles

Week	Autumn/winter				Spring/summer			
	Date of harvesting	Average temperature (^oC)		Rainfall (mm)	Date of Harvesting	Average temperature (^oC)		Rainfall (mm)
		Max	Min			Max	Min	
1	31 - 03 - 05	22.7	15.8	52.5	27 - 10 - 05	29.2	15.3	0.0
2	07 - 04 - 05	22.6	15.1	0.0	03 - 11 - 05	28.0	15.6	0.8
3	14 - 04 - 05	22.0	12.5	7.4	10 - 11 - 05	27.1	16.5	34.5
4	21 - 04 - 05	23.6	14.2	15.3	17 - 11 - 05	26.6	16.1	1.5
5	28 - 04 - 05	21.7	11.2	0.0	24 - 11 - 05	29.0	15.7	17.6
6	05 - 05 - 05	21.1	9.1	0.0	01 - 12 - 05	25.3	16.0	24.7
7	12 - 05 - 05	24.2	10.5	0.0	08 - 12 - 05	27.0	15.6	6.4
8	19 - 05 - 05	21.9	8.2	0.0	15 - 12 - 05	27.0	15.7	0.8
9	26 - 05 - 05	21.9	9.7	0.0	22 - 12 - 05	28.6	16.9	1.8
10	02 - 06 - 05	20.8	6.0	0.0	29 - 12 - 05	28.8	16.6	5.5
11	09 - 06 - 05	24.1	6.9	0.0	05 - 01 - 06	24.7	16.9	36.4
12	15 - 06 - 05	20.8	6.9	0.0	12 - 01 - 06	26.1	17.9	32.8
13	23 - 06 - 05	21.3	5.4	0.0	19 - 01 - 06	25.3	18.2	4.5
14	30 - 06 - 05	20.2	7.1	0.0	26 - 01 - 06	26.0	17.4	98.5
15	07 - 07 - 05	19.6	7.0	0.0	02 - 02 - 06	25.0	17.4	69.8
16	14 - 07 - 05	19.3	4.0	0.0	09 - 02 - 06	25.8	14.7	31.7
17	21 - 07 - 05	20.5	5.4	0.0	16 - 02 - 06	25.3	18.1	39.0
18	28 - 07 - 05	23.1	6.8	0.0	23 - 02 - 06	28.2	17.7	2.7

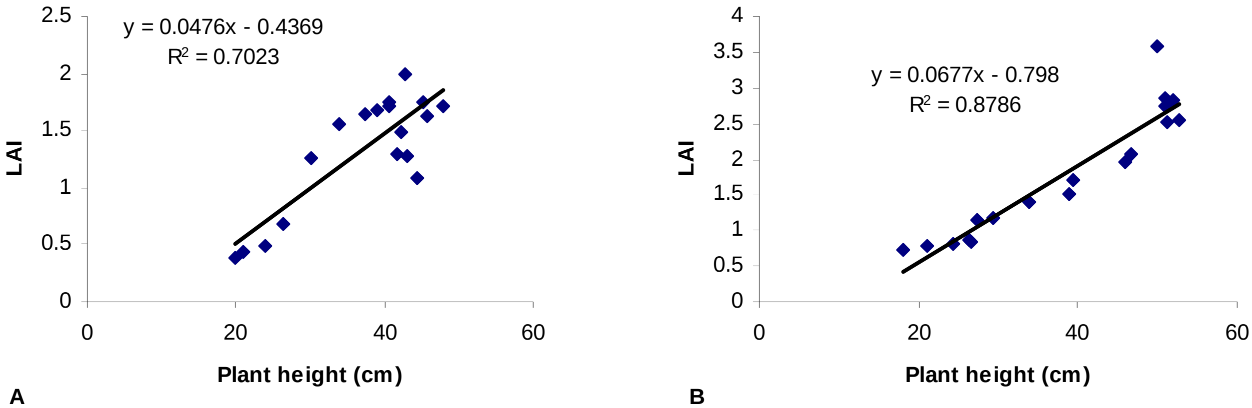


Figure A1: Relationship between LAI and plant height in A) autumn/winter experiment and B) spring/summer experiment

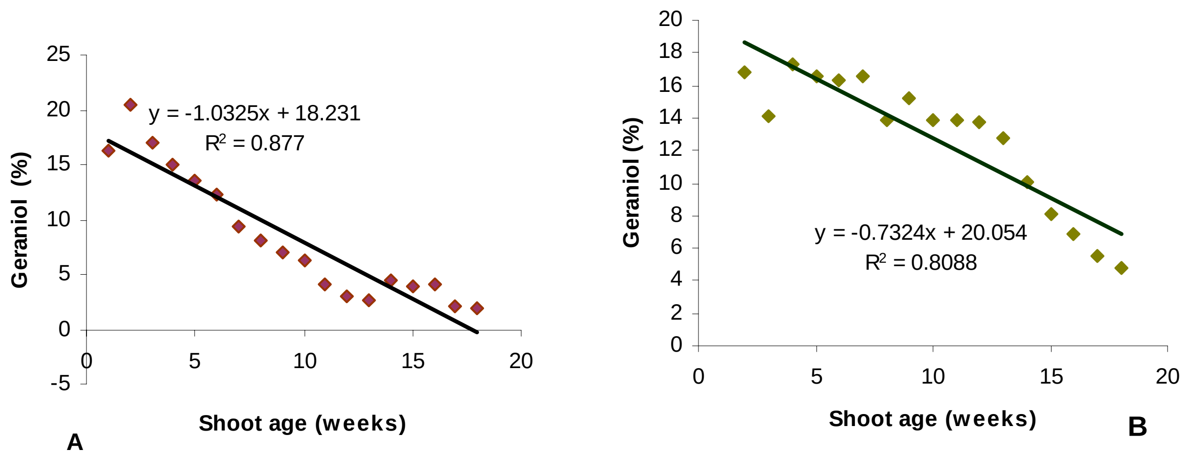


Figure A2: Relationship between plant shoot age and geraniol concentration in A) autumn/winter experiment and B) spring/summer experiment

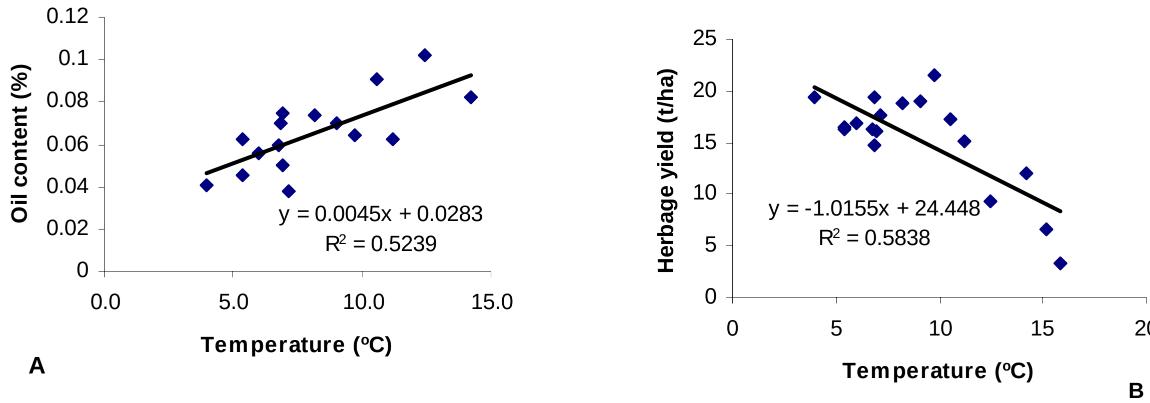


Figure A3: Relationship between minimum temperatures and (A) oil content (B) herbage yield in the autumn/winter experiment

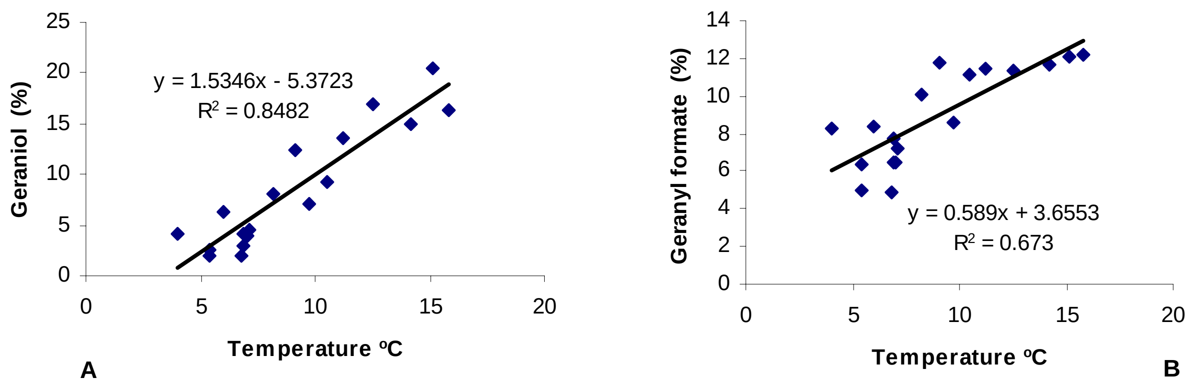
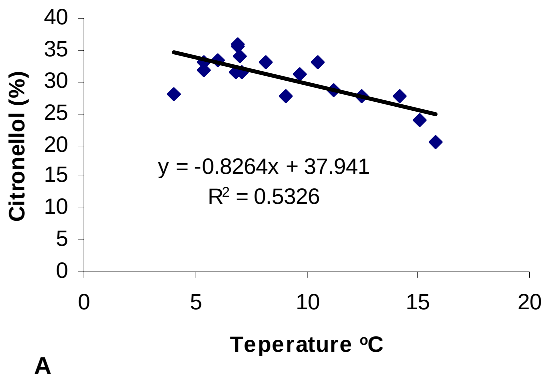


Figure A4: Relationship between minimum temperature and (A) geraniol concentration, (B) geranyl formate in the autumn/winter experiment



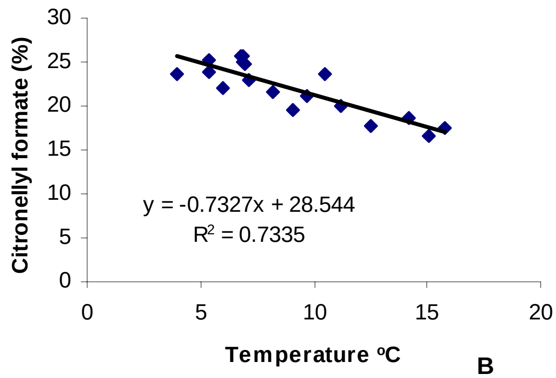


Figure A5: Relationship between minimum temperature and (A) citronellol concentration or (B) citronellyl formate concentration in the autumn/winter experiment

Table A2: Summary of ANOVA table for plant growth, herbage yield and oil yield of rose-scented geranium in the autumn/winter experiment

Source	DF	Mean square						
		Herbage yield (t/ha)	Oil yield (kg/ha)	LAI	Fresh mass (kg/plant)	Dry mass (kg/plant)	Moisture content (%)	Plant height (cm)
Treatment	17	66.75**	39.59**	0.77**	0.83**	0.16**	2972.71**	148.28**
Rep	2	0.14^{NS}	0.05^{NS}	0.002^{NS}	0.006^{NS}	0.24^{NS}	3212.30**	0.77^{NS}
Error	34	0.13	0.05	0.004	0.004	0.04	363.43	0.85

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability

Table A3: Summary of ANOVA table for plant growth, herbage yield and oil yield of rose-scented geranium in the spring/summer experiment

Source	DF	Mean square						
		Herbage yield (t/ha)	Oil yield (kg/ha)	Plant height (cm)	LAI	Fresh mass (kg/plant)	Dry mass (kg/plant)	Moisture content (%)
Treatment	17	1041.03**	879.74**	451.37**	2.35**	2.018**	0.52**	6865.02**
Rep	2	11.60^{NS}	12.99^{NS}	0.39^{NS}	0.02**	0.18^{NS}	0.05^{NS}	428.10^{NS}
Error	34	46.01	45.81	3.21	0.003	0.15	0.08	789.35

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability

Table A4: Summary of AVOVA table for oil composition of rose-scented geranium harvested in the autumn/winter experiment

Source	DF	Mean square						
		Linalool	Iso-menthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Guaia-6,9-diene
Treatment	17	0.31^{**}	4.23^{**}	48.42^{**}	103.94^{**}	27.39^{**}	19.29^{**}	2.50^{*}
Rep	2	0.12^{**}	16.25^{**}	17.25^{**}	14.22^{**}	0.22^{NS}	10.89^{**}	0.22^{NS}
Error	34	0.0015	0.05	0.0	0.22	1.05	0.42	1.05

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability

Table A5: Summary of AVOVA table for oil composition of rose-scented geranium harvested in the spring/summer experiment

Source	DF	Mean square						
		Linalool	Iso-menthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Guaia-6,9-diene
Treatment	16	0.32^{**}	11.79^{**}	22.94^{**}	50.73^{**}	6.17^{**}	3.79^{**}	3.21^{**}
Rep	2	0.09^{**}	2.98^{*}	18.02^{**}	13.24^{**}	17.00^{**}	9.94^{**}	13.20^{**}
Error	32	0.002	0.69	0.02	0.24	0.00	0.44	0.24

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability

Table B1: Weather parameters during the period of experimentation

Month	Temperature (°C)		Rainfall (mm)
	Min	Max	
Sept 05	12.1	27.9	0.0
Oct 05	14.8	28.7	12.9
Nov 05	16.3	27.6	81.8
Dec 05	15.9	27.6	67.8
Jan 06	17.7	25.4	281.1
Feb 06	17.0	25.9	194.2
Mar 06	14.9	23.3	11.3
Apr 06	12.6	21.4	31.6
May 06	6.4	19.4	0.9
Jun 06	5.8	18.6	0.0
Jul 06	6.9	19.4	0.0

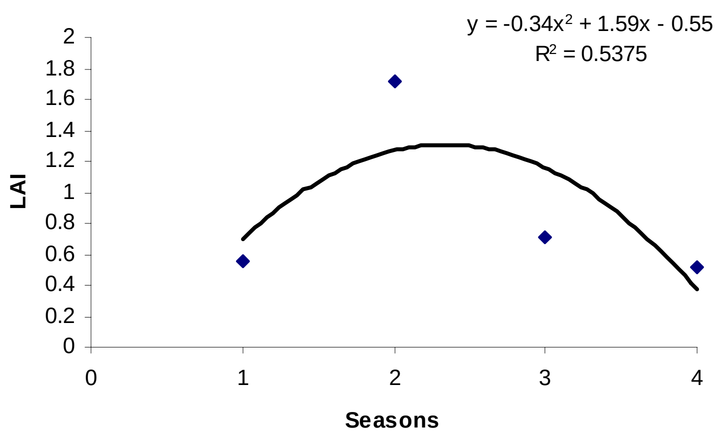


Figure B1: The polynomial relationship between LAI seasonal changes

Table B2: Summary of ANOVA table for the two months harvesting frequency

Source	DF	Mean square					
		Plant height (cm)	Fresh mass (kg/plant)	Dry mass (kg/plant)	LAI	Herbage yield (t/ha)	Oil yield (kg/ha)
Treatment	3	612.93**	1.056**	0.03**	1.30**	137.15**	102.89**
Rep	3	2.56^{NS}	0.017^{NS}	0.00^{NS}	0.00^{NS}	1.108^{NS}	0.81^{NS}
Error	9	1.06	0.01	0.00	0.02	1.48	0.91

^{z}F value significant ant 5% level of probability (*) or highly significant at 1% level probability (**)

Table B3: Summary of ANOVA table for the three months harvesting frequency

Source	DF	Mean square					
		Plant height (cm)	Fresh mass (kg/plant)	Dry mass (kg/plant)	LAI	Herbage yield (t/ha)	Oil yield (kg/ha)
Treatment	2	272.77**	0.08^{NS}	0.04**	0.71**	343.04**	115.43**
Rep	3	0.35^{NS}	0.00^{NS}	0.00^{NS}	0.00^{NS}	4.69^{NS}	1.87^{NS}
Error	6	1.77	0.02	0.00	0.01	3.41	1.48

^{z}F value significant ant 5% level of probability (*) or highly significant at 1% level probability (**)

B4: Summary of ANOVA table for the four months harvesting frequency

Source	DF	Mean square					
		Plant height (cm)	Fresh mass (kg/plant)	Dry mass (kg/plant)	LAI	Herbage yield (t/ha)	Oil yield (kg/ha)
Treatment	1	946.13**	14.85*	0.25*	5.45^{NS}	1827.10**	2207.80**
Rep	3	8.13^{NS}	0.06^{NS}	0.02^{NS}	1.09^{NS}	10.49^{NS}	12.73^{NS}
Error	3	11.46	0.19	0.01	0.95	1.57	1.98

^{z}F value significant ant 5% level of probability (*) or highly significant at 1% level probability (**)

Table B5: Summary of ANOVA table showing seasonal changes and harvesting frequency

Source	DF	Mean square					
		Plant height (cm)	Fresh mass (kg/plant)	Dry mass (kg/plant)	LAI	Herbage yield (t/ha)	Oil yield (kg/ha)
Harvesting frequency	2	645.02**	3.58**	0.15**	2.19**	562.51**	949.45**
Season	3	1014.77**	3.75**	0.12**	2.97**	871.82**	647.58**
Season*harvesting frequency	3	95.39**	2.31**	0.01*	0.62^{NS}	103.05**	268.19**
rep	1	0.36^{NS}	0.02^{NS}	0.01^{NS}	0.12^{NS}	0.03^{NS}	0.17^{NS}
Error	26	3.36	0.04	0.00	0.24	3.36	2.66

^{z}F value significant ant 5% level of probability (*) or highly significant at 1% level probability (**)

Table B6: Summary of ANOVA table for oil composition as influenced by seasonal changes and harvesting frequency

Source	DF	Mean square						
		Linalool (%)	Iso-menthone (%)	Citronellol (%)	Geraniol (%)	Citronellyl formate (%)	Geranyl formate (%)	Guaia-6,9-diene (%)
Harvesting frequency	2	0.85^{**z}	4.74**	78.48**	221.67**	21.20**	48.50**	5.74**
Season	3	0.82**	6.38**	77.70**	39.66**	20.24**	20.31**	2.42**
Season*harvesting frequency	3	1.42**	2.46**	15.88**	37.58**	11.56**	7.43**	1.86**
rep	2	0.00^{NS}	0.00^{NS}	0.18^{NS}	0.14^{NS}	0.04^{NS}	0.14^{NS}	0.00^{NS}
Error	16	0.003	0.00	0.62	0.11	0.04	0.11	0.001

^{z}F value significant ant 5% level of probability (*) or highly significant at 1% level probability (**)

Table B7: Summary of ANOVA table for oil composition as influenced by seasonal changes and harvesting frequency in the 2005 growing season

Source	DF	Mean square						
		Linalool (%)	Iso-menthone (%)	Citronellol (%)	Geraniol (%)	Citronellyl formate (%)	Geranyl formate (%)	Guaia-6,9-diene (%)
Harvesting frequency	2	0.53^{**}	20.38^{**}	57.89^{**}	3.10^{NS}	58.80^{**}	35.250^{**}	2.71^{NS}
Season	2	0.44^{**}	0.62^{NS}	9.40^{**}	87.14^{**}	6.35^{**}	45.71^{**}	1.66^{NS}
Season*harvesting frequency	2	0.08^{**}	11.89^{**}	3.22^{NS}	23.55^{**}	2.15^{NS}	8.67^{**}	2.80^{NS}
rep	2	0.02^{NS}	2.38^{*}	1.29^{NS}	0.14^{NS}	3.86^{*}	1.00^{NS}	0.22^{NS}
Error	12	0.019	0.61	0.95	1.14	0.60	1.00	0.85^{NS}

[z]F value significant at 5% level of probability (*) or highly significant at 1% level of probability

Table B8: Summary of ANOVA table showing seasonal changes and harvesting frequency in the 2005 growing season

Source	DF	Mean Square	
		Herbage yield (t/ha)	Oil yield (kg/ha)
Harvesting frequency	2	768.55**	254.99**
Season	2	1269.24**	641.25**
Season*harvesting frequency	2	262.46 **	142.09**
rep	3	5.28NS	3.65NS
Error	18	8.81	4.31

z F value significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table C1: An ANOVA table for oil gland density of rose-scented geranium from the three months harvesting frequency

Source	DF	Mean square		
		Large oil glands	Small oil glands	Total no. of glands
Harvesting frequency	2	1014.00**	1843.63**	5514.13**
Rep	1	261.94*	126.38NS	316.38NS
Surface	2	1066.67**	737.04NS	3577.04**
Surface* Harvesting frequency	3	367.1*	270.29NS	604.04NS
Error	15	67.84	175.04	304.41

***F value significant (*) at 5% or highly significant (**) at 1% level of probability**

Table C2: An ANOVA table for oil gland density of rose-scented geranium from the four months harvesting frequency

Source	DF	Mean square		
		Large oil glands	Small oil glands	Total no. of glands
Harvesting frequency	2	417.88*	996.54*	2668.67*
Rep	1	183.11NS	22.33NS	321.89NS
Surface	2	2281.50**	266.67NS	4108.17*
Surface* Harvesting frequency	3	35.38NS	199.29NS	67.17NS
Error	15	100.58	260.13	498.12

***F value significant (*) at 5% or highly significant (**) at 1% level of probability**

Table C3: An ANOVA table for oil gland density of rose-scented geranium from the two months harvesting frequency

Source	DF	Mean square		
		Large oil glands	Small oil glands	Total no. of glands
Harvesting frequency	2	2192.79**	6550.54**	16297.13**
Rep	1	172.93NS	217.50NS	254.60NS
Surface	2	1584.380*	6016.67**	13776.04**
Surface* Harvesting frequency	3	58.63NS	1371.29**	1995.29*
Error	15	202.13	207.80	498.60

***F value significant (*) at 5% or highly significant (**) at 1% level of probability**

Table C4: An ANOVA table for the effect of leaf position and harvesting frequencies on oil gland density of rose scented geranium

Source	DF	Mean square		
		Large oil glands	Small oil glands	Total no. of glands
Harvesting frequency	2	315.19NS	1899.08NS	719.53NS
Harvesting frequency *rep	9	411.99NS	244.14NS	595.24NS
Leaf position	2	6433.69**	15732.33**	42261.19**
Harvesting frequency *position	4	407.82NS	1524.54NS	3349.32NS
Error	18	290.19	698.14	1507.46

***F value significant (*) at 5% or highly significant (**) at 1% level of probability**