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An investigation of the rate of biogenic volatile organic carbon emissions from commercial crop and plantation species in the Mpumalanga Province

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CHAPTER 1: INTRODUCTION

Volatile Organic Compounds (VOCs) are emitted into the atmosphere in substantial quantities from anthropogenic and biogenic sources (Fehsenfeld *et al*. 1992; Singh and Zimmerman, 1992). The importance of Biogenic Volatile Organic Compounds (BVOCs) in plant physiology and ecology has grown over the past twenty years. The production and emission of BVOCs by plants is one of the ways in which plant processes influence the functioning of the atmosphere (Lerdau *et al*. 1997). The global annual BVOC emission rates are estimated to be ~10^{15} g y^{-1} (Penuelas and Llusia, 2003), which constitutes ~80 % of the chemically reactive VOCs added to the atmosphere each year. Biogenic Volatile Organic Compounds are extremely reactive which increases their significance in atmospheric chemistry (Brasseur and Chatfield, 1991).

Although chemical researchers of the early 1900s, discovered emissions from plants, they did not consider how these compounds could interact with the climate. However, there is emerging evidence that this influence might be significant at different spatial scales, from local to regional and global, due to the formation of aerosols which directly or indirectly interact with the greenhouse effect (Hyden, 1998). Thus, BVOCs are important for several reasons. The organic compounds take part in O_3 formation in the troposphere, and react with OH, O_3 and NO_x, which affects the oxidative capacity of the atmosphere and air quality (Chameides *et al*. 1992; Fehsenfeld *et al*. 1992; Atkinson and Arey, 2003). The BVOCs are also considered to play a role in global climate warming through:

- increased temperatures, which affects BVOCs and the reactions forming tropospheric O_3 (ozone formation only occurs during the day time and at night the loss of ozone through titration of BVOCs becomes a dominant process (Wang *et al*. 2008);
- altered weather patterns such as precipitation, and clouds;
- increased anthropogenic emissions from increasing energy demand (Bernard *et al*. 2001; McCarthy *et al*. 2001).

Increases in NO_x and BVOCs can result in decreased O_3 levels (Seciufeld and Pandis, 1998). The photochemical reactions that take place during ozone formation are complex. In the presence of sunlight, O_2 reacts with NO and VOCs to produce ozone (Houweling *et al*. 1998).

Plants have the capacity to synthesize a plethora of low molecular weight compounds that have no obvious role in growth and development, and are therefore called secondary metabolites (Winer *et al*. 1992). Terpenes, the largest class of plant secondary metabolites, have many volatile representatives, including hemoterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), and even some diterpenes (C_{20}). Graedel (1979b) listed 367 different compounds known to be emitted by vegetation. Twenty one compounds were identified as being emitted from trees and grasses, including six oxygenated terpenoids (three alcohols, one aldehyde, one ether and one ketone), (Graedel, 1979a). The other 346 compounds were volatile substances from essential oils of foliage and flowers (Helas *et al*. 1997). Isoprene and monoterpenes dominate biogenic emissions, however, other highly reactive chemical species, such as methanol, acetone, aldehydes and organic acids are now also recognized to be emitted by terrestrial vegetation at significant levels (Kirstine *et al*. 1998). The estimated amounts of annual global emissions of isoprene are between 175-503 Tg C y^{-1}, of monoterpenes are between 127-480 Tg C y^{-1} and for the other BVOCs ~260 Tg C y^{-1}. Isoprene is produced mainly by deciduous trees, whereas the monoterpenes, are usually emitted from conifers (Helas *et al*. 1997). The first global estimates of BVOCs were published by Went (1960) to be175 Tg C Rasmussen and Went (1965) later increased the estimate to 200-400 Tg C yr^{-1} and (Guenther *et al*. 1995) increased it again to 1150 Tg C yr^{-1}.

Tropical and subtropical regions cover a large land surface area and have a high productivity and leaf area index, making them important in terms of vegetative emissions. Estimates of BVOC emissions from southern Africa, as a subtropical region, have been limited to relatively few plant species found in the savanna regions. Extrapolation of these emissions provided an estimated annual emission of 80 Tg C (Otter *et al*. 2002). Harley *et al*. (2003) derived a land-scape scale isoprene emission capacity for South Africa which was based on measured emissions,

species composition and biomass data (Scholes *et al*. 2001) Approximately 400 species in Africa have been screened either for total VOC emissions using a branch enclosure and a photoinization detector (PID), or a leaf chamber (Guenther *et al*. 1996; Klinger *et al*. 1998; Otter *et al*. 2002). Inventories of BVOCs from South Africa are important in order to improve the understanding of regional and global atmospheric chemistry. High temperatures and photosynthetic active radiation (PAR) fluxes make this bioclimatic region a large potential source of BVOCs. Guenther *et al*. (1995) proposed that southern African savannas have an isoprene emission capacity of almost 10 mg C m^{-2} $hr,^{-1}$ whereas monoterpene emission capacity, assumed by the global model capacity, is lower.

Mpumalanga is the second smallest province of South Africa, however, it is the region with the highest level of productivity. There are three vegetation biomes in Mpumalanga, forest, grassland and savanna biomes. It is also a major exotic forestry production region covering 79 590 km^2 planted mainly with *Eucalyptus* (38 %) and *Pinus* (46.6 %), in addition it is a prime cropping area (covering 1 450 km^2) (Godsmark, 2008).

All the previous work on BVOC emissions in South Africa has been conducted on indigenous species. The rate of land use change is increasing in the Mpumalanga province driven by increasing population and demand for commodities. The aim of this project was to measure the rate of emissions from those species in the Mpumalanga province, which had not been previously investigated, for example, forestry plantations and subtropical crops, and to understand the factors that control the rates of the emissions. Leaf level studies were conducted using a leaf cuvette. One of the goals of leaf-level studies of emissions is to understand the ecophysiological mechanisms that underlie emissions. This mechanistic understanding provides insight into the possible functional roles for production and is essential in developing regional models of isoprene emissions that can be used to predict emissions across large spatial and temporal scales (Guenther *et al*. 1995).

HYPOTHESIS AND KEY QUESTIONS

Hypotheses

- The emission rates of BVOCs from the plantation species are higher than from the crop species due to differences in carbon allocation.
- The emission rates of BVOCs from the species are higher in the hot, wet season than in the dry season.

Key questions

- How do emission rates of BVOCs differ between the plantation species and the crop species?
- How do the BVOC emission rates change over the seasons?
- How does leaf nitrogen content affect the BVOC emission rate?

CHAPTER 2: LITERATURE REVIEW

2.1 The Role of BVOCs in the Atmosphere

Biogenic volatile organic carbons play a number of important roles in the atmosphere.

2.1.1 BVOCs and Ozone

The biogenic emission of isoprene plays an important role in atmospheric chemistry because of its reactivity with other gases (Fuentes *et al*. 2000). Isoprene oxidation in the atmosphere can give rise to ozone and smog if nitrogen oxides are present (Haagen-Smit, 1952; Chameides *et al*. 1988; Daum *et al*. 2000). Houweling *et al*. *(*1998) estimated that globally, VOCs contribute approximately 40 % to the net photochemical ozone production, creating a 17 % increase in the tropospheric ozone column. The photochemical reactions that take place during ozone formation are complex. Simply stated, in the presence of sunlight, oxygen reacts with nitrogen oxide and VOCs to produce ozone. Isoprene can also react directly with ozone. This reaction breaks down isoprene primarily to methyl vinyl ketone methacrolein and formaldehyde, also yielding moderate amounts of hydrogen peroxide and other oxidative species (Sauer *et al*. 1999; Fuentes *et al*. 2000; Ruppert and Becker, 2000). Ozone and its reaction products (OH, O_2 and H_2O_2) are toxic to plants (Pell *et al*. 1997) because ozone concentrations cause the peroxidation and denaturation of membrane lipids at high temperatures (Maccarrone *et al*. 1992; Ranieri *et al*. 1996; Wellburn and Wellburn, 1996; Ederli *et al*. 1997; Pell *et al*. 1997).

The reaction of monoterpenes with OH can significantly suppress OH concentrations in the troposphere (Brasseur and Chatfield, 1991; Fehsenfeld *et al*. 1992). Hydroxyl radicals initiate nearly all the oxidation paths in the troposphere and change markedly with season (Singh and Zimmerman, 1992). Suppressing OH concentrations will therefore influence the lifetimes and concentrations of many important chemical species, such as CH_4 and CO (Brasseur and Chatfield 1991;

Fehsenfeld *et al*. 1992). Together with anthropogenic NO_x emissions, the natural VOCs can act as precursors in photochemical oxidant (Trainer *et al*. 1987; Roselle 1994) and aerosol formation (Zhang *et al*. 1992; Odum *et al*. 1996), and hence might contribute to regional-scale air pollution. In sunlight, VOCs, which typically come from sources such as trees, gasoline, solvents, and paint, recycle NO_x by reacting with nitrogen oxide, which reacts with O_3.

$$VOCs + NO \rightarrow NO_2 \quad \textbf{which leads to} \quad NO_2 + O_2 \rightarrow O_3 + NO$$

The carbonyl compounds can undergo further photochemical reactions that will result in additional production of organic and hydrogen radicals and, in turn, produce more ozone. Volatile organic carbons and NO_x are referred to as ozone precursors. In 1960, Went identified the blue haze phenomenon associated with volatile isoprenoids from plants. These are particles acting as cloud condensation nuclei. Raindrop size is also affected by the concentration of cloud condensation nuclei. These particles that are formed from monoterpenes also scatter light. When the particles are very small, blue light is scattered more than other colours, giving rise to the natural blue haze (Went, 1960).

One isoprene molecule can lead to the formation of many ozone molecules when the NO_x levels are high. At low NO_x level, destructive reactions can dominate and isoprene emissions from plants can reduce ozone in the atmosphere (Trainer *et al*. 1987).

2.1.2 BVOCs and the Greenhouse effect

BVOC emissions from plants increase with increasing ambient temperatures. These emissions may produce both negative and positive feedbacks on climate warming through aerosol formation and direct and indirect greenhouse effects (Penuelas and Llusia, 2003). Chemical transformations of isoprene and other BVOCs reduce the concentration of OH radicals which are required for the degradation of methane (Penuelas and Llusia, 2003; Bell *et al*. 2003). This amount of isoprene is almost equal to the amount of methane present in the atmosphere, and the latter gas largely

contributes to the greenhouse effect. Although isoprene itself is not a greenhouse gas, it competes with methane and other terpenoids for the oxidation potential of the atmosphere and, thereby, prolongs the lifetime of methane and other organic substances.

2.2 BVOCs and Plant Function

Biogenic volatile organic carbons appear to attract pollinators and herbivore predators, and to communication with other plants and organisms (Penuelas ,1995). Given that plants universally emit induced volatiles in response to herbivory, it is apparent that undamaged neighbors could benefit from exploiting this information (Fig. 2.1).

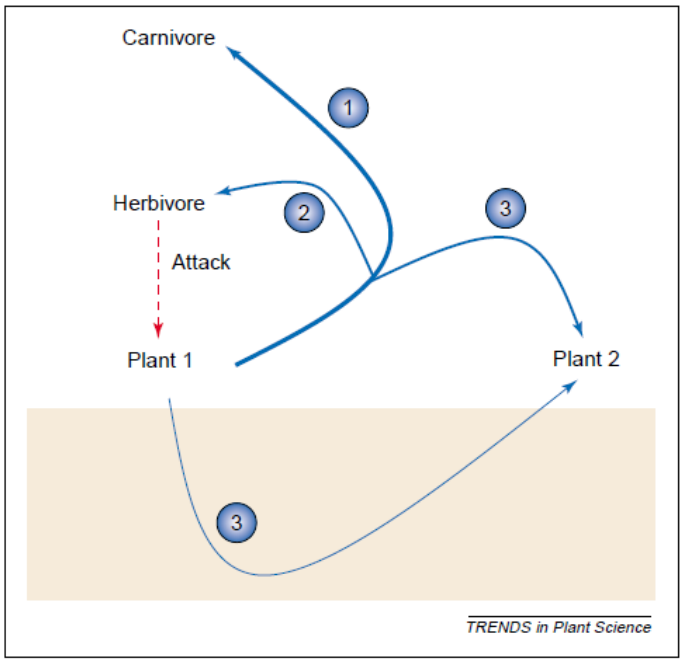

Figure 2.1 Plants respond to herbivory with the emission of chemical cues above and below ground, which can elicit responses in (1) carnivorous enemies of the herbivores, (2) herbivores and (3) neighbouring plants. The plant–plant interactions comprise interactions among conspecifics and heterospecifics. The red broken arrow represents herbivore attack on a plant; the blue arrows represent the emission of chemical cues that affect other organisms. The thickness of the blue arrows indicates the relative knowledge of the interactions (Dicke and Van Loon, 2000)

Plants that are able to activate their defenses in response to the information from their neighbour might time their defense more effectively and thus gain a selective

advantage over plants that are deaf to the information of their neighbour (Dicke and Van Loon, 2000)

Plant BVOCs convey signals between neighbors, whereby defense mechanisms are induced in undamaged plants because of volatiles produced by nearby infested plants. These infochemicals comprise of specific plant volatiles, methyl salicylate (MeSA), methyl jasmonate (MeJA), and cisjasmonate (Ton *et al*. 2001).

2.2.1 Repellents and Attractants

Monoterpenes such as α–pinene and β-pinene, limonene and camphene are photochemically reactive biogenic hydrocarbons that are released in significant quantities from certain plant species and are stored within plant tissues in considerable quantities. These compounds can be produced as a result of biotic and abiotic stresses (Pare and Tumlison, 1997) and generally serve as defense mechanisms against grazing and insect pests (Lerdau, 1991; Banthorpe, 1991). In addition, many of these compounds, play roles as attractants, for example linalool, which is involved in attracting pollinators, and is produced primarily in the petals of flowers (Pichersky and Gang, 2000).

A number of BVOCs produced in unspecialized plant tissues also play a role in defending plants against disease and herbivores (Harborne, 1993). These compounds can act by repelling pests or attracting predators. They may effectively deter insect and herbivore feeding and block the colonization of wounding by bacteria and fungi. Microorganisms or insects may trigger a series of defense reactions, initiating the production of different compounds such as jasmonic or salicylic acid, coumarin, different terpenes or ethene, acting as a signal or a chemical defense component (Enmos and Swales, 1988; Kauss, 1991; Hopke *et al*. 1994). Plants respond to insect feeding damage by releasing a variety of volatiles from the damaged site, and the profile of the volatiles emitted is markedly different from those of undamaged or mechanically damaged plants (Loughrin *et al*. 1994). Altered chemical emissions can influence interactions with organisms in the environment of the plant, resulting for example, in a modified susceptibility to insects and pathogens (Hildebrand and Grayburn, 1991).

Plant-to-plant signaling in the field has failed over distances >10 cm, yet signals have the capacity to attract predators and parasitoids (Slobodkin and Lerdau, 2002) over distances greater than 10 cm, for example, *Nicotiana attenuate* plants are able to reduce herbivore damage by 90 % by attracting predators when the plants are grown 3–5 cm apart, but herbivore damage is reduced by only 20 % when the plants are grown >20 m apart (Kessler and Baldwin, 2001).

Leaves produce their repellent odors in a variety of different structures (such as trichomes, idioblasts, cavities, and ducts) depending on the species. In contrast, flowers usually produce their attractive fragrance in osmophores or in conical cells located in the petals. These cells do not store VOCs but release them into the air. The importance of VOCs in species interactions has recently been investigated at the molecular level (Dudareva and Pichersky, 2000; Kolosova *et al*. 2001a; Pichersky and Gershenzon, 2002), but surprisingly little information is available on the exact cellular location or transport and emission of VOCs into the surrounding air (Hudak and Thompson, 1997; Jasinski *et al*. 2001; Goodwin *et al*. 2003). Emissions of monoterpenes and oxygenated non-methane volatile organic compounds (NMVOC) can increase 100-fold when some plant tissues are damaged (Fall, 1999). Dufay *et al*. (2003), found some repellent VOCs which are produced in the leaves, whilst other attractant VOCs are produced by different structures in flowers. Leaves of *Chamaerops humilis L*. (*Arecaceae*), the Mediterranean dwarf palm, produce volatile compounds that attract their species-specific pollinating weevil (*Derelomus chamaeropsis*), whereas the flowers are almost scentless. This volatile production is limited to anthesis and may have a function similar to that of floral scents.

2.2.2 Disease

Many plants produce and store terpenoid compounds within specialized tissues that act as physical barriers to insects and pathogens (Guenther *et al*. 2000). The differences in monoterpenes between healthy and diseased tree graft combinations indicate that the increases in monoterpenes in diseased trees are a general response to infection (Kossuth, 1984). Plant pathogens induce the production of BVOCs that

inhibit the spread of pathogens into plant tissues because of their antimicrobial activities (Cardoza *et al*. 2003; Gouinguene *et al*. 2003).

2.2.3 Membranes and Heat Stress

There are several hypotheses as to why isoprene has a protective action on membrane (Sharkey, 1996; Sharkey and Yeh, 2001). Isoprene stabilizes the membrane lipid bilayer, which is often denatured by exposure to high temperatures (Gounaris *et al*. 1984). Moreover, isoprene may provide substrates for protein prenylation, one of the main mechanisms that anchors proteins to the lipids in biological membranes (Yalovsky *et al*. 1999).

The lipophylic isoprenoids interact with lipids in the membranes to strengthen their tolerance to stress (Sharkey and Yeh, 2001). However, isoprenoids may also react in the intercellular spaces with toxic reactive oxygen species, decreasing pressure and potential damage to the membranes. Velikova *et al*. (2005) have shown that isoprene may interact with nitrogen oxide formed in stressed leaves and be responsible for starting the signaling cascade which leads to programmed cellular death.

2.3 A Description and Synthesis of Isoprene and Monoterpenes

There are many volatile organic carbons that are emitted from plants, especially trees. Dumas proposed in 1866 the name "terpene", derived from turpentine, instead of camphor for crystalline oxygenated substances extracted from essential oils. The structure of camphor was established by Bredt in 1893, that of pinene by Wagner in 1894 and that of citral by Tiemann in 1895. The period since 1945 has seen an explosion in natural product chemistry due to the advent of chromatographic and spectroscopic techniques. The discovery of the isoprene unit is the basis of the concept of the "isoprenic rule" edited in 1953 by Ruzicka and completed by (Lynen *et al*. 1959 and Bloch *et al*. 1959).

2.3.1 Isoprene

History

Isoprene (2-methyl-1,3-butadiene) was first discovered as a cell metabolite in the mid-1950's at the Department of Anatomy and Plant Physiology in the Institute of Botany, Georgian Academy of Sciences, Georgia (Sanadze, 1961). The biosynthesis of isoprene and its emission from leaves was initially thought to be a light-dependent process. The light-dependent biosynthesis of isoprene was strongly correlated with changes in CO_2 partial pressure in the atmosphere, inside a glass experimental chamber containing a leaf (Sanadze, 1959, 1964). Subsequent studies revealed that isoprene could be synthesized without light, both in autotrophic leaf cells and heterotrophic cells of microorganisms, plants, animals, and humans (Deneris *et al*. 1984).

Structure

Isoprene (Fig. 2.2) is a linear hydrocarbon molecule containing five carbon atoms and two double bonds. The structural formula was identified in 1882 (Saltman, 1985). Isoprene emission rates are often expressed in units of micrograms of isoprene per gram of dry leaf mass per hour (Sharkey, 1991). The chemical lifetime for isoprene ranges from 1.3 hours to 34 hours depending on the ambient ozone and hydroxyl concentrations (Altshuller, 1983).

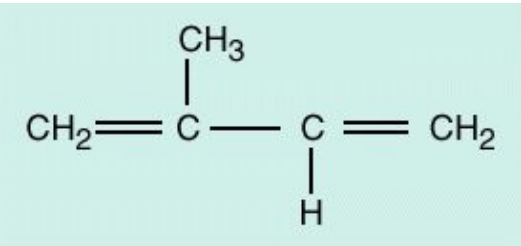

Figure 2.2 Structure of isoprene

Isoprene emissions from selected plant families

Guenther *et al*. (1995) proposed that Southern African savannas have an isoprene emission capacity of almost 10 mg C m^{-2} hr^{-1}. Isoprene emissions has been measured from several poplar (*Populus*) species, (Miller *et al*. 2001; Sasaki *et al*. 2005; Sharkey *et al*. 2005) and kudzu (*Pureria lobata*) (Sharkey *et al*. 2005). In forests, most genera are low isoprene emitters (< 0.1 mg C g^{-1} (foliar) hr^{-1}), but for the tree genera *Casuarina, Eucalyptus, Liquidambar, Populus* and *Quercus,* isoprene emissions may be as high as 70 mg C g^{-1} hr^{-1} (Geron *et al*. 1994).

2.3.2 Monoterpene

History

In 1818 the analysis of turpentine oils was conducted by Houston de la Billardière and further research led to the name "terpene", which was used to define the hydrocarbons with the general formula $C_{10}H_{16}$ found in complex plant products. These terpenes are frequently found in plant essential oils which contain the "*Quinta essentia*", the plant fragrance. More than 1000 monoterpenes, 7000 sesquiterpenes and more than 3000 diterpenes have been described.

Structure

Monoterpenes may be linear (acyclic) or contain rings. Most monoterpenes have cyclic structures and are synthesised by so-called monoterpene synthases or cyclases. Monoterpenes generally contain at least one unsaturated carbon-carbon bond and often have one or more rings in their structure. In the case of pinene, one of these rings contains just four carbon atoms, which introduces strain on the carbon bonds and so increases the reactivity of the molecule (Sanderson, 2002). The lifetime of monoterpenes in the atmosphere lies in the range of 1 minute to several hours. Their lifetime is much shorter when compared to the lifetime of VOC emissions that result from anthropogenic activity (Atkinson and Arey, 2003). The short lifetimes of the biogenic hydrocarbons indicate that localized

atmospheric transformations will occur and that significant concentrations of secondary aldehydes and ketones will be generated close to the BVOC source.

Monoterpene emissions from selected plant families

Monoterpene production has been found in forty six families of flowering plants and all conifers (Lerdau, 1991). Monoterpenes are highly variable in their relative composition within a single species.

2.4 Biosynthesis of Isoprene and Monoterpenes

In the 1980s and 1990s, BVOCs were found to be synthesized in plant tissues by a variety of specific metabolic processes (Sharkey *et al*. 1991).

2.4.1 Location of synthesis and synthesis pathways

Isoprene and monoterpene production consume a considerable amount of the carbon fixed in photosynthesis (Monson and Fall, 1989), as well as additional energy in the form of Adenosine Triphosphate (ATP) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) that is consumed in the synthesis. Biosynthesis of other terpenoids is often restricted to specific tissues at their sites of utilization. Biosynthesis of isoprene in higher plants occurs through the pathways located in the cytoplasm or in the plastids (Lichtenthaler, 1999), and can be regulated either by the supply of substrates and availability of energy, or by enzyme activities in the metabolic branching points of the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, also known as the non-mevalonate pathway or mevalonic acid independent pathway (Bohlmann *et al*. 1998). The source of DMAPP within the chloroplast is the MEP pathway (Rohmer *et al*. 1993). In this pathway, pyruvate and glyceraldehyde 3-phosphate are the initial substrates starting a reaction resulting in 1-deoxy-D-xylulose 5-phosphate (DOXP). The next step is the formation of MEP. In three further reaction steps, MEP is converted to 2-C-methyl-D-erythritol 2, 4-cyclodiphosphate (MECDP). To date this is the last known reaction leading to isopentil diphosphate (IPP) and dimethylallyl diphosphate (DMADP). The final step is the formation of isoprene where the conversion of

DMADP to isoprene and pyrophosphate is catalysed by isoprene synthase (Schnitzler *et al*. 2002). Regulation of isoprene emissions by light may be due to light-dependent activation of isoprene synthase and light-dependent production of DMADP from photosynthetic intermediates (Fig. 2.3).

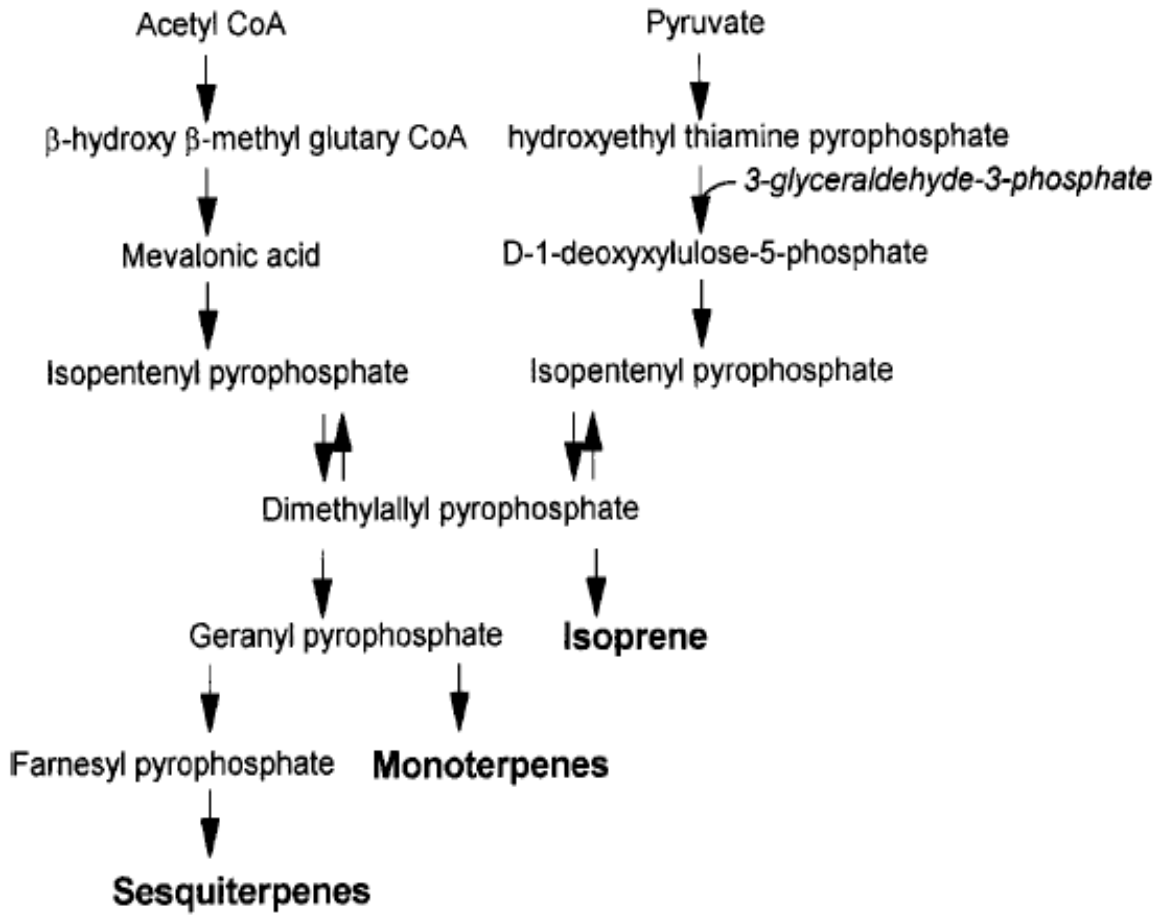

Figure 2.3 Schematic compilation of the biosynthesis of the volatile and semi-volatile isoprenoids: isoprene, monoterpenes and sesquiterpenes in plants. Upper left part describes the classical acetate/mevalonate pathway, whereas the upper right part summarizes the recently described novel pathway via pyruvate by introducing 3-glyceraldehyde-3-phosphate (GAP), both leading to the key compound isopentenyl pyrophosphate (Rohmer *et al*. 1993)

A light-attributed increase in DMADP concentration could be attributed to light-induced increase in the fluxes of intermediates through the plastidial MEP-pathway leading to increased stromal DMADP (Schnitzler *et al*. 2002). Isoprene biosynthesis is strongly correlated with the formation of the first stable product of photosynthetic CO_2 fixation, 3-phosphoglyceric acid (Logan *et al*. 2000).

The biosynthesis of monoterpenes uses the same pathway as that of isoprene. An interesting characteristic of monoterpene synthases is their ability to produce more than one enzymatic product (for example, pinene), although, all monoterpene synthase enzymes use a similar reaction mechanism. There are a number of inhibitors that inhibit the process of terpene biosynthesis and photosynthesis, one inhibitor in this class is methyl viologen, which catalyzes the pseudocyclic electron transport pathway (Loreto and Sharkey, 1990). Photosynthesis and isoprene are not always simultaneously inhibited (Sharkey and Singsaas, 1995).

In higher plants, linalool, like other monoterpenoids, is produced from Isopentenyl diphosphate (IPP) via the universal isoprenoid intermediate geranyl pyrophosphate, through a class of membrane-bound enzymes named monoterpene synthases. One of these, linalool synthase (LIS), has been reported to produce (S)-linalool in several floral tissues. Limonene is formed from geranyl pyrophosphate (GPP), via cyclisation of a neryl carbonation . The final step involves the loss of a proton from the cation to form the alkene, α-pinene and β-pinene; both produced from GPP, via cyclisation of linaloyl pyrophosphate followed by the loss of a proton from the carbonation equivalent.

2.4.2 Storage of BVOCs

While all plants exchange non-organic volatiles during photosynthesis or respiration, most of them also have the ability to emit BVOCs from different organs such as flowers, fruits, stems and leaves (Fig. 2.4). Where large amounts of hydrophobic terpenoids are produced and accumulated, specialized secretory structures are usually required.

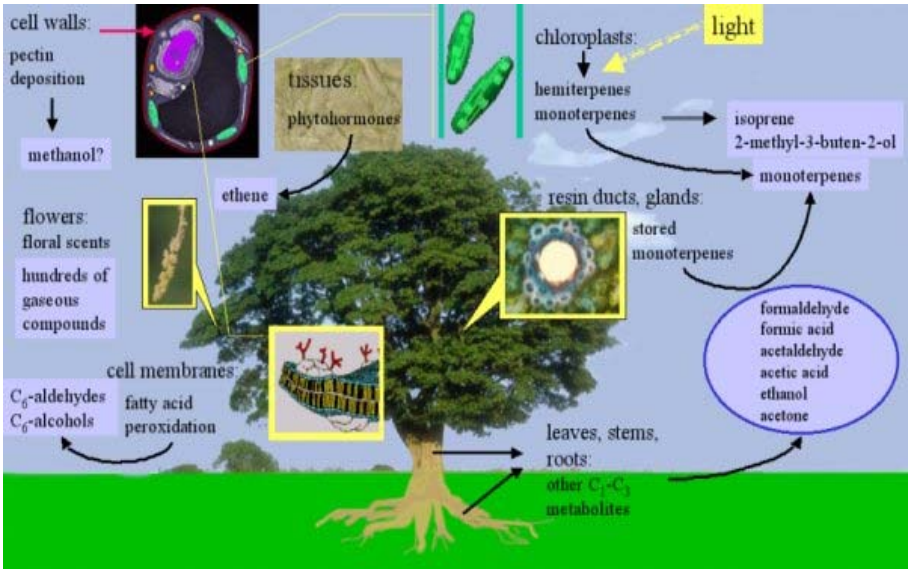

Figure 2.4 BVOC emissions from woody plant species (Loreto *et al*. 1996a)

BVOC emitting species also possess two different types of monoterpene storage pools. A dynamic, fast responding temporary pool of monoterpenes is located in chloroplasts and in the intercellular spaces of the mesophyll tissue (Loreto *et al*. 1996a). This storage pool is much larger than the temporary pool, yet it changes in size significantly, only if there is foliar damage, which induces both *de novo* synthesis and increases emissions (Holopainen, 2004). Many compounds have very low volatility or they are stored in plant structures that present substantial barriers to emission. As a result, there are probably less than 40 compounds emitted at rates that significantly influence the chemical composition of the atmosphere (Guenther *et al*. 2000). Physicochemical controls are represented by limited volatility and diffusion. Volatility primarily affects the diffusion gradient, which is mainly controlled by compound molar size, leaf internal structure and stomatal aperture. Volatility is determined by compound equilibrium partitioning among the leaves and the atmosphere within leaf storage (Kesselmeier, 2000).

The capacity for isoprene emission from leaves of different species varies over several orders of magnitude (Harley *et al*. 1999). Isoprene production participates in the regulation of chloroplast metabolism by maintaining appropriate pool sizes for various intermediates. Upon darkening, the isoprene emission rate from leaves begins to decline immediately, demonstrating that the internal pool of isoprene, or

its precursors, is small and that the instantaneous emission rate is tightly coupled to the rate of synthesis (Monson *et al*. 1991 a; b). Isoprene emitting plants typically expend 0.1 % - 2.0 % of their net photosynthesis on isoprene emission (Monson *et al*. 1994). The emission rate relates to the photosynthate pool and therefore, to the photosynthetic rate (Loreto and Sharkey, 1993).

Emissions of monoterpenes are dependent on the pool size and the vapor pressure of each monoterpene (Tingey *et al*. 1981). These storage structures vary within plant taxa such as the glandular hairs on Mints; the glandular dots in leaves of the Citrus family, *Rutaceae*; and the storage cavities in *Eucalyptus* leaves (Lerdau *et al*. 1997). The lipophilic nature of monoterpenes suggests possible liberation routes directly through the cuticle (Guenther *et al*. 1991). This implies that the emissions are practically independent of stomatal conductance and suggests that monoterpene emissions may occur homogeneously from the leaf surface (Back *et al*. 2005). In addition to the complex regulation of the biosynthetic pathway influencing the terpenoids emissions, many of the terpenoids forming the constitutive defense of conifer needles are mainly synthesized in leucoplasts of the resin ducts (Lerdau and Gray, 2003). Monoterpenes contained in resin ducts have constitutive or induced defensive functions (Gershenzon and Croteau, 1991).

Common among the conifers are systems of resin ducts and blisters (Penhallow, 1907; Fahn, 1979), and the relative complexity of these structures closely parallels their potential for monoterpene production. In angiosperms that produce high levels of monoterpenes, sesquterpenes, or diterpenes, the biosynthetic machinery is often sequestered in specialized glandular structures (Fahn, 1979). The monoterpene leakage from the large, permanent storage pool located in resin ducts, to the mesophyll pool, is considered to be stable under normal conditions. Thus the changes in the monoterpene emissions, originate from the temporary mesophyll storage pool (Back *et al*. 2005).

2.5 Factors Influencing BVOC Emissions

Emissions are highly reactive and have a short half-life which is strongly influenced by environmental conditions such as temperature, light and humidity (Guenther, 1999).

2.5.1 Temperature

The influence of temperature on the rates of isoprene emissions has been well documented, with much less emphasis on other volatile species. Temperature can affect emission rates by altering the isoprene vapour pressure which increases the concentration gradient driving diffusion from the plant (Tingey, 1979).

Emission rates increase with temperature reaching a maximum between 35 ^{o}C – 45 ^{o}C (Tingey *et al*. 1979, 1987; Jobson *et al*. 1994; Lerdau *et al*. 1997) depending on the plant, the area where the plant is growing and the peak activity of isoprene synthesis (Fig 2.5).

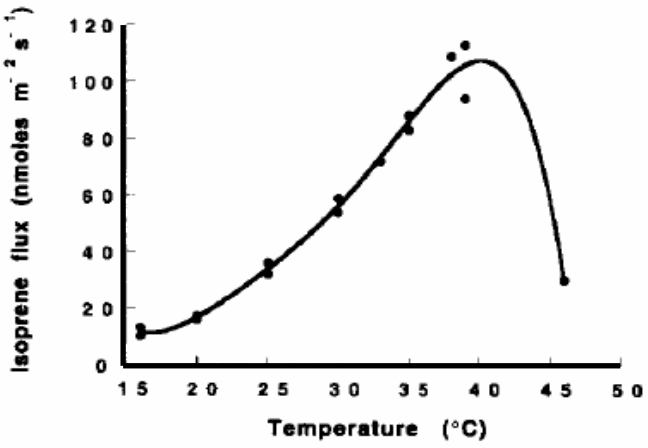

Figure 2.5 Isoprene emissions in White Oak leaves as a function of temperature (Lerdau *et al*. 1997)

Heat stress may interfere with *de novo* protein biosynthesis which inhibits enzyme activity and induces degradation of existing proteins (Nover, 1991). Because isoprenoids are volatile, their concentration inside the leaf and hence their

thermotolerance, changes rapidly with fluctuations in light and leaf temperature. Temperature affects respiration, indirectly regulating the amount of pyruvate available to form isoprenoid intermediates in the chloroplast (Gray, 1999).

An attractive idea is that the thylakoid membranes become leaky at moderately high temperatures (Pastenes and Horton, 1999). Possible mechanisms could include changes in the hydrophobicity of membranes due to the presence of isoprene or a blockage in the formation of water channels (Gounaris, 1984). Another alternative is that high temperature excursions could allow for large membrane-bound protein complexes (for example photosystem II) to fragment (Singsaas *et al*. 1997).

Growth under high-temperature regimes yield higher isoprene emissions; some isoprene-emitting species, maintained at cool temperatures, even fail to emit any isoprene until exposed to high temperatures (Kuzma and Fall, 1993; Monson *et al*. 1992, 1994, 1995; Loreto and Sharkey, 1993). Monson *et al*. (1994), showed that in spring, isoprene emissions are delayed for up to a month after leaf emergence, however these leaves could be induced to emit isoprene when exposed to warm temperatures (32 ºC) (Monson *et al*. 1994). Otter *et al*. (2002) reported that isoprene and monoterpene emissions are highly seasonal, with emissions declining to near zero during winter months when the leaf area index (LAI) and foliar density is low due to the vegetation losing its leaves at this time.

2.5.2 Light

Isoprene emissions have been shown to be triggered by light, due to the link between isoprene emission and the synthesis of photosynthetic products. As no large isoprene pool exists, synthesis and hence emission will cease within minutes under dark conditions (Tingey *et al*. 1981; Evans *et al*. 1982; Guenther *et al*. 1991; Sanadze, 1991; Sharkey *et al*. 1991; Monson *et al*. 1991a). Isoprene production has been found to be photodependent. For the first 10 min after illumination the rate of production is low (Rasmussn and Jones, 1973), but reaches a maximum after approximately 20 min of being exposed to light. These processes are known as inductive synthesis. Light availability determines the amount of the isoprenoid

precursor G3P. Light also controls the availability of ATP and NADPH, which, for highly reduced volatile compounds, are required to a greater extent than they are for sugar synthesis (Read, 2000) and can limit the formation of many volatiles (Wu *et al*. 2000; Gray, 2002). Light-dependent BVOC emissions are often the most abundant BVOC emissions from ecosystems on daily or seasonal time-scales. In most cases, isoprene emissions saturate at the same light level as photosynthesis, but in some cases isoprene emissions increase with escalating light intensity after photosynthesis is saturated (Sharkey and Loreto, 1993; Harley *et al*. 1996; Lerdau and Keller, 1997) (Fig.2.6). Laboratory studies on isoprene-emitting plants showed that growth under high-light regimes resulted in increased emission capacities (Sharkey *et al*. 1991b; Sharkey and Loreto, 1993; Harley *et al*. 1994; Monson *et al*. 1995; Litvak *et al*. 1996).

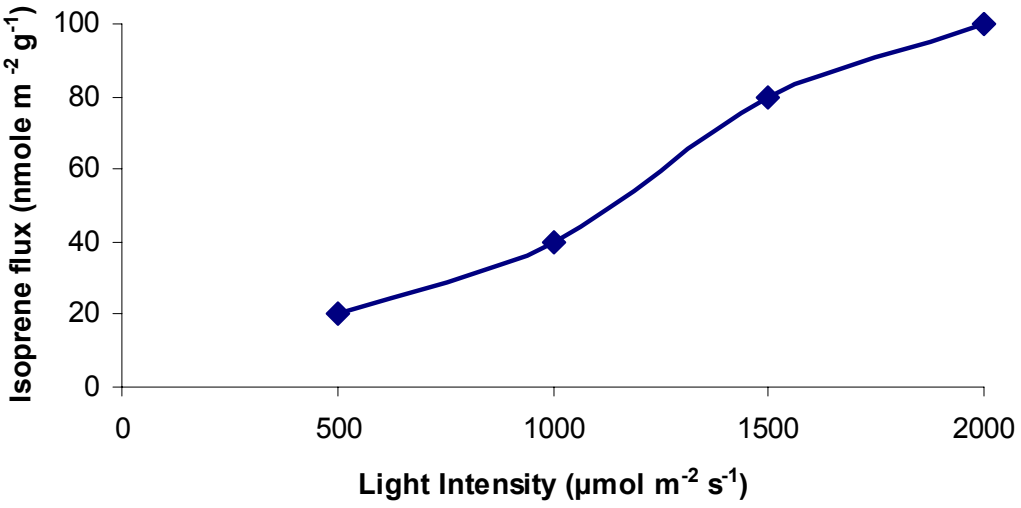

Figure 2.6 Isoprene emissions in tropical deciduous forest leaves as a function of light intensity (Lerdau and Keller, 1997)

The rates of production vary widely across and within species, depending on, among other factors photosynthetic active radiation, temperature of ambient air, age and canopy position (Harley *et al*. 1999; Sharkey *et al*. 1999; Petron *et al*. 2001).

In contrast to the light-dependency shown by isoprene emissions, monoterpene emissions are usually independent of light. However, young needles on conifers can have both light-dependent and temperature-dependent monoterpene emissions (Seufert *et al*. 1995). However, in *Quercus*, monoterpenes are produced and emitted in a light-dependent manner directly to the atmosphere, without any storage (Loreto *et al*. 1996a).

Many taxa produce both light-dependent and light-independent VOC's, for example, *Eucalyptus* produces both isoprene and light-independent monoterpenes, and several *Pinus* species produce methylbutenol and light-independent monoterpenes (Pio *et al*. 1996; Street, 1997).

2.5.3 Water stress and humidity of ambient air

There have been a number of reports in which emission rates have been shown to be impacted by humidity. Dement *et al*. (1975) noted that emissions were directly proportional to the relative humidity. Other studies, however, suggest that humidity does exert a slight positive influence on emissions, for example monoterpene emissions from Sage, Mint, and Oak (Dement *et al*. 1975; Croteau, 1977) and isoprene emissions from *Eucalyptus* and aspen (Monson and Fall, 1989). In contrast, Yokouchi and Ambe (1984), Juuti *et al*. (1990) and Janson (1992) concluded from their studies on Pine, Spruce, and *Eucalyptus* foliage, that relative humidity does not affect monoterpene emissions.

Guenther *et al*. (1991) showed that isoprene emissions depended on humidity, a 2.3 % increase in emission was measured for every 10 % increase in relative humidity. Emission rates are usually normalized by the observed isoprene emission rate at a relative humidity of 40 %. A similar phenomenon has been observed with isoprene emission rates from aspen leaves (Monson and Fall, 1989). Loreto *et al*. (2001) noted that monoterpene emissions were elevated during an increase in humidity. A number of researchers have shown that wetting leaves by means of rain, dew or fog increases monoterpene emissions (Lamb *et al*. 1985; Janson, 1992; Helmig *et al*. 1998; Shade *et al*. 1999). Schade *et al*. (1999) suggested that water

adsorbs to the leaf surface in response to changing humidity levels which potentially increases the cuticular permeability. Hence, monoterpene fluxes from the leaf can either increase as a result of water adsorption or by physical displacement from the cuticular surface.

Stomates of many, but not all species, are highly sensitive to atmospheric humidity (Tibbitts, 1979). They close when the difference between the vapor content of the air and that of the intercellular spaces exceeds a critical level. As water availability decreases, water stress increases and the stomata close. This effect can override low CO_2 levels and bright light. Alternatively, plants undergoing stress for multiple days may increase carbon import from root and stem storage into leaves, which could account for the greater incorporation of alternative carbon into isoprene in water-stressed plants (Funk, 2004). Croteau (1977) noted an increase in monoterpene emissions from plants placed under extremely humid atmospheres. This increase may be explained by a rise in the cuticular conductance due to significant moisture hydration. This is not the same as the hydration of secretory glands on conifer needles. Under conditions of moderate water stress, the percentage of carbon converted to isoprene may increase slightly, but the total isoprene emission remains about the same (Fall, 1999). Water deficit stress is known as a further key abiotic factor profoundly influencing plant metabolism. It alters carbon allocation between roots and shoots (Teskey *et al*. 1987) and changes nutrient uptake ratios and nutrient circulation (Schulze, 1991), with major implications on volatile emissions and the ecology of the system (Takabayashi *et al*. 1994).

Sharkey and Loreto (1993) observed that isoprene emissions increased from kudzu (*Pueraria lobata* (Willd) Ohwi.) leaves that were water stressed. This can be explained by the low stomatal conductance during the dry season, which results in higher leaf temperatures. Pegoraro *et al*. (2005) also showed that drought or water stressed conditions not only increased isoprene emissions as a result of partial stomatal closure, but also dramatically increased isoprene production as a result of reduced soil uptake of water. Plant water stress may occur if there is a lack of water availability for the root system or when there is a high rate of evapotranspiration

resulting in a temporary deficit. Emissions from plants under drought conditions are reduced, due to restricted carbon acquisition (Staudt *et al*. 2002), in well-watered plants, 84 % - 88 % of the carbon in isoprene was derived from photosynthate. This percentage dropped significantly in water-stressed plants to 61 % (Funk, 2004).

2.5.4 Leaf nitrogen content

The effect of nitrogen availability or leaf nitrogen concentration on isoprenoid emissions is not well understood: a positive correlation between emission capacity and one of these parameters was found in most of the investigated plant material, although not always (Monson, 1994, 1995; Harley *et al*. 1994; Lerdau *et al*. 1995; Litvak *et al*. 1996; Ekberg *et al*. 2009). Many of the studies focused on gradients of leaf area (LA), light and nitrogen through the canopy (Hirose *et al.* 1988). Several studies conducted under controlled conditions have shown that specific leaf area (SLA) and leaf nitrogen content (LNC) per unit leaf mass were significantly higher in annuals that in wild herbaceous perennials (Muller and Garnier, 1990; Garnier, 1992; Roumet *et al*. 1996; for SLA; Garnier and Vancalyzeele, 1994; Arendonk and van Poorter, 1994; Roumet *et al*. 1996; for LNC). Data obtained in the laboratory on grasses show that LNC decreases with increases leaf density (Arendonk and Poorter, 1994; Garnier and Vancalyzeele, 1994; Garnier and Laurent, 1994).

Levels of nitrogen nutrition clearly influence photosynthesis and isoprene emissions expressed on a leaf-area basis. Photosynthesis and emissions increase as leaf nitrogen concentration increases. Harley *et al*. (1994) also observed that isoprene emission rates increased with increases in leaf nitrogen concentration irrespective of whether plants were grown at high or low photon flux density (PFD). Alternatively, increased leaf nitrogen concentration could have resulted in greater concentrations of isoprene synthase, independently of the concentration of photosynthetic enzymes. As the carbon/nitrogen ratio of leaf tissue increases, a higher percentage of fixed carbon escapes the leaf as volatile emissions. This result may reflect an accumulation of photosynthate due to nitrogen – limited growth

sink, some of which is shunted into volatile carbon emissions. Thus, although a lower amount of isoprene is synthesized and emitted as leaf nitrogen concentration decreases, it represents a greater proportion of available photosynthate (Harley *et al*. 1994). Chazdon and Field (1987) reported significant correlations between leaf nitrogen content and the leaf level light environment among leaves in different canopy positions in several species of rain forest trees in the genus *Piper*.

Givnish (2002) reported that in temperate regions, evergreen leaves with a long life span finish their development in early summer and are then exposed to large changes in light and temperature over the rest of the season. Leaf nitrogen content is low during summer, but increases from autumn to winter. If the leaves are very thin, leaves cannot increase the number of chloroplasts in winter. However, evergreen leaves are generally thicker than those of deciduous and herbaceous plants. Remobilization of leaf nitrogen (LN) in autumn is larger in early successional species than in late successional species (Oguchi, 2008). The chloroplast thickness and thickness of the mesophyll layer were highest in winter, similar to the seasonal change in nitrogen area (Koike *et al*. 2004). Givnish (2002) also found that herbaceous ephemerals have the highest levels of photosynthesis and LN and shorter leaf life-spans, while evergreen woody plants – especially conifers – have the lowest photosynthetic rates, LNC, and longest leaf life-spans.

2.5.5 Stomatal conductance

If compounds are not able to diffuse through the cuticle, they have to pass through the stomata before they reach the atmosphere. If pools are localized inside the leaf, as for example as resin ducts, inner hairs or glands, the emission from these pools is under stomatal control. If the compounds are stored outside on plant surfaces, their emission is not controlled by the stomatal aperture (Kesselmeier *et al*. 1997). Stomatal controls have been explained by low foliar VOC air-phase partial pressures, which readily increase in response to decreasing stomatal conductance, and thereby balance the decrease in conductance by an enhanced diffusion gradient from the leaf intercellular air-space to ambient air (Sharkey, 1991; Fall and Monson, 1992; Kesselmeier and Staudt, 1999). The light could be acting on

mesophyll cells, which would send a message to the guard cells. This opinion is supported by experimental observations indicating the lack of significant stomatal control over the emission rate of isoprene (Monson and Fall, 1989) and α-pinene (Loreto *et al*. 1996a). Indeed, Tingey *et al*. (1991) have shown that stomatal conductance plays a minor role as compared with mesophyllic conductance, when stomata close.

Isoprene emissions are not controlled by stomatal closure (Monson, 1989). The rate of emission is controlled by isoprene synthesis rather than by stomatal opening and emissions can occur through closed stomata (Fall and Monson, 1992). Surprisingly, the stomata that control carbon dioxide uptake and water loss from leaves have no effect on the isoprene emission rate, even though isoprene is emitted through stomata (Anezo and Vries, 2003). When stomata close tightly under the influence of abscisic acid, the intraleaf concentration of isoprene increases to a level that is sufficient to overcome the diffusion resistance of stomata and in addition establish an equilibrium between the rate of isoprene biosynthesis and its release from the leaf (Fall and Monson, 1992). As stomatal conductance fluctuates, the concentration of isoprene inside the leaf alters until the flux from the leaf is equal to the rate occurring before stomatal conductance changes. Since changes in isoprene concentrations inside the leaf do not affect the rate of emission, there must be no feed-back of isoprene on its own synthesis (Gong and Xiao, 1998). As stomata close, the isoprene concentration in the leaf increases (Fall and Monson, 1992), because stomatal closure reduces transpiration rate and increases leaf temperature. Several studies have indicated that increasing plant water stress modifies stomatal response to humidity. A long-term decrease in water potential, however, progressively increases stomatal conductance and the responsiveness of stomata to humidity (Johnson and Ferrell, 1982).

This literature review highlighted the lack of data from tropical areas and from crop plants. Seasonal studies were also very limited. These gaps in the literature helped focus the experimental design of this project.

CHAPTER 3: METHODS AND MATERIALS

3.1 Site Description

The study was conducted during the 2007-2008 growing season in the White River and Hazyveiw areas, Mpumalanga (Fig. 3.1). Measurements were carried out in the southern Mpumalanga Provinces at 25° 06'S, 31° 04'E using Banana, Avocado, Mangoes, *Eucalyptus* and *Pinus*. The five study areas are located close to each other.

Figure 3.1 Mpumalanga Province with arrows indicating study areas

The study areas lie within the subtropical climate zone, where rain falls predominantly in summer. The sites experience a mean annual rainfall of 600 mm and have a pronounced dry season between May and September, while the wet periods are between September and April. The climate is subtropical, with hot, wet summers and warm, dry winters. On average, January and February are the wettest

months. Daily rainfall data for the sampling period (2007-2008) were collected from the Nelspruit weather station.

3.2 Sampling Time

Foliar VOC emissions were measured from crop and plantation species during May-2007 to Aug-2008. Vegetation BVOC emission rates were estimated using two field-portable enclosure systems. The first system, referred to here as the screening Photoionization Detector (PID) system, provides an immediate, quantitative measure of total isoprene and terpene concentrations as an instantaneous measure of emissions. The second system is a leaf cuvette enclosure system used to measure leaf emission rates associated with specific light and temperature conditions.

Results from the literature demonstrate that emission rates are affected by season, due to variability in temperatures and humidity of ambient air. Therefore, the five study periods were chosen to investigate the differences in emission rates at the start, peak and end of the rainy season as well as at the start and end of the dry season. Warmest temperatures were recorded at the start of the rainy season and coolest temperatures at the end of the dry season.

3.3 Study Species

About 40 % of the forestry plantation area in South Africa occurs in Mpumalanga. Crop species and plantation species were the focus of this study. Plant species diversity is extremely high globally and it would be impossible to study each of these species. To facilitate understanding plants have been divided into functional types which cluster the phonological and metabolic attributes. In this study we could refer to two functional groups with the crop species being one and the plantation species the other. Two plantation species, which are common in these areas and commercially important were used: *Eucalyptus* and *Pinus.* Subtropical fruits are also extensively grown in the area. Three commercially grown crops: Mangoes, Banana and Avocado were used in this study.

Figure 3.2 Pine plantation site

The *Eucalyptus* plantation (*Eucalyptus grandis* x *Eucalyptus camaldulensis L'Hér*), compartment number U001, was planted in April 2004. It was previously planted with *Eucalyptus grandis*. The coordinates for this compartment are: 25°20'S 03°91'E, elevation is 1,124 meters asl. The *Eucalyptus grandis* x *Eucalyptus camaldulensis* plantation received fertilization at planting, 125 g per tree of NPK (2:3:3 (22 %) + 0.5 % Zn), 15 cm from the stem (Fig. 3.3).

Figure 3.3 Eucalyptus plantation site

The Mango (*Mangifera indica L.*) plantation, compartment Mg25, was planted in May 2001 following a Tobacco crop and has had no fertilizer applied for the past

three years (Fig. 3.4). The coordinates for this compartment are: 25°04'S 03°14'E and the elevation is 512 meters asl.

Figure 3.4 Mango site

The Avocado (*Persia Americana Mill.*) plantation (Fig. 3.5), compartment Av19, was planted in 2003 following a Banana crop. The coordinates for this compartment are: 25°07'S 03°74'E and the elevation is 775 meters asl. This crop received (20 g per tree – P), (40 g per tree – N) and potassium chloride (KCl – 200 g/tree) twice a year and limestone ammonium nitrate (40 g LAN) four times a year. Both the Mango and Avocado plantations are irrigated once a week at 140 l hr^{-1} per tree.

Figure 3.5 Avocado site

The Banana (*Musa acuminate L.*) plantation (Fig. 3.6), compartment Bn17, which was previously natural bush, was planted in 1998. The cultivation of Banana plants in Mpumalanga is severely affected by *Fusarium* wilt, caused by the fungus *Fusarium oxysporum.* The parent root stock was planted in 1998 and has been maintained while the suckers are clearfelled every three years. Therefore the Banana plants in this study are older than the other sampled species. The coordinates for this compartment are: 25°06'S 03°04'E and the elevation is 800 meters asl. Every year fertilizer (40 g LAN) was applied to each plant every month from September to April and 200 g KCl was applied per plant in September, November and February. The plants are irrigated once a week at 140 l hr^{-1} per tree.

Figure 3.6 Banana site

3.4 Experimental Design and Sampling

Techniques for VOC measurements in South Africa are not well developed and many of the techniques used in this research project had to be developed, tested and implemented by the author of this dissertation.

The project was conducted in two phases:

3.4.1 First phase: Screening

The purpose of this phase was to establish which of the five species emit isoprene and terpenes and at what concentrations. Field screening results were used to evaluate whether the five selected species were producing VOCs. The 2020ppbPRO (manufacturer), which was used in this study (Fig. 3.7), is a microprocessor-controlled air monitor for measuring the presence of photoionizable compounds in air at ppb levels.

Figure 3.7 Handheld Photoionization Detector (model 2020ppb PRO Photovac, Inc.)

The flow diagram in Fig. 3.8 shows the main components of the 2020ppbPRO. The PID converts the concentration of photoionizable compounds in the sample into an electrical signal. The microprocessor subtracts any background from the signal and divides this signal by a lamp sensitivity obtained by calibrating with a standard gas of known concentration. The PID consisted of a 10.6 eV (1 electron volt=1.60217646×10^{-19} Joules) UV lamp and a pump attached to draw air samples past the detector at a flow rate of no less than 300 ml min^{-1}. The pump creates a sub ambient pressure in the measurement chamber that prevents condensation of water on the detector window. The PID is sensitive to all unsaturated carbon-carbon bonds and is used to develop a response factor (RF) to distinguish isoprene (RF= 0.6) from the terpene (RF=1) signal. The display can show readings in ppb,

ppm or mg m^{-3}. For the purpose of this study the meter was used to display concentrations from 10 ppb to 40 000 ppb.

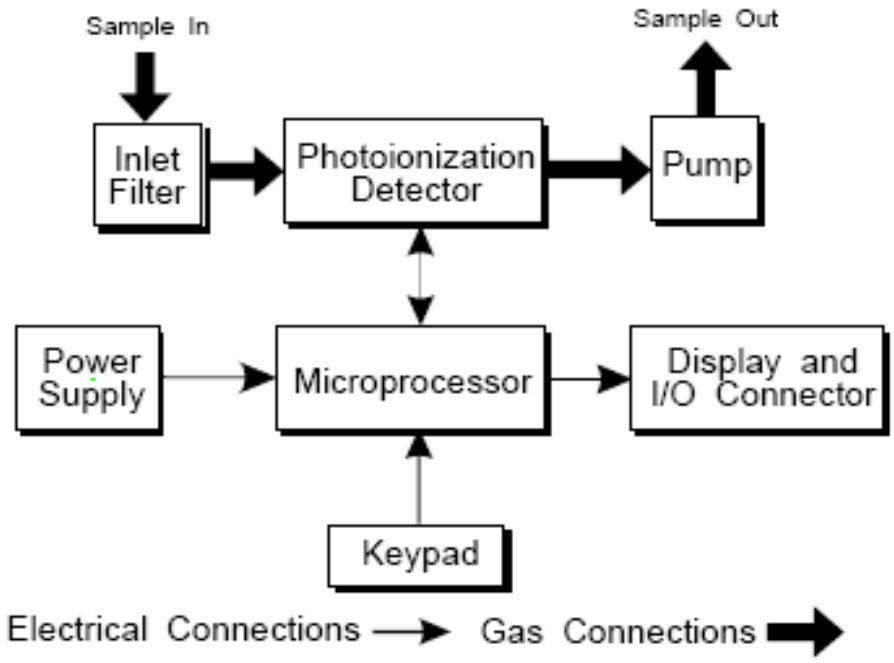

Figure 3.8 Handheld Photoionization Detector (model 2020ppb PRO) flow diagram

The 2020ppbPRO does not distinguish one type of compound from another, but indicates the total concentration of all photoionizable compounds in a sample. The PID is used to screen samples following which more detailed studies can be conducted (for example, using canisters and gas chromatography (GC)).

Calibration

During calibration, the 2020ppbPRO is first exposed to zero air (void of any ionizable gases or vapours, 99.9 % free of VOC) and then to the span gas. The microprocessor subtracts the zero signal from the span gas signal and divides the difference by the user-entered span gas concentration. This value is then multiplied by the response factor and displayed. The display also indicates the operating temperature range, the humidity range and the altitude range.

Field screening

The screening procedure was conducted between 23-Aug-2007 to 30-Aug-2007 and daily between 11 am and 3 pm. Thirty individuals from the five chosen species were randomly selected from within the study area and screened for isoprene and terpene emissions. Branches from the upper third of the canopy were selected and placed in a Teflon bag together with the 2020ppbPRO and exposed to direct sunlight for 60 sec. The concentrations of isoprene and terpene were recorded for each of the individuals sampled (Table 3.1 a data table was prepared for each species sampled).

Table 3.1: Example of the data table for *Eucalyptus* for recording the concentration of BVOCs (ppb)

Name of species Eucalyptus	**Date**	**RH (%)**	**T (C^o)**	**Time start (00:00)**	**Time end (00:00)**	**Isoprene(ppb)**	**Terpene (ppb)**
Rep 1							
Rep 2							
.							
.							
Rep 30							

3.4.2 Second phase: Seasonal field sampling

Leaf sampling and emission measurements

This phase of the study was to determine the effects of temperature and light on the emission rates of BVOCs, using a GC with cryogenic preconcentration and a flame ionization detector (FID) for analysis. Leaf samples for BVOC emissions were measured from two plantation species (*Pinus* and *Eucalyptus*) and three crop species (Bananas, Mangoes and Avocados). This phase of the project was conducted between 16^{th}-20^{th} Sept-2007, 8^{th} -10^{th} Feb-2008, 4^{th} -7^{th} Apr-2008, 6^{th} -7^{th} –Jun-2008, 4^{th} -7^{th} Aug-2008 and daily between 10 am and 3 pm.

Structure of the leaf cuvette

The leaf cuvette (653 cm^3) (Fig. 3.9) is made of Delrin-acetal resin with a glass window. The top of the chamber is secured to the bottom with spring-loaded clamps. The cuvette system includes a flow sensor, a PAR sensor, a leaf thermocouple, and humidity sensors upstream and downstream of the cuvette, and a data logger. There are two to three bulkhead sampling ports for air intake and sample withdrawal. The cuvette is airtight when sealed. Power for the pump is supplied from a 12 V battery. The air flow through the leaf cuvette is held constant at 900 ml min^{-1}. The leaf being sampled is secured within the chamber by placing it between the removable top and the body of the chamber, both of which are fitted with closed-cell neoprene foam to provide an air-tight seal.

Figure 3.9 A diagram of the leaf cuvette showing the leaf temperature sensor and the PAR sensor

Detailed leaf cuvette analyses

Before leaf sampling began, measurements were taken from an empty cuvette system to ensure that the cuvette was not releasing any hydrocarbons and was sealing properly. Five individual plants of each of the five species were randomly selected from within the study area. A representative leaf, exposed to full sunlight,

from each selected plant was carefully placed into the leaf cuvette containing the temperature and PAR sensors. Leaves were held under constant conditions in the leaf cuvette for 20 min to allow the emission rates to stabilise. After 20 min, an air sample was drawn into a canister. Sampled air was collected using 1 liter and 0.4 liter electropolished, stainless steel canisters (Fig. 3.10). Stainless steel canisters are used as they do not react with the target compounds thereby protecting against sample contamination. The canisters have a single shut-off valve which allows for air to be sampled. Each sampling session resulted in five canisters from each of the selected species and an ambient air sample per selected species. The leaf in the cuvette was sampled for leaf area and additional leaves were collected for leaf nitrogen analyses. During the 20 min, the conditions in the cuvette, leaf enclosure temperature, photosynthetically active radiation (PAR) and general sampling conditions were logged every four seconds on the data logger. Mean temperatures and PAR values were calculated to assess the effect of environmental conditions on the rate of emissions.

Figure 3.10 Canister for sampling leaf air

In addition, the relative humidity and temperature of the ambient air were measured with hydrometer and simple thermometer, which were recorded together with the sampling time for each of sampled individuals (Table 3.2 a data table was prepared for each species sampled).

Table 3.2: Example of the data table for *Eucalyptus* for recording environmental conditions with hydrometer and simple thermometer

Name of species Eucalyptus	Date	Canister Number	RH (%)	T (C^o)	Time start (00:00)	Time end (00:00)
Rep 1						
Rep 2						
.						
.						
Rep 5						

3.4.3 Detailed GC-FID analyses

After sampling and analysis on the GC-FID system, eight canisters at a time are refreshed by a cleaning system comprising of a heating frame at 140 ^oC, compressor and CO reactor. Once the canisters are cleaned, dried and heated, each canister is attached to a vacuum pump to create a vacuum at 80 kPa, ready for sample collection. These rigid canisters are compact and rugged for ease of storage and transportation.

All samples from the canisters were analyzed, within three days of sampling, by GC with cryogenic preconcentration, using FID to quantify concentrations. Calibrations of the GC-FID were done with N-hexane standards. Peak areas were integrated using the software PeakSimple (version 2.83, licensed by SRI Inc.). The GC-FID was housed in an air conditioned room where the temperature was 20 ^oC which is within the optimum system operating conditions.

The samples were analyzed using a SRI 8610-C GC equipped with a FID. The FID responds to any molecule with a carbon-hydrogen bond. The hydrogen (40 psi) mix supports a diffusion flame at the jet's tip which ionizes the analyte molecules. Positive and negative ions are produced as each sample component is eluted into the flame (Fig. 3.11). Helium is the carrier gas at a flow rate of 10 ml min^{-1} through the column, with a make up gas of 20 ml min^{-1} leading to a flow rate of 30 ml min^{-1} at the detector.

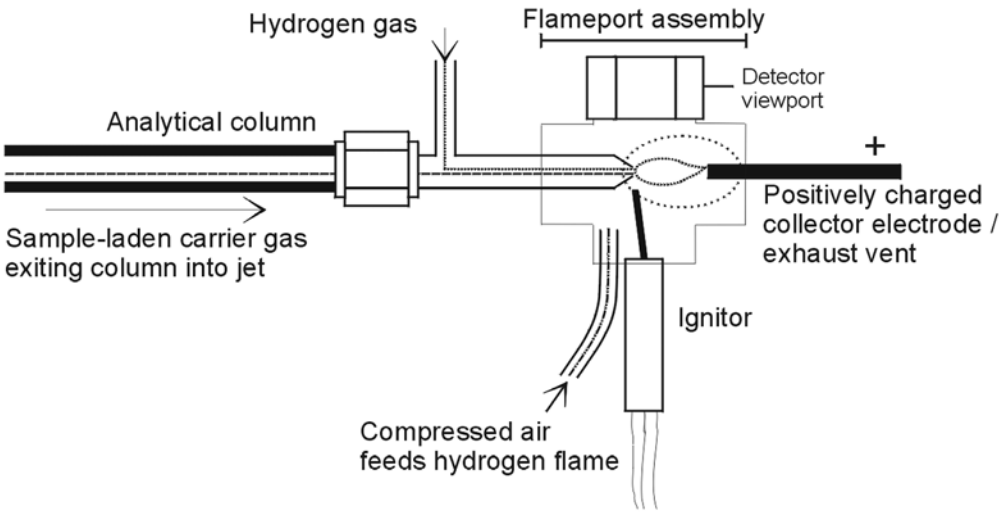

Figure 3.11 Flame ionization detector

SRI Gas Chromatographs (Fig. 3.12) are designed to use both packed and capillary columns. The Model 8610 has an oven which allows for columns coiled on a 7" (175 mm) diameter or smaller.

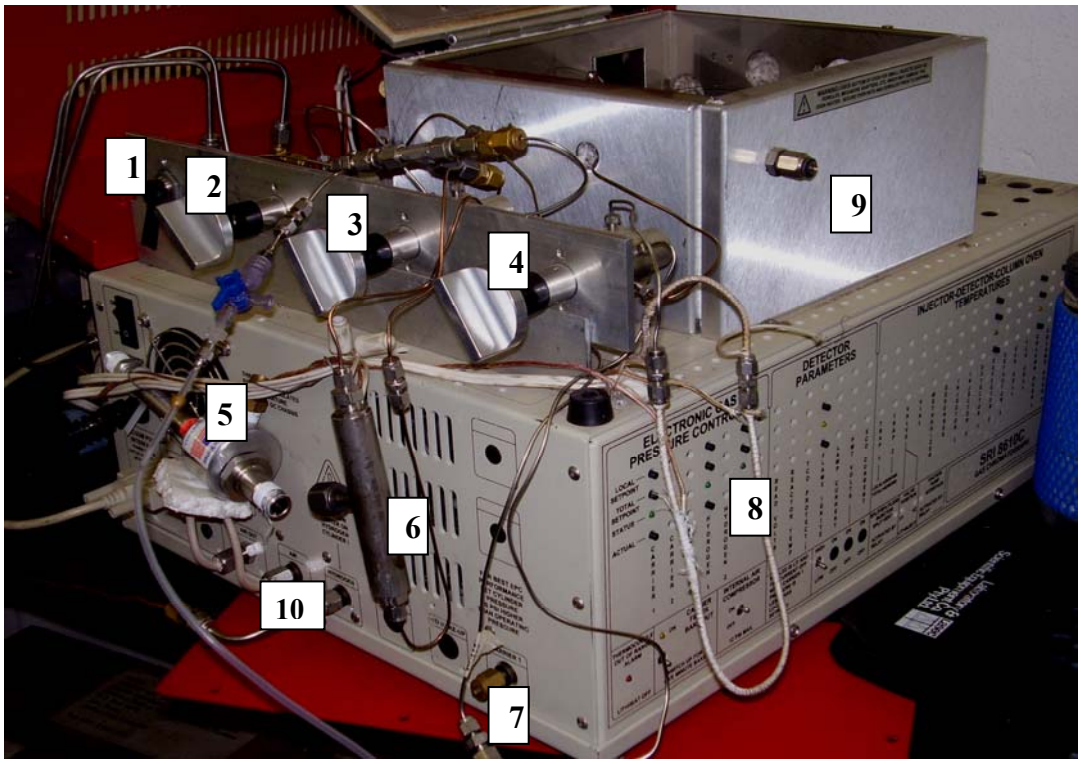

Figure 3.12 GC-FID equipment (Gas chromatography with flame ionization detector) 1-Vacuum valve; 2-3 - valves; 4- the sample to the column; 5-sample injection port; 6-trap 1; 7- helium; 8- trap 2; 9- oven; 10- hydrogen.

Diameter SRIs normally have the columns coiled to a 3.5" (90 mm) diameter so they fit easily in either the 8610 or 310 column oven. The volatile components of the sample are carried through the GC column by the mobile phase, the ultra-pure helium. The inside surface of the column has a thin coating of a stationary phase designed to interact with the components of the mixture passing through the column.

For any individual hydrocarbon, the amount of time taken to traverse the length of the column retention time (RT) is determined by the component's affinity for the stationary phase. Controlled variation of column temperature over the course of the analysis (temperature program) also affects the separation of the compounds, the retention time for each compound, and the total analysis time for the sample. Compounds are identified by their characteristic retention times. The samples are kept in the gaseous state by using an oven to keep the column temperature above the boiling point of the samples. Air circulation is driven by a fan. BVOCs are present at trace levels (ppb) in the atmosphere; therefore preconcentration traps and valves are normally attached to the GC system in order to detect these low concentrations. The first trap is cooled to 0 ^{o}C after which the sample is transferred to the second trap, which is cooled in liquid nitrogen to -200 $^{\circ}$C. The rate at which the mobile phase moves through the column can vary, higher rates are associated with faster analysis times, but with results of lower resolution.

VOC separation was carried out using a fused silica MTX-624 capillary column (30 m ×0.52 µm × 1.4 µm, Restek Corp., Bellefonte, PA) with helium (UHP, AFROX, SA) and hydrogen as carrier gases at a flow rate of 5 ml min^{-1} and a temperature program (initial temperature 40 ^{o}C for 5 mins, increased to 200 ^{o}C at a rate of 15 ^{o}C per min^{-1}, then held for 2 mins). Samples are pre-concentrated through two traps before moving onto the column. The samples are introduced to the first stage trap and cooled to 0 ^{o}C. The second trap is cooled with liquid nitrogen to -200 ^{o}C for 3 mins 55 secs then reheated to 200 ^{o}C. 3 mins 55 secs was chosen as the upper bound because that is the length of time needed for coupled swing column systems. Full details of the analytical procedure are described by (Greenberg *et al*. 2003).

Calibration

Each of the commercial standards (99.9 %, isoprene, limonene, β-pinene, α-pinene, limonene and linalool) were introduced into the liquid phase. However, the volumes injected onto the GC-FID were introduced in the gas phase (1-5 ml), which was taken from the top of each individual bottle of standard and injected onto the GC-FID, using zero air (zero-grade air with <0.1 ppm total hydrocarbon content). Chromatographic retentions and quantifications of limonene, isoprene, pinenes and linalool were repeated three to four times. After each individual standard calibration the GC-FID column is heated to 200 ^{o}C three to four times, until the PeakSimple shows a straight line, which indicates that the column is clean. Compound identification was calculation by retention time comparison with known standards.

Frequency of Calibration

- The commercial standards were used once for each set of compounds at the start of the analyses. The five chemical species chosen were those that could be easily identified using the gas chromatograph, that appeared to be present in the highest concentrations and for which calibration standards were available.
- The secondary standard (N-hexane – 99.9 %) was run before each analysis.

Calculation of emission rates

The GC integrator recorded the results from the GC analysis. A calibration curve was setup so that the integrator could calculate the concentrations (in ppb) in each sample. The emission rates were calculated from these values using an equation which was modified from Guenther (1995). Terpene emission rates were calculated from flow rate, leaf area, and concentrations as described in Singsaas *et al*. (1997).

The concentration of each compound (ppb) was determined from the area of the chromatographic peak (in mV) by using the following equations derived from the calibration curves, the volume of the sample and the response factor.

$$Concentration(ppb) = ((RF \times area) \div Volume) \times (1 \div 10) \quad \textbf{[1]}$$

$$Volume = -1.78322 + (4.08042 \times (2\,pressure - 1\,pressure)) \quad \textbf{[2]}$$

Where:

RF - is a correction factor which is calculated from incorporating the N-hexane standard and not a specific compound standard

Area – is the area of the compound peak given in the GC, which is the total area within the retention time range. The peak is the integration of the mass per unit volume with respect to time. Peak area is proportional to the mass of solute. The peak area is obtained as the product of the peak height and the peak width.

Volume- is the volume of sample injected onto the column

The number "**10**" is the number of carbon atoms in monoterpenes and this number will vary with compound.

Final and initial pressure = of the GC

These concentrations were converted to micrograms of carbon per cubic meter ($\mu g\ m^{-3}$) using the following equation:

$$\textbf{Concentration (µgrams C per m}^{3}\textbf{)} = ppb \times mol.weight(compound) \div 24.45m^{3} \quad \textbf{[3]}$$

24.45 – the volume occupied by 1 mol of the VOC at 25 ºC and 1 atm. standard pressure.

$$\textbf{Emission rate (E) (µg C m}^{-2}\textbf{ hr}^{-1}\textbf{)} = Conc \times (F \div A) \quad \textbf{[4]}$$

Where:

F – flow rate through cuvette 900 ml min^{-1} = 0.0054 m^3 hr^{-1}

Conc (µgrams C per m^3) - from equation (3) (sample – blank)

LA – leaf area (m^2)

3.4.4 Other methods

Soil moisture content

Soil samples were collected from beneath each selected plant during all five sampling periods, from a depth of 0-10 cm. A 10 g subsample of the soil was dried at 60 ^oC for 48 hours and then re-weighed to determine the gravimetric soil moisture content.

Calculation

$$\theta_g = (m_{wet} - m_{dry}) \div (m_{dry} - m_{container}) \quad \textbf{[5]}$$

$$\%moisture = \theta_g \times 100 \quad \textbf{[6]}$$

$$X = (wet(g) \times \%moisture) \div 100 \quad \textbf{[7]}$$

$$Dry(g) = wet(g) - X \quad \textbf{[8]}$$

Soil pH

The soil pH was measured in a 1:1 extract with 1 (M) KCl and H_20. The comparison of the pH in KCl with the pH in H_2O provides an assessment of the nature of the net charge on the colloidal system.

Soil texture

The particle size distribution of the sampled soils was determined using the hydrometer method (Anderson, 1993). Once the sand, silt, and clay percentages of a soil are known, the textural class can be read from the textural triangle (Fig. 3.13)

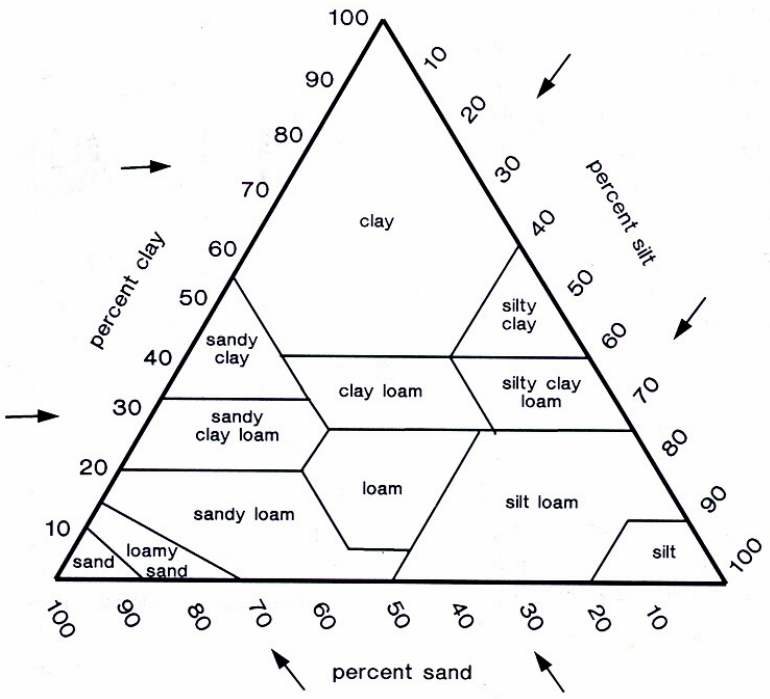

Figure 3.13 A soil textural triangle is used to determine soil textural class from the percentages of sand, silt, and clay in the soil

Leaf nitrogen content

Leaves or needles from the upper third of the canopy were collected from each sampled plant at each the five sampling periods (Sep-2007, Feb, April, Jun and Aug-2008). The samples were oven dried at 60 ^{o}C for 72 hours, ground and analyzed in the laboratory using the CHNS-932 LECO nitrogen analyzer.

Leaf area

Each of the leaves used in the cuvette, were measured for leaf area (cm^2) using a LI-3100 area meter. The LI-3100 area meter is designed for biological or industrial applications that require rapid precise projected area measurements. Samples are placed between the guides on the lower transparent belt and allowed to pass through the instrument. As the leaf travels under a 15 watt fluorescent light source, the image is reflected by a system of three mirrors to a solid state scanning camera within the rear housing. The leaf area meter was calibrated before use for the sampled leaves.

Statistical analyses

Analyses of variance (ANOVA) were used to determine the significance within and between species and across time. Significance was tested at the 95 % confidence level. For factors that were measured seasonally, factorial ANOVAs were used. Post-hoc Tukey tests were used to determine which groups differed from each other. Exponential regressions and Pearson's correlation coefficients were used to find the relationship between the emission rates and the environmental factors. All analyses were conducted using Statgraphics version 6.0.

CHAPTER 4: RESULTS

Emissions are presented in both tables and figures for the screening phase and the cuvette studies.

4.1 Screening phase

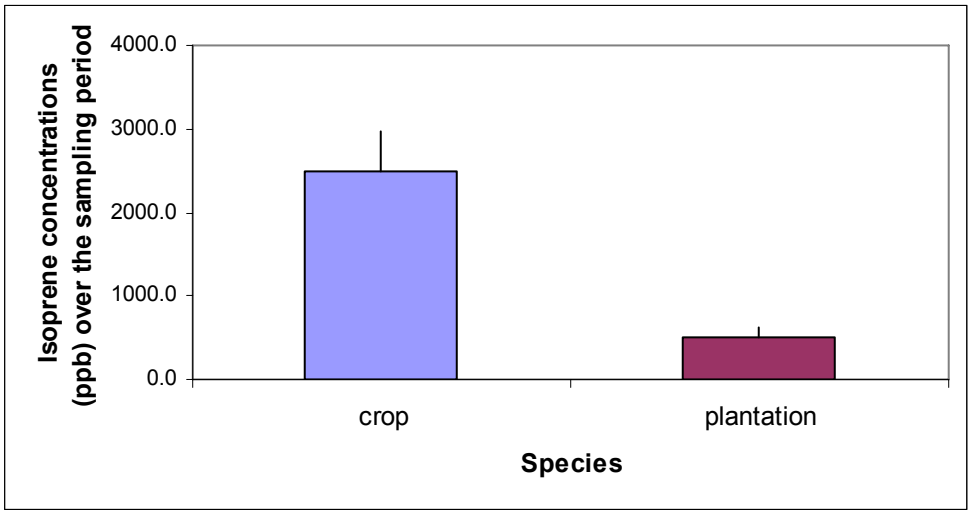

Figure 4.1a Isoprene concentrations (ppb) (crop species n=90, plantation species n=60, mean and standard error)

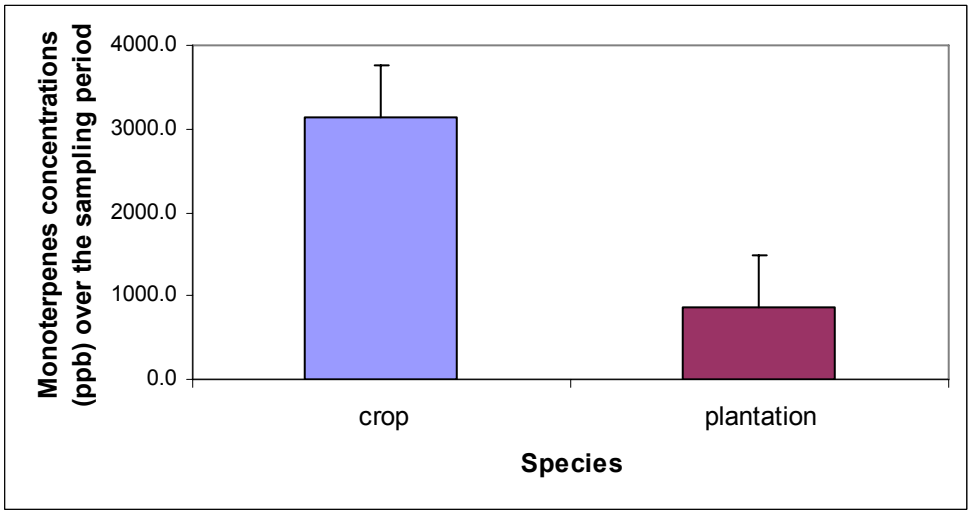

Figure 4.1b Monoterpenes concentrations (ppb) (crop species n=90, plantation species n=60, mean and standard error)

In Figure 4.1a and b, the concentrations of the emissions from the screening procedure were grouped as either isoprene or monoterpenes for each of the sampled species. Crops emitted significantly higher concentrations of isoprene ($p=0.001$) and monoterpenes ($p=0.004$) than the plantation species.

Table 4.1: Mean values and (±Standard Error) of the isoprene and monoterpene concentrations (ppb) for the five study species (n=30)

Species	Isoprene	Monoterpenes
Mango	7073.7 ± 108.7^{a}	8895.8 ± 140.4^{a}
Banana	169.3 ± 28.3^{c}	171.8 ± 27.5^{d}
Avocado	152.9 ± 45.8^{c}	238.6 ± 48.8^{c}
Pine	835.1 ± 207.7^{b}	1553.9 ± 351.9^{b}
Eucalyptus	185.0 ± 54.6^{c}	149.5 ± 38.5^{d}

Values with the same letter are not significantly ($p>0.05$) different for each element within the column for each of the species

The screening phase proved that all the chosen species are BVOC emitters and therefore the seasonal study continued to focus on these five species.

4.2 Seasonal Leaf Cuvette Study

4.2.1 Ambient Air Temperature

The ambient air temperature, measured between 10 am-3 pm, varied across all selected sites over the study period. The highest temperatures were experienced in September and February and the lowest temperatures in August (Table 4.2). The temperatures in the different months differed statistically from each other. In September, the temperatures were significantly higher than temperatures in April, June and August ($p<0.0001$). August was significantly cooler than all the other months.

Table 4.2: Mean (± Standard Error) values of the temperature (ºC) of ambient air (10 am-3 pm) at the five study sites across the five study periods

2007 September	2008 February	2008 April	2008 June	2008 August
36.9±1.3[a]	33.2±0.9[ab]	29.0±1.2[bc]	27.8±1.3[c]	20.8±1.5[d]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling month

The sites at which Mangoes and Pines grew experienced significantly higher temperatures than the site at which Eucalyptus grew (p=0.004). There were no significant differences in ambient temperature across the sites at which Mangoes, Bananas, Avocados and Pines grew (Table 4.3)

Table 4.3: Mean (± Standard Error) values of the temperature (ºC) of ambient air at the five study sites

Mango	Banana	Avocado	Pine	Eucalyptus
32.4±1.5[a]	27.9±1.7[ab]	29.4±1.7[ab]	31.4±1.3[a]	26.5±1.6[b]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling month

4.2.2 Rainfall

The study sites were situated in the lowveld region of the north-eastern Mpumalanga Province where the rainfall distribution is seasonal. The year is divided into dry (May 2008 – August 2008) and wet seasons (September 2007 – April 2008) (Fig. 4.2 a; b).

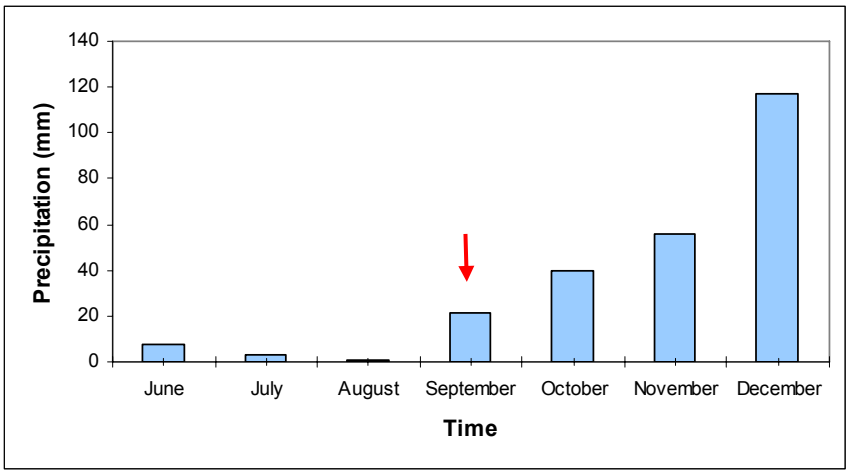

Figure 4.2a Total monthly rainfall (mm) for the period of sampling (2007) (Arrows in the figure indicate the sampling months)

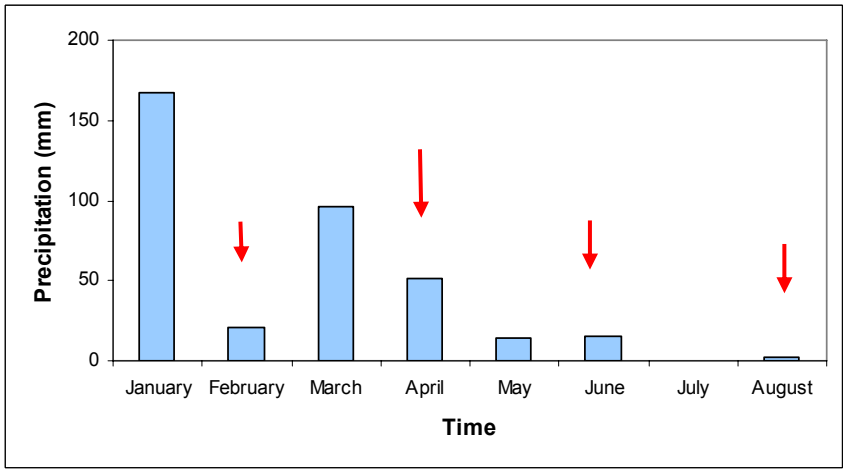

Figure 4.2b Total monthly rainfall (mm) for the period of sampling (2008) (Arrows in the figure indicate the sampling months)

Table 4.4: Mean values of the rainfall (mm) for the period of sampling (2007-2008)

Month	n (days with rain)	Total rainfall (mm)
June (2007)	5	17.6
July (2007)	3	3.2
August (2007)	1	1.0
September (2007)	4	21.6
October (2007)	11	39.8
November (2007)	7	56.0
December (2007)	15	117.2
January (2008)	17	167.0
February (2008)	4	20.8
March (2008)	9	96.0
April (2008)	11	51.2
May (2008)	2	13.8
June (2008)	3	15.2
July (2008)	1	0.4
August (2008)	3	2.0

The total rainfall from June – December 2007 was 256.4 mm and from January – August for 2008 was 366.4 mm.

4.2.3 Relative humidity (%) of ambient air

Relative humidity, measured between 10 am-3 pm, varied significantly within the sampling year ($p \leq 0.0001$) and across the sites ($p \leq 0.0001$). The relative humidity of ambient air in June (63.9 %) and August (65.6 %) was significantly higher than at other sampling times, with April being similar to February, June and August (Table 4.5). As the temperatures increased in September the relative humidity decreased.

Table 4.5: Mean (± Standard Error) values of relative humidity (%) at the five study sites across the five study periods (10 am-3 pm)

2007 September	2008 February	2008 April	2008 June	2008 August
42.5±1.3[c]	51.2±1.9[bc]	57.9±4.5[ab]	63.9±5.1[a]	65.6±2.1[a]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling month

Avocados grew at the site with the highest relative humidity. All the other sites showed similar relative humidity values. (Table 4.6).

Table 4.6: Mean (± Standard Error) values of relative humidity (%) at the five study sites

Mango	Banana	Avocado	Pine	Eucalyptus
53.4±3.1[bc]	60.2±3.1[ab]	66.7±3.9[a]	46.8±3.7[c]	53.9±3.8[bc]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling species

4.2.4 Photosynthetic active radiation (PAR) (µmol m^{-2} s^{-1})

Results showed that the PAR did not varying significantly over the sampling period, this is probably due to the low cloud cover and the constant daily sampling time (10 am-3 pm) (Table 4.7 and 4.8). However, the highest PAR was experienced in the summer (September) and the lowest in the autumn (June) (Table 4.7).

Table 4.7: Mean (± Standard Error) values of PAR (µmol m^{-2} s^{-1}) at the five study sites over the five study periods (10 am-3 pm)

2007 September	2008 February	2008 April	2008 June	2008 August
1652±40[a]	1594±32[ab]	1555±30[ab]	1501±39[b]	1623±28[ab]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling month

The PAR at each of the sampled sites did not vary significantly (Table 4.8).

Table 4.8: Mean (± Standard Error) values of PAR (μmol m^{-2} s^{-1}) at the five study sites

Mango	Banana	Avocado	Pine	Eucalyptus
1630±31[a]	1543±46[a]	1632±22[a]	1604±28[a]	1516±39[a]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling species

4.2.5 Soil properties

Texture of soil

The soil particle size distributions of the five sampling sites are shown in Table 4.9. The soils in which the Bananas and the Avocados grew have high clay contents with a medium amount of silt and low sand contents, whereas the Mango site had the highest sand content.

Table 4.9: Soil texture (%) from each of the study sites, 0-10 cm depth

Study sites	% Clay (<2μm)	%Silt (2-50 μm)	% Sand (>50 μm)	Soil texture class
Mango	24.0[d]	20.4[d]	55.5[a]	Loam
Avocado	53.0[b]	36.1[c]	10.8[cd]	Clay
Banana	64.0[a]	31.2[c]	4.6[d]	Clay
Pine	35.0[c]	41.6[b]	23.3[b]	Silty Clay Loam
Eucalyptus	27.0[d]	53.9[a]	18.9[c]	Silty Clay Loam

Values with the same letter are not significantly (p>0.05) different for each element within the column for each of the sampling species

As the sites do show a different soil texture, one would expect there to be differences in nutrient and water availability. The interpretation of the data on water availability needs to consider those species that are irrigated compared with the rain-fed species. The amounts of water provided by the irrigation will be more

tightly held in the Avocado and Banana soils, which have a higher clay content when compared with the soils in which the Mangoes grew, which were mostly sandy.

Soil water content

September had the lowest soil water content of the measured periods, probably as a result of the sample being taken at the end of the dry season. The samples taken in April and June were significantly wetter ($p \leq 0.0001$), which is probably due to slower water extraction by the plants as a result of cooler temperatures (Table 4.10). The crop species were irrigated weekly making the interpretation of soil water data difficult.

Table 4.10: Mean (± Standard Error) values of soil water content (%) at the five study sites across the five study periods

2007 September	2008 February	2008 April	2008 June	2008 August
0.35 ± 0.02^{d}	0.47 ± 0.02^{bc}	0.54 ± 0.02^{a}	0.52 ± 0.02^{ab}	0.45 ± 0.02^{c}

Values with the same letter are not significantly ($p>0.05$) different for each element within the row for each of the sampling month

Irrigation seemed to have a greater effect at the sites where Bananas and Avocados grew as these sites were significantly wetter than the other sites ($p<0.0001$). The soil texture at the site where the Mangoes grew was predominantly sandy again making the interpretation of the soil water content difficult. (Table 4.11).

Table 4.11: Mean (± Standard Error) values of soil water content (%) at the five study sites

Mango	Banana	Avocado	Pine	Eucalyptus
0.39 ± 0.02^{c}	0.51 ± 0.02^{ab}	0.56 ± 0.02^{a}	0.42 ± 0.02^{c}	0.44 ± 0.02^{bc}

Values with the same letter are not significantly ($p>0.05$) different for each element within the row for each of the sampling species

Soil pH

The pH measured in water was close to neutral, except for the soils under the pine plantation which was acidic, probably as a result of cation removal by the tress and acidic decomposition products in the litter (Table 4.12). The Banana and the Avocado plants received LAN which would have raised the pH in the soil, it is interesting that the soils at the Mango site had the highest recorded pH. There is no documentation of fertilizer application at the Mango site over the last three years but no records were available for periods prior to 2005. The pH measured in KCl is lower than in H_2O because of the displacement of OH^- ions by Cl^- ions. The values showed that there are indications of mixed mineralogy. In general, most agricultural crops perform better on soils that are not too acid, the pHs measured in this study would be ideal for plant growth.

Table 4.12: Soil pH values at each of the selected sites

Species	pH (KCl)	pH (H_2O)
Avocado	5.6^{b}	7.1^{ab}
Mango	6.3^{a}	7.8^{a}
Banana	6.1^{ab}	6.7^{bc}
Eucalyptus	4.7^{c}	6.0^{c}
Pine	4.1^{d}	4.9^{d}

Values with the same letter are not significantly ($p>0.05$) different for each element within the column for each of the sampling species

4.2.6 Plant Properties

Leaf nitrogen content

There were significant differences in leaf nitrogen content between the species ($p<0.00$) and within the sampling year ($p<0.00$). The lowest leaf nitrogen content was measured in February with the highest being measured in June (Table 4.13).

Table 4.13: Mean (± Standard Error) values of leaf nitrogen content (%) for the five study species (n=30) over the five study periods

2007 September	2008 February	2008 April	2008 June	2008 August
1.5 ± 0.1^{b}	1.3 ± 0.2^{c}	1.4 ± 0.2^{c}	1.9 ± 0.2^{a}	1.7 ± 0.2^{ab}

Values with the same letter are not significantly ($p>0.05$) different for each element within the row for each of the sampling month

Bananas and Avocados have higher leaf nitrogen contents than the other species. This could be as a result of the fertilizer applied to these two species. There were no differences in leaf nitrogen content between the two plantation species (Table 4.14).

Table 4.14: Mean (± Standard Error) values of leaf nitrogen content (%) for the five study species (n=30)

Mango	Banana	Avocado	Pine	Eucalyptus
1.1 ± 0.1^{c}	2.8 ± 0.1^{a}	1.8 ± 0.1^{b}	0.9 ± 0.1^{c}	1.0 ± 0.1^{c}

Values with the same letter are not significantly ($p>0.05$) different for each element within the row for each of the sampling species

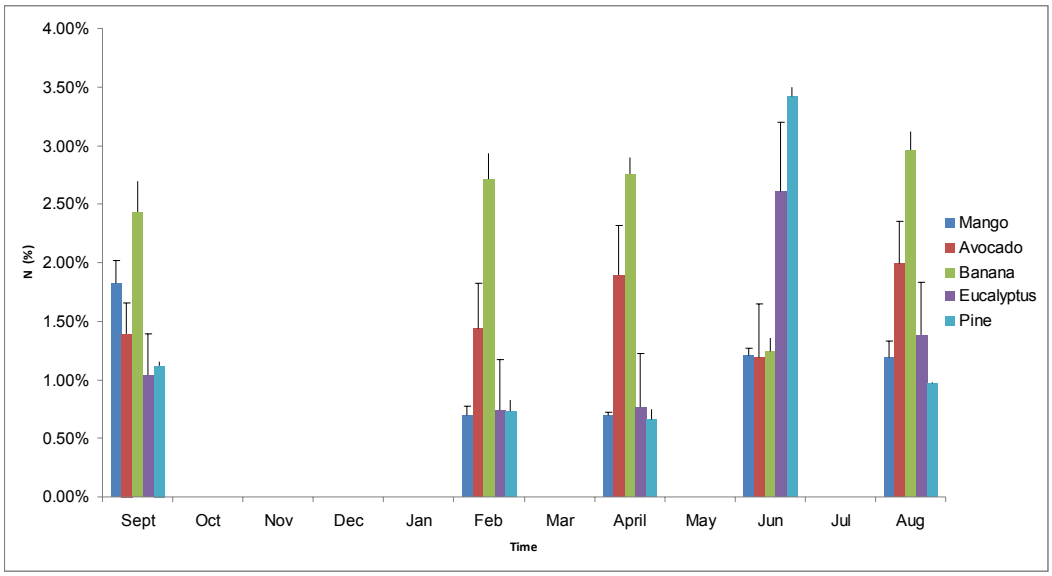

Figure 4.3 Leaf nitrogen content (%) for each of the sampled species at each sampling time (crop species n=15, plantation species n=10, mean and standard error)

Results showed that leaf nitrogen content changed seasonally across the sampled species (Fig. 4.3). Results from this research demonstrated that leaf nitrogen content rapidly increased in winter in the Pines and Eucalyptus and decreased in summer, this may be related to the proportion of structural and nonstructural carbohydrates in the leaves during the different seasons. The high leaf nitrogen contents in the Bananas and Avocados could be due to fertilization with LAN, however, Mangoes did not receive any fertilization over the sampling periods.

4.3 Emission rates

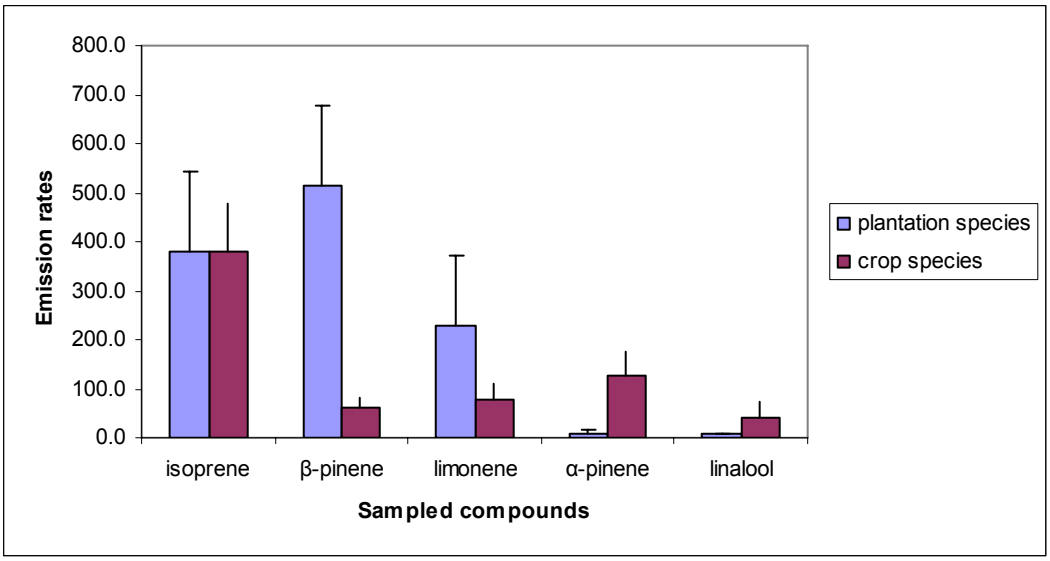

Figure 4.4 The emission rates of the sampled chemical species (µg C m^{-2} hr^{-1}) from the crop and plantation species (crop species n=75, plantation species n=25, mean and standard error)

The emission rates were expressed on a per leaf area basis. The data for the emission rates were grouped for the compounds and species. Figure 4.4 shows that the rates of the β-pinene emissions from plantation species were significantly higher (p=0.000) than from crop species. Sampled crop species for α-pinene emission rates were significantly higher (p=0.03) than from plantation species. However, there were no differences between averages of isoprene emission rates for the sampled crop and plantation species. The averages for the emission rates of limonene were two magnitudes higher for the sampled plantation species than for the crop species (Fig. 4.4).

The published literature shows that β-pinene is the main compound emitted from coniferous trees. This is supported by the results from the Pine trees in this study. Linalool is emitted in high quantities during flowering and fruit ripening. This may account for the linalool emissions from Mangoes.

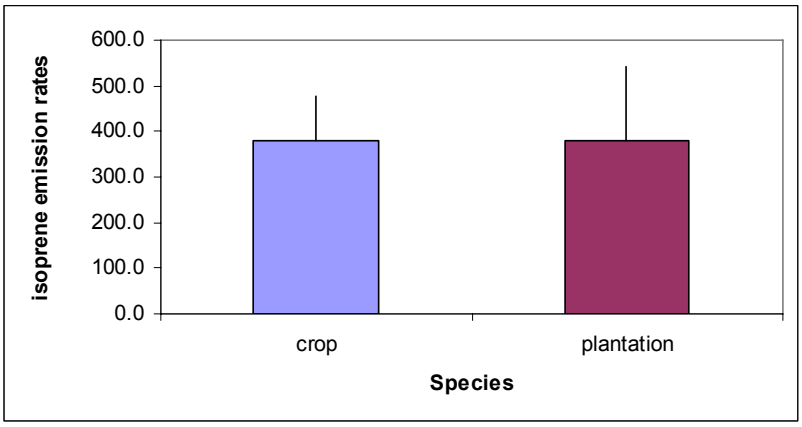

Figure 4.5a The emission rates of isoprene (µg C m^{-2} hr^{-1}) from the crop and plantation species (crop species n=75, plantation species n=25, mean and standard error)

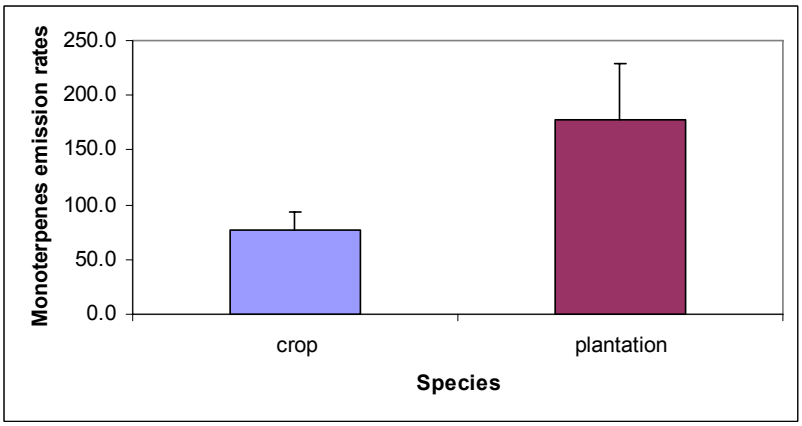

Figure 4.5b The emission rates of monoterpenes (µg C m^{-2} hr^{-1}) from the crop and plantation species (crop species n=75, plantation species n=25, mean and standard error)

In Figure 4.5 a and b, the emission rates of isoprene and monoterpenes from the leaf cuvette study were grouped as either being from the crop or plantation species. There was no significant difference between isoprene emission rates from the crop and plantation species (p=0.03), however, monoterpene emission rates were significantly higher (p=0.00) from sampled plantation species than from sampled crop species.

The different rates measured during the screening study and during the leaf cuvette study can be explained in at least two ways: 1) the screening study took place in August and the data were dominated by the exceedingly high emissions of isoprene

and monoterpenes from Mangoes. This could be related to the fact that the Mangoes were flowering and the carbon in the tissue was being remobilized; and 2) the photoinization detector measured the total value for all monoterpenes whereas in the leaf cuvette study only a limited number of monoterpenes were measured. β-pinene was the dominant monoterpene emitted from Pine trees and this resulted in the plantations having higher emissions.

4.4 Isoprene emission rates (µg C m^{-2} hr^{-1})

Mangoes had the highest emission rates with Bananas having the lowest rates.

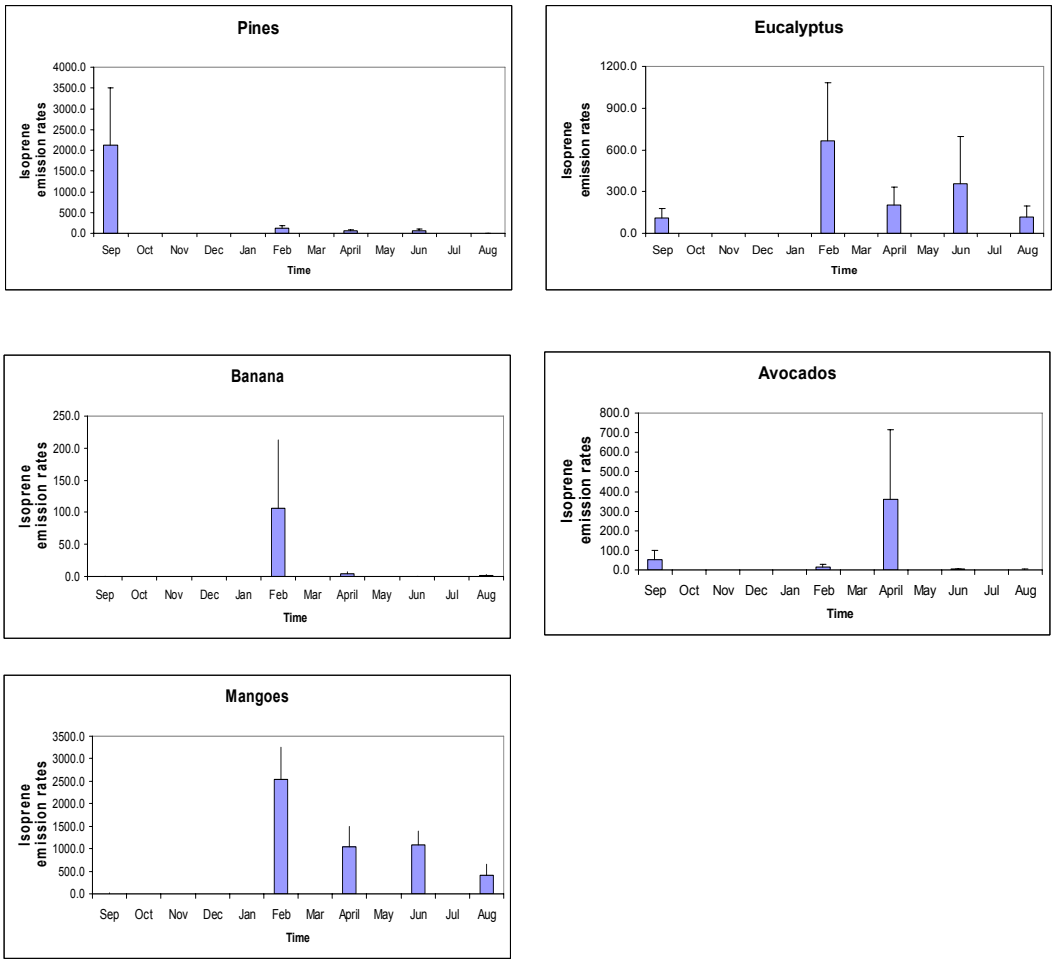

Figure 4.6 Mean values (n=5 for each sampling period) and standard error of isoprene emission rates (µg C m^{-2} hr^{-1}) for each of the sampled species over the sampling time

Figure 4.6 shows that three of five sampled species had significantly higher emission rates of isoprene (p=0.002) in February, when compared with the other species. However, for Pines, high emissions were measured in September following the onset of the spring rains. Emissions from Avocados were only evident in April when the Avocados were bearing fruit. The emission rates for Pines and Mangoes are an order of magnitude higher than the other species.

The published literature shows that Avocado and Banana were observed as non emitter species for isoprene emissions. Mangoes and Pines showed almost negligible values, ranging between 0.01 and 0.05 μg C m^{-2} hr^{-1}. Eucalyptus trees were found to have the highest isoprene emission rates which ranged between 1.5 to 5.4 μg C m^{-2} hr^{-1}.

The emission rates in February were significantly higher (p=0.002) than in all the other sampling months, with the rates dropping to a minimum in August. It appears that isoprene emissions respond to the onset of the spring rains in September and the warmer temperatures (Table 4.15).

Table 4.15: Mean (± Standard Error) values of isoprene emission rates (μg C m^{-2} hr^{-1}) for all species over the five study periods (n=25)

2007 September	2008 February	2008 April	2008 June	2008 August
462.6±305.2^{b}	695.2±245.8^{a}	334.1±131.4^{c}	301.2±119.2^{cd}	106.5±56.6^{d}

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling month

The mean of the isoprene emission rates ranged from 26.1 to 1016.9 μg C m^{-2} hr^{-1} (Table 4.16). The isoprene emission rates showed significant differences across the sampled species (p=0.004), with Mangoes having a significantly higher rate than all the other species. The emission rates from Bananas were particularly low.

Table 4.16: Mean (± Standard Error) values of isoprene emission rates (µg C m^{-2} hr^{-1}) for each of the species for all of the five study periods (n=25)

Mango	Banana	Avocado	Pine	Eucalyptus
1016.9±243.5[a]	26.1±21.2[e]	94.4±71.1[d]	471.6±304.8[b]	290.6±110.6[c]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling species

The emission rates of isoprene were negatively correlated with leaf nitrogen (%) in Mangoes. No significant correlations were found for emission rates and soil water content, relative humidity and temperature of ambient air and photosynthetic active radiation.

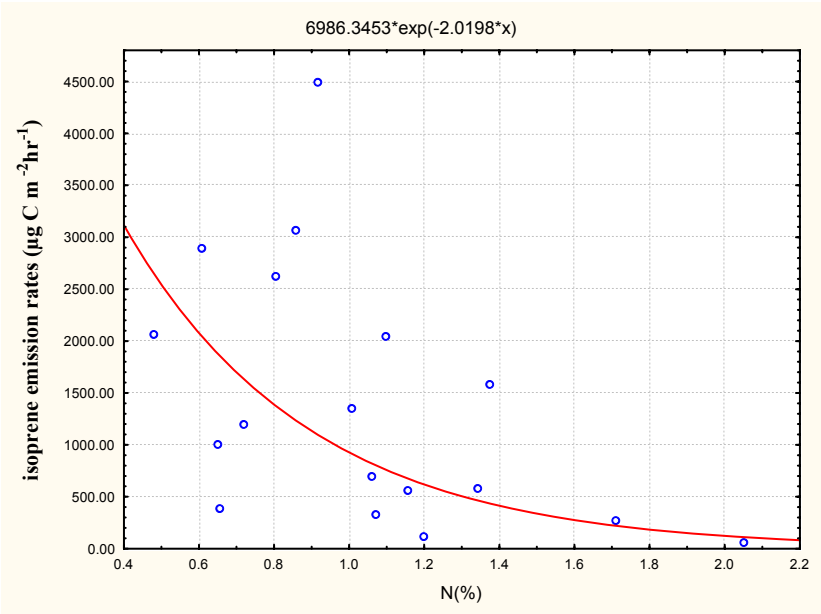

Figure 4.7 The relationship of isoprene emission rates (µg C m^{-2} hr^{-1}) and leaf nitrogen content (%) (Mangoes) (R=-0.42; p=0.03; n=25)

Figure 4.7 shows a negative correlation between isoprene emission rates (µg C m^{-2} hr^{-1}) and leaf nitrogen content (%) from Mangoes. The published literature provides evidence for both positive and negative correlations between emissions and leaf nitrogen. In this study, Mangoes was negatively correlated whereas the other species showed no relationships.

4.5 Monoterpenes emission rates (µg C m^{-2} hr^{-1}) (β-pinene, limonene, α-pinene and linalool)

Previous work in the southern African region demonstrates that there has been very limited work on estimation of monoterpene emission rates. The total monoterpene emission rates for the 5 sampling species in this study were estimated as very low and ranged between 0.0001 to 0.01 mg C m^{-2} hr^{-1}.

4.5.1 β-pinene emission rates (µg C m^{-2} hr^{-1})

β-pinene emission rates for all the five sampled species, followed the same seasonal pattern as was observed for isoprene, with significant emissions after rainfall and close to detection limits in winter. Pines had the highest emission rates, being two orders of magnitude higher than for Eucalyptus, which had the lowest emission rates.

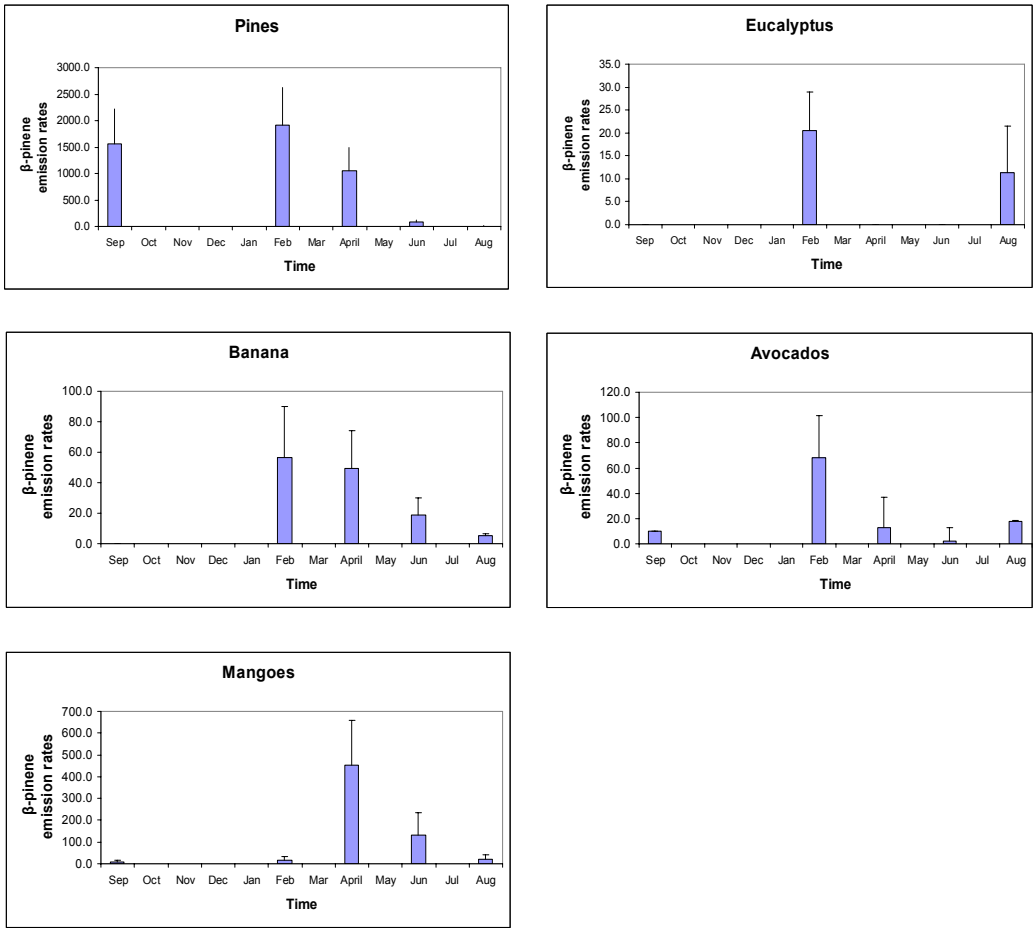

Figure 4.8 Mean values (n=5 for each sampling period) and standard error of β-pinene emission rates (µg C m^{-2} hr^{-1}) for each of the sampled species over the sampling time

Figure 4.8 shows that four of the five sampled species had significantly higher emission rates of β-pinene (p=0.002) in February. In addition, in the Pines, high emissions were measured in September following the onset of the spring rains.

Analysis of variance shows the significance of β-pinene emission rates (µg C m^{-2} hr^{-1}) across the sampling period (p=0.002) and for all the sampled species (p<0.000) (Table 4.17 and 4.18).

The emission rates in September were significantly higher (p=0.002) than all the other months, due to the extremely high values measured for Pines, with the rates dropping to a minimum in August 2008. It appears that β-pinene emissions respond to the onset of the spring rains in September and the warmer temperatures (Table 4.17).

Table 4.17: Mean (± Standard Error) values of β-pinene emission rates (μg C m^{-2} hr^{-1}) for all species over the five study periods (n=25)

2007 September	2008 February	2008 April	2008 June	2008 August
469.9±253.2[a]	339.2±190.1[b]	350.4±139.0[b]	44.6±24.8[c]	12.0±5.3[d]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling month

The mean of the β-pinene emission rates ranged from 7.9 to 921.6 (μg C m^{-2} hr^{-1}) (Table 4.18). The β-pinene emission rates showed significant differences across the sampled species (p<0.000), with Pines having a significantly higher rate than all the other species. However, the rates from Eucalyptus were particularly low.

Table 4.18: Mean (± Standard Error) values of β-pinene emission rates (μg C m^{-2} hr^{-1}) for each of the species for all of the five study periods (n=25)

Mango	Banana	Avocado	Pine	Eucalyptus
126.0±58.7[b]	28.4±10.2[c]	22.1±10.7[c]	921.6±264.3[a]	7.9±3.9[d]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling species

The emission rates of β-pinene were correlated with temperature of ambient air, relative humidity and photosynthetic active radiation, for some of the sampled species. No significant correlations were found between emission rates and soil water content and leaf nitrogen content.

Figure 4.9 shows that as the ambient temperature increased the β-pinene emission rates for Mangoes decreased, which is surprising as a positive response was expected.

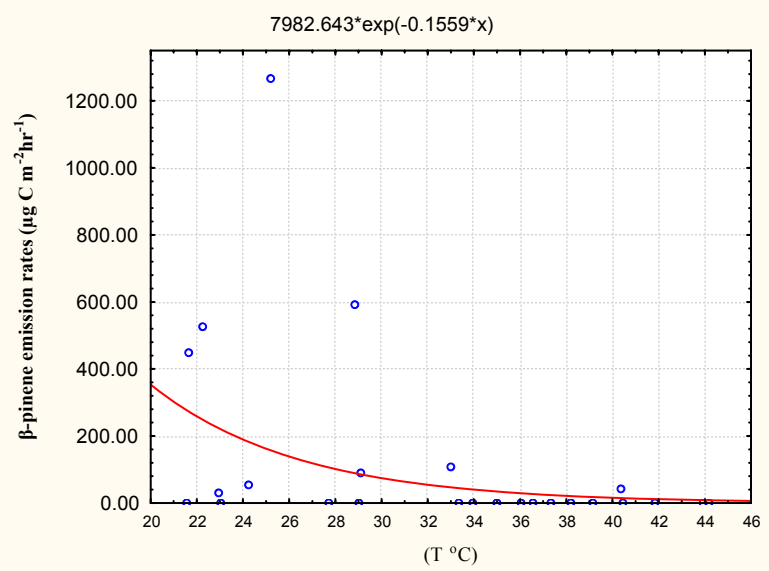

Figure 4.9 The relationship of β-pinene emission rates (µg C m^{-2} hr^{-1}) and temperature of ambient air (T °C) (Mangoes) (R=-0.42; p=0.04; n=25)

However, a positive response was found for Pines (Fig 4.10).

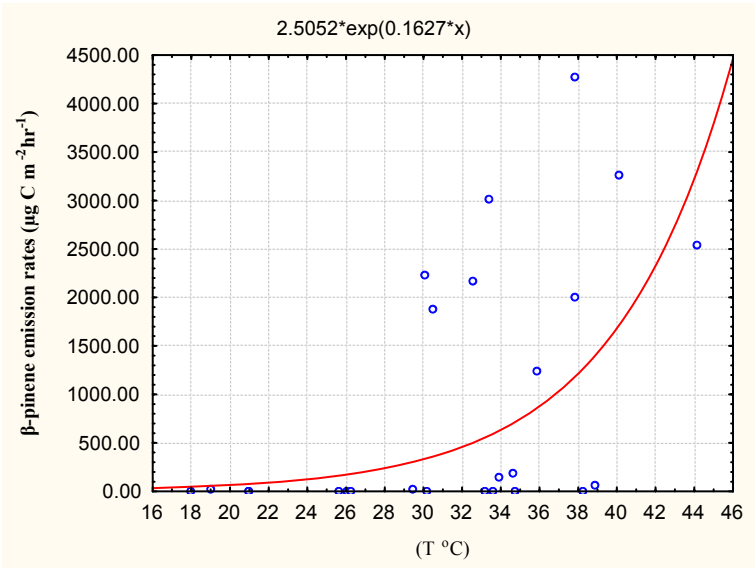

Figure 4.10 The relationship of β-pinene emission rates (µg C m^{-2} hr^{-1}) and temperature of ambient air (T °C) (Pines) (R=0.51; p=0.009; n=25)

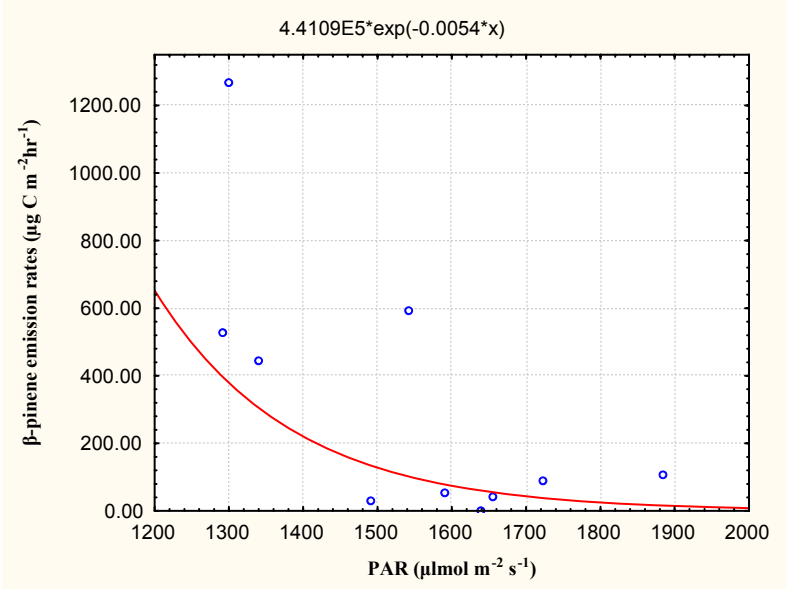

Figure 4.11 The relationship of β-pinene emission rates (µg C m^{-2} hr^{-1}) and PAR (µlmol m^{-2} s^{-1}) (Mangoes) (R=-0.67; p=0.0003; n=25)

Figure 4.11 shows a negative correlation between β-pinene emission rates (µg C m^{-2} hr^{-1}) and PAR (µlmol m^{-2} s^{-1}) in Mangoes whereas in Pines a positive correlation was shown (Fig. 4.12).

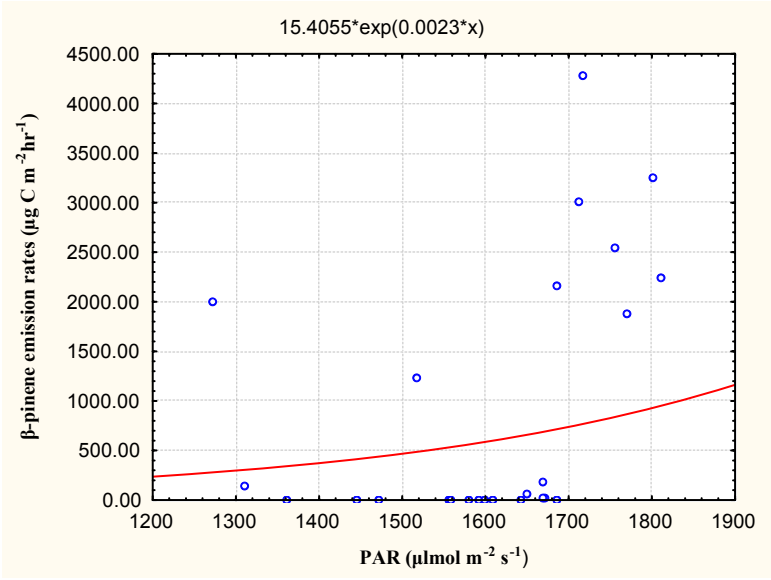

Figure 4.12 The relationship of β-pinene emission rates (µg C m^{-2} hr^{-1}) and PAR (µlmol m^{-2} s^{-1}) (Pines) (R=0.43; p=0.03, n=25)

Published literature supports the idea that the rate of monoterpene emissions are strongly dependent on temperature and do not show a response to light intensity. The results from this study were variable showing both negative and positive responses to photosynthetically active radiation and temperature of ambient air. Figure 4.13 shows a very weak positive correlation between β-pinene emission rates (µg C m^{-2} hr^{-1}) and relative humidity of ambient air (%).

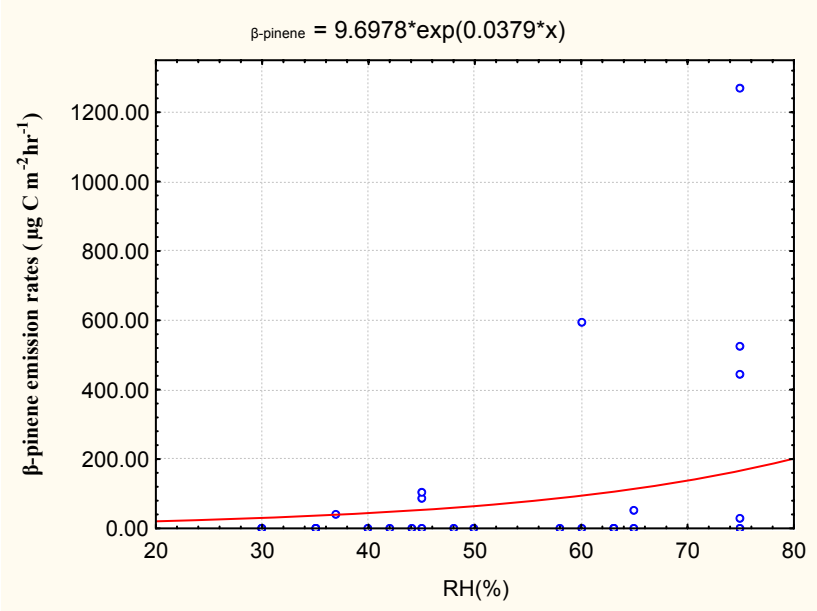

Figure 4.13 The relationship of β-pinene emission rates (µg C m^{-2} hr^{-1}) and RH (%) (Mangoes) (R=0.48; p=0.01; n=25)

4.5.2 Limonene emission rates (µg C m^{-2} hr^{-1})

Pines had the highest limonene emission rates with Eucalyptus having low limonene emission rates.

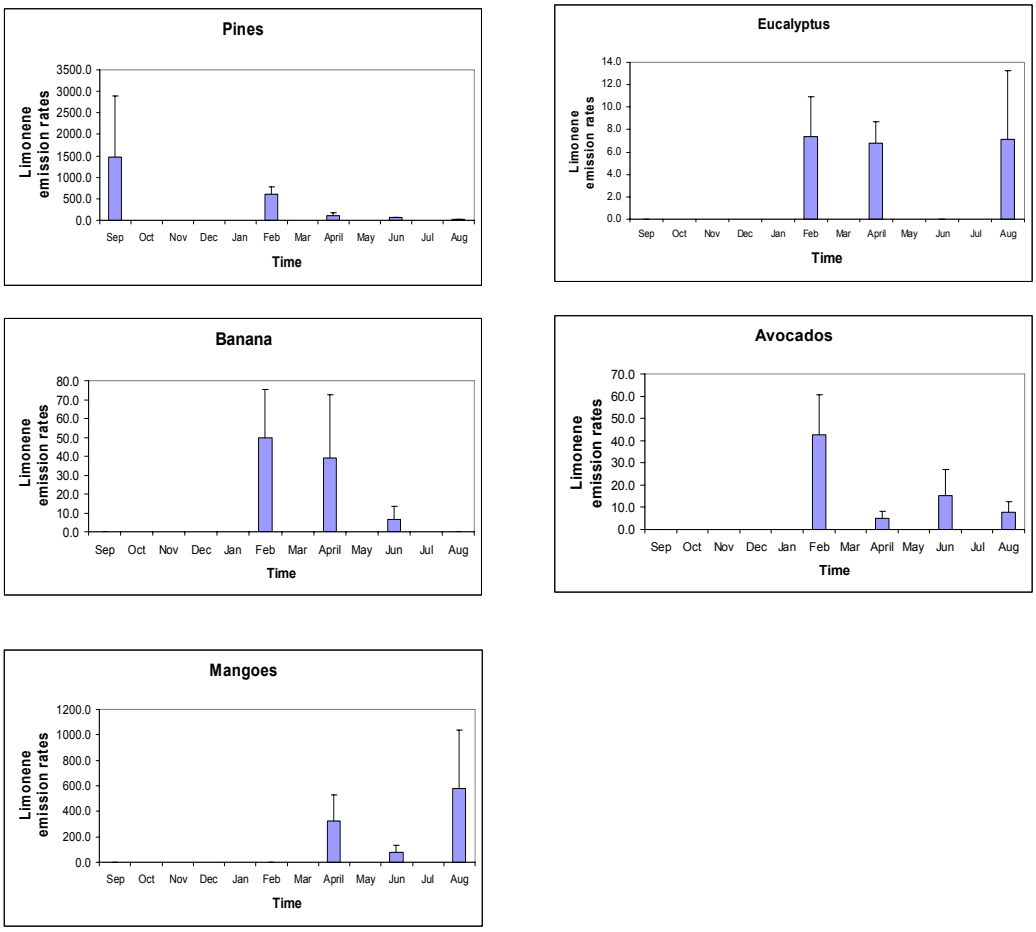

Figure 4.14 Mean values (n=5 for each of the sampling period and standard error) of limonene emission rates (µg C m^{-2} hr^{-1}) for each of the sampled species over the sampling time.

Figure 4.14 shows that three of the five sampled species had significantly higher emission rates of limonene ($p \leq 0.0001$) in February, when compared with the other sampled species. However, for Pines, high emissions were measured in September following the onset of the spring rains.

Analysis of variance shows that emission rates of limonene varied significantly over the sampling period ($p \leq 0.0001$) and across the sampled species ($p < 0.000$) (Table 4.19 and 4.20).

The Table 4.19 shows that September was the dominant month for emission rates of limonene (μg C m^{-2} hr^{-1}) and the lowest emission rates of limonene (μg C m^{-2} hr^{-1}) were recorded in June.

Table 4.19: Mean (± Standard Error) values of limonene emission rates (μg C m^{-2} hr^{-1}) for all species over the five study periods (n=25)

2007 September	2008 February	2008 April	2008 June	2008 August
295.2 ± 285.2^{a}	139.6 ± 57.1^{b}	98.2 ± 46.5^{c}	38.3 ± 15.6^{d}	120.9 ± 96.4^{bc}

Values with the same letter are not significantly ($p > 0.05$) different for each element within the row for each of the sampling month

In Table 4.20 limonene emission rates (μg C m^{-2} hr^{-1}) were significantly different between species, with Pines species, having the highest and Eucalyptus having the lowest limonene emission rates (μg C m^{-2} hr^{-1}).

Table 4.20: Mean (± Standard Error) values of limonene emission rates (μg C m^{-2} hr^{-1}) for each of the species for all of the five study periods (n=25)

Mango	Banana	Avocado	Pine	Eucalyptus
194.8 ± 103.7^{b}	19.2 ± 8.8^{c}	14.1 ± 5.1^{c}	452.3 ± 284.1^{a}	5.3 ± 1.8^{d}

Values with the same letter are not significantly ($p > 0.05$) different for each element within the row for each of the sampling species

There were no relationships between limonene emission rates with relative humidity of ambient air, photosynthetic active radiation, leaf nitrogen content and soil water content. The only exception was for Pine (Fig. 4.15) while there was a positive relationship between temperature of ambient air and emission rates of limonene.

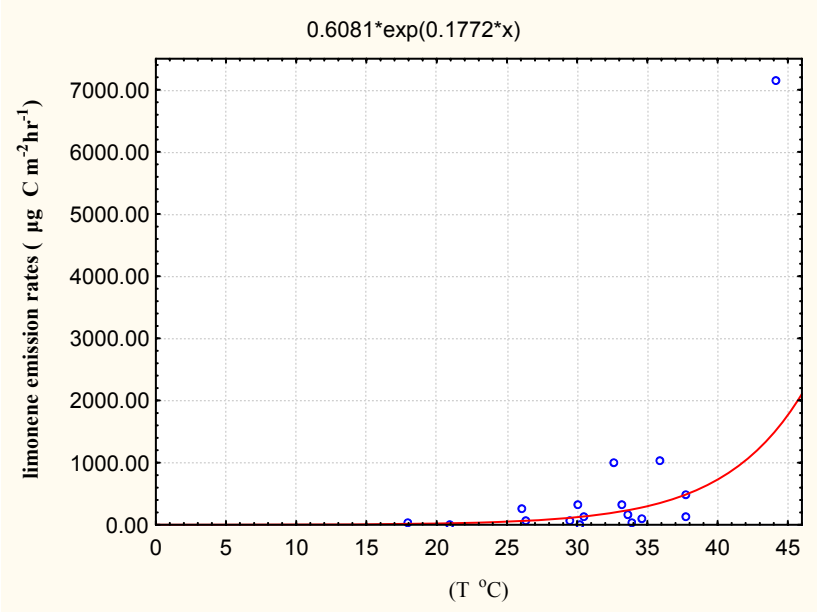

Figure 4.15 The relationship of limonene emission rates (µg C m^{-2} hr^{-1}) and temperature of ambient air (T ^{o}C) (Pines) (R=0.42; p=0.03; n=25)

4.5.3 α-pinene emission rates (µg C m^{-2} hr^{-1})

Measurements of emissions over the sampling year were limited to particular months. Mangoes had high α-pinene emission rates with Eucalyptus having low rates. Detectable rates for Pines, Bananas and Avocados were measured in only a single month, emissions in all species may be limited to the time of flowering as α-pinene is known to attract pollinators.

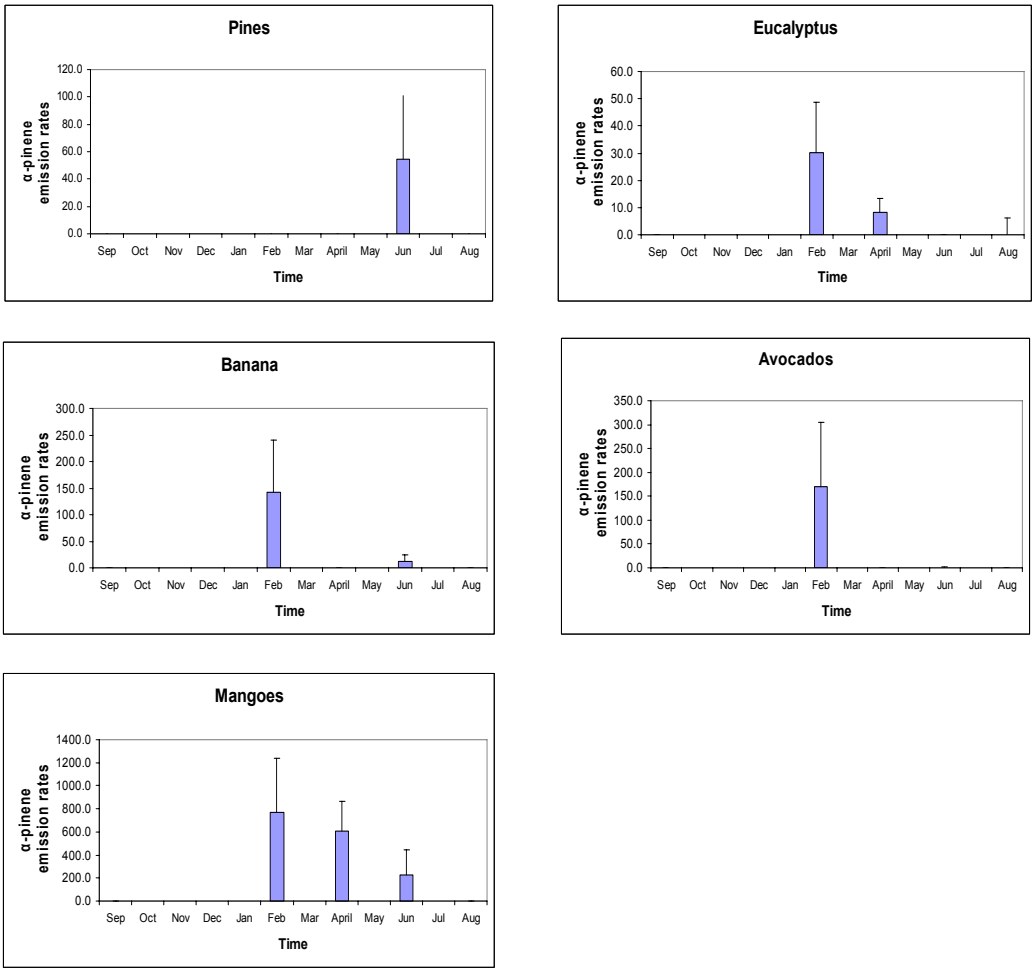

Figure 4.16 Mean values (n=5 for each sampling period) and standard error of α-pinene emission rates (μg C m^{-2} hr^{-1}) for each of the sampled species over the sampling time.

According to the literature, coniferous species are low emitters of α-pinene. In Table 4.21, the α-pinene emission rates (μg C m^{-2} hr^{-1}) were higher in February then in all the other months. In August and in September the emission rates were undetectable.

Table 4.21: Mean (± Standard Error) values of α-pinene emission rates (μg C m^{-2} hr^{-1}) for all species over the five study periods (n=25)

2007 September	2008 February	2008 April	2008 June	2008 August
0	218.3±108.1[a]	122.5±68.7[b]	63.2±43.7[b]	0

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling month

In Table 4.22, the α-pinene emission rates (μg C m^{-2} hr^{-1}) from Mangoes were significantly (p=0.0003) higher than all the other species. The plantation species had the lowest emission rates.

Table 4.22: Mean (± Standard Error) values of α-pinene emission rates (μg C m^{-2} hr^{-1}) for each of the species for all of the five study periods (n=25)

Mango	Banana	Avocado	Pine	Eucalyptus
320.5±123.9[a]	30.7±21.4[b]	34.3±28.1[b]	10.9±9.5[b]	7.7±4.2[b]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling species

There was a weak negative relationship between leaf nitrogen content and α-pinene emission rates for Mangoes. There were no relationships for any of the other environmental factors – relative humidity and temperature of ambient air, photosynthetic active radiation and soil water content or any of the species (Fig. 4.17).

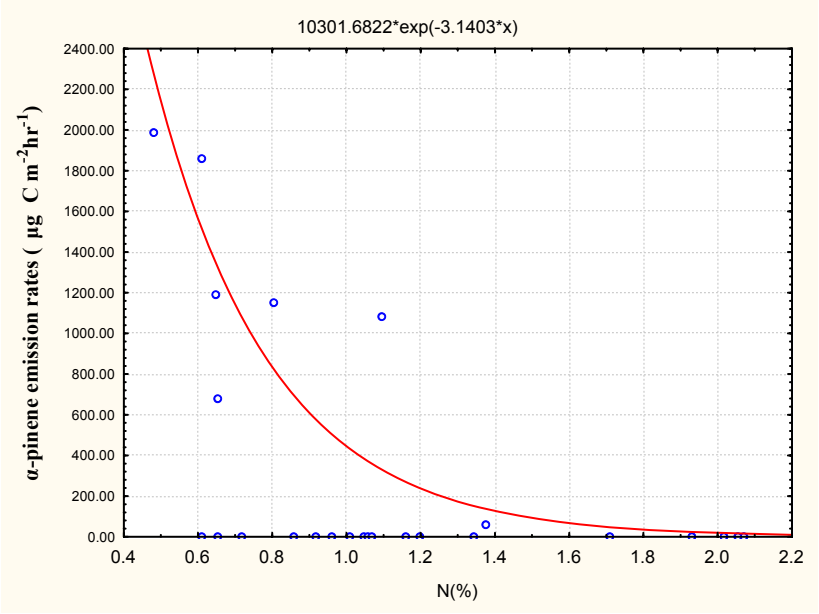

Figure 4.17 The relationship of α-pinene emission rates (µg C m^{-2} hr^{-1}) and leaf nitrogen content (%) (Mangoes) (R=-0.48; p=0.01; n=25)

4.5.4 Linalool emission rates (µg C m^{-2} hr^{-1})

There were very few detectable emissions (µg C m^{-2} hr^{-1}) over the sampling year for all the plant species, with the emissions being limited to April and August. Only four of the five sampled species emitted linalool, with Bananas having zero emissions. Linalool is usually associated with fruit ripening but in this case only the Avocados had fruit in April.

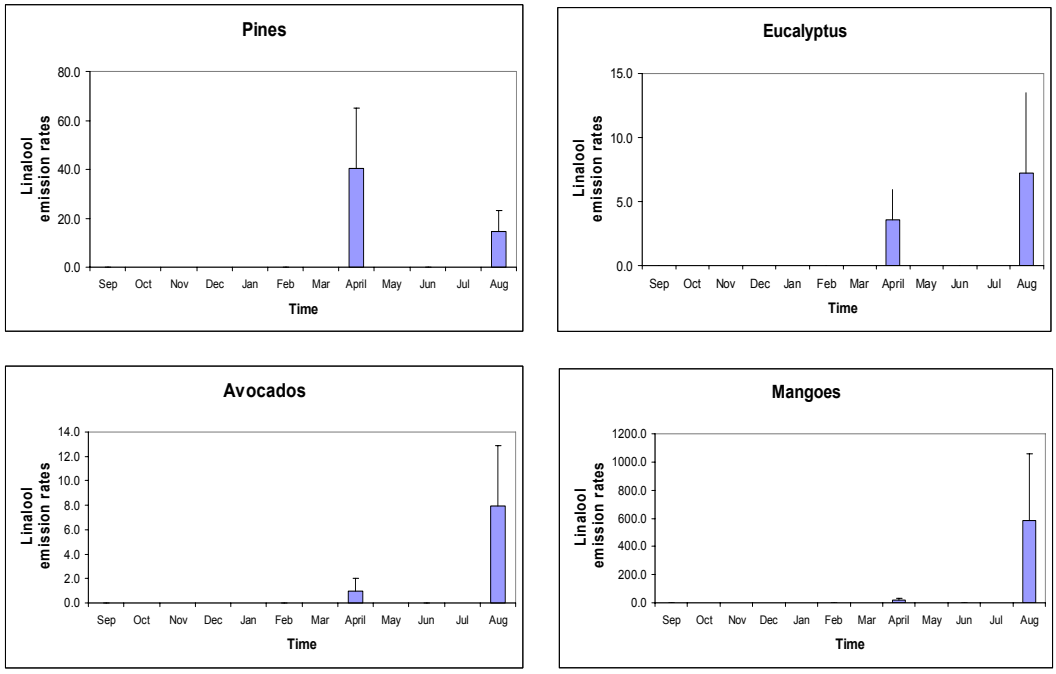

Figure 4.18 Mean values (n=5 for each sampling period) and standard error) of linalool emission rates (µg C m^{-2} hr^{-1}) for each of the sampled species over the sampling time

Table 4.23 shows that the maximum linalool emission rates (µg C m^{-2} hr^{-1}) occurred in August, with a minimum in April.

Table 4.23: Mean (± Standard Error) values of linalool emission rates (μg C m^{-2} hr^{-1}) for all species over the five study periods (n=25)

2007 September	2008 February	2008 April	2008 June	2008 August
0	0	13.2±5.9[b]	0	122.7±97.8[a]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling months

Table 4.24: Mean (± Standard Error) values of linalool emission rates (μg C m^{-2} hr^{-1}) for each of the species for all of the five study periods (n=25)

Mango	Banana	Avocado	Pine	Eucalyptus
120.9±97.9[a]	0	1.8±1.1[a]	10.9±5.8[a]	2.2±1.4[a]

Values with the same letter are not significantly (p>0.05) different for each element within the row for each of the sampling species

The mean values ranged from 0 to 120.9 (μg C m^{-2} hr^{-1}), with Mangoes being the highest emitter. There was a weak negative relationship between leaf nitrogen content and linalool emission rates for Pines. There were no relationships for any of the other environmental factors – relative humidity and temperature of ambient air, photosynthetic active radiation and soil water content or any of the species (Fig 4.19).

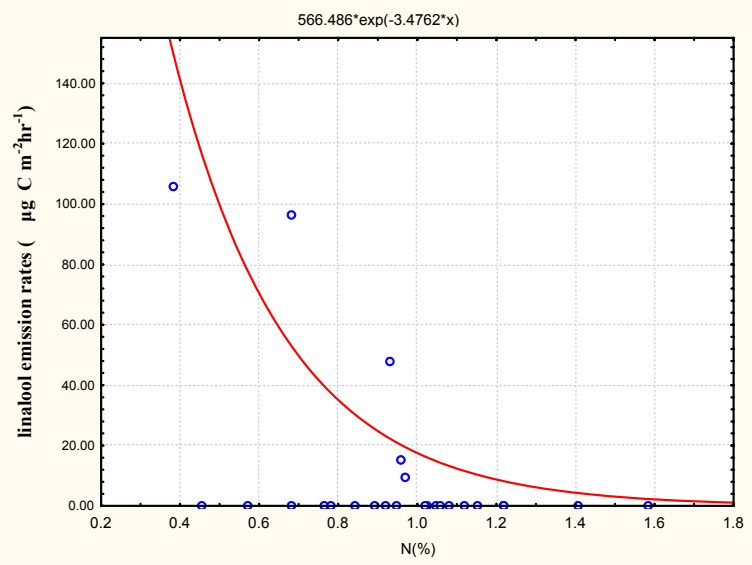

Figure 4.19 The relationship of linalool emission rates (µg C m^{-2} hr^{-1}) and leaf nitrogen content (%) (Pines) (R=-0.45; p=0.02; n=25)

CHAPTER 5: DISCUSSION

This chapter discusses the seasonal patterns and species differences of the BVOCs measured in relation to the published literature. The chapter ends by outlining possible directions for future research in the field of BVOCs.

5.1 Impacts of Biogenic Volatile Carbons on Atmospheric Chemistry

The species sampled all emitted BVOCs which could potentially contribute to the changed aerosol and cloud condensation nuclei status of the atmosphere in Mpumalanga. The extent of the impact would depend on the sources and concentrations of other compounds, especially OH and NOx in the atmosphere. It is difficult to predict what the resultant changes in ozone chemistry would be. Isoprene and β-pinene were the dominant species emitted. The emission rates, especially from Mangoes, Eucalyptus and Pines, were high but limited in duration. Sampling occurred five times over the year, with the emissions being highest at the start of the rainy season but very low during the winter. The integrated values by species over the seasons would probably indicate that the impact on atmospheric chemistry will be small but this needs to be further discussed. The wide range of plantation and crop species grown in the Mpumalanga province make this an ideal study area. This study contributes data on the emissions from subtropical crops and plantation species which can be used to estimate landscape scale emissions. The BVOCs emission rates for five plant species were characterized and correlated with environmental factors. Detailed emission information, from Pines, Eucalyptus and Mangoes, have been reported in the literature. Some screening studies have been conducted on Bananas and Avocados.

5.2 The Emission Rates of Selected Compounds for the Plantation and Crop Species

Isoprene was the dominant BVOC measured in this study. Measurements of seasonal isoprene emission patterns from tropical and subtropical plant species are extremely limited (Lerdau and Keller, 1997; Otter *et al*. 2002; Kuhn *et al*. 2004). Isoprene emissions from studies in Africa have reported an emission capacity of between 0.5 - 10.0 mg C m^{-2} hr^{-1} for different savanna species (Guenther *et al*. 1995; 1996; Otter *et al*. 2002 and Harley *et al*. 2003). These researchers showed that maximum isoprene emissions were recorded during summer with minimum rates being measured during the winter, similar patterns were found in this study. These results demonstrate the strong dependence of isoprene emissions on temperature. In this study isoprene emission rates were highest in September (for Pines) and February (for Eucalyptus) when both the temperature and the humidity were high. However, the literature states that isoprene emissions are only slightly affected by humidity (Guenther *et al.* 1991).

Terpene emissions from studies in Africa have reported an emission capacity of between 2.4 - 3.0 mg C m^{-2} hr^{-1} for different mopane woodlands (Guenther *et al*. 1996; Otter *et al*. 2002). In Mpumalanga, β-pinene was the dominant monoterpene, being emitted predominantly from the Pine plantations. This finding is similar to other published work where β-pinene has been found to mostly be associated with coniferous trees (Helas, 1997). Work done by Sharkey (1996) and Delfine *et al*. (2000) observed that monoterpenes have a thermotolerance function in leaves. In this study, the highest emission rates for β-pinene, α-pinene and limonene were measured in September, February and April, when temperatures and rainfall were high.

The majority of studies on BVOCs have been conducted in the Northern Hemisphere, where light is often limiting. Therefore, an emphasis on light as a controlling factor on emissions is found in the literature. For example, Steinbrecher (1989) showed that α-pinene emissions from spruce were light induced. In sub-tropical and tropical studies, light is usually non-limiting.

α-pinene emission rates from the present study demonstrated a greater dependence on temperature and relative humidity than light. Temperature and relative humidity dependence have also been reported by Loreto (1996a) in *Quercus ilex.*

Limonene has been reported to be emitted from more than 300 plant species (Burdock, 1995). However, the role of limonene is still unclear, but several experiments have shown that limonene might have a role in protecting plants from thermal damage (Sharkey and Singsaas, 1995; Loreto *et al*. 2001; Sharkey and Yeh, 2001). Potential reasons why limonene, and monoterpenes in general, provide thermotolerance are the increase of fluidity, the stabilization and the protection of plant membranes and their lipids due to its lipophility and antioxidant capacity similarly to what occurs with isoprene. All five sampled plant species were found to emit limonene, with Pines emitting the highest recorded value. Results showed that limonene emission rates were only slightly affected by ambient air temperature and humidity (Fig. 4.13), where, the highest values were recorded in summer and the lowest in winter. Limonene emissions were high in winter from Mangoes and it is suggested that this may be associated with flowering. There is no evidence in the literature to support these findings.

Linalool accumulation takes place in the leaf tissue during fruit ripening, the linalool is then relocated into the developing fruits (Ronen *et al*. 1999). A significant fraction of the isoprenoid pool, in the plastids, is diverted into S-linalool in the fruits during ripening. In addition, Bergstrom (1991) and Knudsen and Tollsten, (1993) concluded that linalool is a typical component of flowering fragrances, attracting pollinators. Release of linalool emissions from Avocados and Mangoes could possibly be explained by the biosynthesis of linalool during flowering and fruit ripening in April and August, however, there was no evidence that linalool was emitted by Bananas. Pines and Eucalyptus showed high emissions of linalool in August coinciding with cone and seed production respectively in the species.

5.3 The Emission Rates from Sampled Plantation and Crop Species

The high variability in isoprene and monoterpene emission rates from different plant species was the focus of a study published by Benjamin *et al*. (1996). Crop plants, especially annuals, are considered to be low emitters. In some cases, they can even be regarded as a sink for some compounds for example short-chain organic acids (Kesselmeier *et al*. 1998). In this study it was hypothesized that plantation species would have higher emission rates than the crop species. This hypothesis was not accepted for isoprene where the rates from the two functional groups of plants were approximately equal. However, it was accepted for the monoterpenes, where the plantations produced higher rates (Fig 4.5 a; b). The crop and plantation isoprene emissions appear similar as a result of the very high emissions from the Mangoes. The differences between the remaining crops (Avocado and Banana) and the plantations are substantial.

It is acknowledged that the sample size in this study was small and the variability high but there was a tendency for the crop species, except for Mangoes, to have low emission rates. All of the species in this study display the C_3 photosynthetic pathway. The majority of C_3 plants, particularly woody species, display low photosynthetic rates (Larcher, 1995). However, some woody agricultural C_3 crops, especially those grown under irrigation and fertilization may have elevated photosynthetic rates, similar to those measured in C_4 species (Larcher, 1995). The carbon assimilated in the crop species is predominantly allocated to vegetative growth and fruit production and away from BVOC emissions, whereas in plantation species, the leaf age is greater than in the crop species. As leaves age, the photosynthetic capacity declines, resulting in the need for more intense reallocation of carbon to growing tissues and wood production. It is well known that when the carbon is solubilised for relocation, leaks can occur. These leaks may result in enhanced BVOC production. This explanation relies more heavily on the idea that BVOC emissions are passive. However there is much evidence that BVOC emissions are active, under the control of specific enzymes. Therefore the enhanced production of emissions from plantation species, as seen in this

study, must invoke all known mechanisms. Mangoes are interesting - it appears that they adopt a growth strategy of rapid assimilation coupled with rapid loss. It must also be remembered that all the crops were irrigated and Avocados and Bananas were fertilized, thereby removing these constraints on photosynthetic capacity.

The literature suggests that Pine and Eucalyptus species frequently emit BVOCs, with isoprene being emitted in higher amounts than terpenes. The range of all BVOC emissions measured from Pine species, from cool, temperate areas is 12.1 μg C g^{-1} hr^{-1} (Scots Pine) (Isidorov *et al*. 1985), 11.5 μg C g^{-1} hr^{-1} (Sand Pine) (Zimmerman, 1979), and 0.2 μg C g^{-1} hr^{-1} (Red Pine) (Yokouchi and Ambe, 1984). Whereas, much higher values (limonene emissions of 1800 μg C g^{-1} hr^{-1} and β-pinene – 400 μg C g^{-1} hr^{-1}) from *Pinus pinea* and *Pinus halepensis*, from the Mediterranean, were measured (Loreto *et al*. 2000). Pines from the current study had rates of 0.92 mg C m^{-2} hr^{-1} for β-pinene and 0.45 mg C m^{-2} hr^{-1} for limonene. The range of published isoprene emissions for Eucalyptus is between 1.5 – 5.4 mg C cm^{-2} hr^{-1} (He *et al*. 2000), 20.4 μg C g^{-1} hr^{-1} (Padhy and Varshney, 2005) and 48-59 μg C g^{-1} hr^{-1} (Nunes and Pio, 2001) The values recorded in our study were 0.29 mg C m^{-2} hr^{-1}. The units of expression for the BVOCs are variable in the literature, in this study all the data was expressed as mg C m^{-2} hr^{-1}, which corresponds to many other published values in the literature. However, other units of expression include μg C g^{-1} hr^{-1}, mg C m^{-2} hr^{-1} and mg C cm^{-2} hr^{-1} and there is no simple conversion between these different units of expression. This does add to the complexity and frustration of trying to compare results from a variety of studies.

Much lower emissions of monoterpenes were recorded from Eucalyptus species: 0.9 μg C g^{-1} hr^{-1} for monoterpenes (Padhy and Varshney, 2005). It is extremely difficult to compare the rates because of the different units that authors choose to publish. The values from this study were expressed as mg C m^{-2} hr^{-1} because these are the units used for other South African studies.

Subtropical evergreen crop species have been poorly characterized for BVOC emissions in the literature. Most of the research was conducted to enhance the understanding of monoterpenes as attractants or repellents in plant pathology. Mangoes have been described as low emitters (with isoprene values of 0.02 μg C g^{-1} hr^{-1} Rasmussen, 1978; Klinger *et al*. 1998, 4.9 μg C g^{-1} hr^{-1} Padhy and Varshney 2005 and 42 μg C g^{-1} hr^{-1} Harley *et al.* 2004) being measured which is in contrast to our research where the values were extremely high (1.02 mg C m^{-2} hr^{-1}). Monoterpene emissions range from 1.2 μg C g^{-1} hr^{-1} to 0.12 mg C m^{-2} hr^{-1} for linalool and 0.32 mg C m^{-2} hr^{-1} for α –pinene emissions (this study).

Avocados and Bananas have been reported in the literature to be non emitters (Rasmussen, 1978; Benjamin *et al*. 1996; Klinger *et al*. 1998). Bananas have been found to store hydrocarbons in the fruits, however, it is not known whether they emit low levels of isoprenoid VOCs when the fruits are forming (Owen and Penuelas, 1995). Results from this study showed very low emissions from Bananas and Avocados.

5.4 Factors Controlling the Seasonal Emissions of BVOCs in Mpumalanga

The second hypothesis of this study addressed the relationship between emission rates and season. There is increasing evidence that season has a great impact on isoprenoid emissions where emissions rapidly decline in winter and increase in summer. (Yokouchi and Ambe, 1984; Monson *et al*. 1994; Lerdau *et al*. 1995; Street *et al*.1997; Guenther, 1997 and Otter *et al*. 2002). Species differences are also pronounced. Significant seasonal changes in the leaf emission capacity of diverse deciduous and evergreen trees have been observed (Ohta, 1986; Yatagai *et al*. 1995; Lerdau *et al*. 1995 and Street *et al*. 1996). Results from the present study also show high seasonal variability in emission rates within and between species and across the sampling period. The results show that emission rates of all compounds increased in summer (September, February and April) and declined in

winter (June and August). Some compounds, α-pinene and linalool, were very close to the detection limit or absent in August (winter).

A detailed study of light, temperature and seasonal effects on isoprene and monoterpene emissions from *Picea glauca* was published by Kemp *et al*. (1996). They reported that isoprene emission values ranged from 0.3 to 20,000 mg C m^{-2} hr^{-1}. Results confirm the hypothesis above. The explanation is based on stomatal closure. Under drought conditions in the dry season, stomata close in the proportion to the degree of stress, progressively limiting CO_2 availability in chloroplasts (Flexas *et al*. 1999). CO_2 estimation is reduced and the CO_2:O_2 ratio drops, thereby increasing photorespiration and Mehler reaction (Medrano *et al*. 1997). These processes consume relatively less ATP than photosynthesis (Osmond *et al*. 1997), but the released energy is enough for photorespiration and evaporation. Therefore, most of assimilated CO_2 will emit from the leaves as BVOCs (primary isoprene) to protect plants from the heat.

5.4.1 Ambient air temperature

Studies of temperature effects on BVOC emissions from subtropical plants are limited. Temperature responses differ among plant species and can even vary between shade and sun adapted leaves within the canopy of an individual tree (Tingey *et al*. 1987; Monson *et al*. 1992; Sharkey *et al*. 1996; Harley *et al*. 1996; Lerdau and Keller, 1997). The emission rates for some of the compounds in this study were correlated with temperature.

The decline in emission rates at high temperatures, above 35 ºC, which were observed in September in this study, could be as a result of the reversible control of 'heatshock proteins' (Vierling, 1991). Singsaas and Sharkey (2000) reported similar findings, where they pointed out that isoprene emission rates started to decrease at 35 ºC and increase when the temperatures were below 32.5 ºC. It has been suggested that emissions act as a thermotolerance mechanism during periods of prolonged high temperature. The emissions of BVOCs as well as other physiological processes, such as the production of heatshock proteins (Vierling,

1991) and changes in xanthophyll epoxidation (Havaux and Tardy, 1996; Havaux *et al*. 1996) are induced after several minutes of elevated temperatures. In addition, Siwko *et al*. (2007) concluded that isoprene stabilizes lipid membranes and provides a mechanistic basis for explaining why isoprene protects plants from damage due to heat spikes. The physiological processes are reversible controlling the increases and decreases of isoprene emissions.

This view is consistent with the theory of leaf protection from rapidly increasing temperatures (Singsaas *et al*. 1997; Singsaas and Sharkey, 1998). The moderate to high temperatures (35 $^{\circ}$C – 45 $^{\circ}$C) can cause thylakoid membranes to become leaky and stimulate cyclic electron flow (Pastenes and Horton, 1999; Bukhor *et al*. 1999; Schrader *et al*. 2007). The cyclic electron flow maintains the proton motive force needed for ATP synthesis and the production of isoprenoid precursors (Schrader *et al*. 2007; Hickler *et al*. 2008)

5.4.2 Relative humidity of ambient air

The evidence in the literature relating to the effect of relative humidity on BVOC emissions is inconclusive. Some reports have shown a positive correlation between humidity and BVOC emissions (Tingey *et al*. 1980; Lamb *et al*. 1985; Staudt and Seufert, 1995; Loreto *et al*. 1996a) whereas others, show slight or no effect (Croteau, 1977; Yokouchi and Ambe, 1984; Monson and Fall, 1989; Juuti *et al*. 1990; Guenther *et al*. 1991; Janson *et al*. 1992 and Loreto *et al*.1996a). The physiological mechanism of how relative humidity affects emissions is still not well understood and the mechanisms may be purely a physical one. Shade *et al*. (1999) suggested that water is absorbed by the leaf surface in response to changing humidity levels, which potentially increase the cuticular permeability to the emissions. He also noted that monoterpene emissions were elevated during and after rain events, as a result of increased humidity.

The relative humidity measured in this study varied from 42-66 % across the year. There was only one set of results (for Mangoes) that showed a weak positive relationship between relative humidity and β-pinene emissions. Physiologically,

increased emissions could be explained by an increase in the conductance of the cuticle due to hydration (Croteau, 1977). In addition, epidermal turgor is affected by humidity (Shackell and Brinckmann, 1985; Frensch and Schulze, 1988) which causes stomatal responses to occur passively (i.e. hydraulically) without any active (i.e. metabolic) involvement. Humidity-induced changes, in steady state stomatal apertures, apparently involve guard cell ion fluxes, though measured changes in guard cell K^+ content, exhibit a delay relative to changes in stomatal aperture (Losch and Schenk, 1978; Laffray *et al*. 1984). Biogenic volatile compounds can accumulate in the tissue during periods of stress, this accumulation is exacerbated by the closure of the stomata. When the stress is relieved and the stomata open, the accumulated volatile compounds will be emitted into the atmosphere in high quantities. Metabolic processes which involve ABA and other inhibitors (Ogunkamni *et al*. 1974; Munns and King, 1988) or Ca^{2+}, or promoters such as cytokinins (Itai and Vaadia, 1965; Blackman and Davies, 1985) or K^+, could mediate stomatal responses to humidity.

It is suggested that relative humidity is not the primary factor controlling emissions but it may be important.

5.4.3 Photosynthetic active radiation (PAR)

There are numerous studies that show a relationship between light intensity and the emissions of BVOCs. The mechanisms of interaction are still not well defined but thought to be directly related to photosynthetic pathways and compensation points. Previous studies show different emission rates from sun and shade adapted leaves. Emissions from temperate species saturate at a light intensity of about 1,000 (μmol m^{-2} s^{-1}), whereas tropical species saturation occurs only at 2,500 (μmol m^{-2} s^{-1}) (Lerdau and Keller, 1997). Sharkey (1996) observed that emissions of isoprene increased with light intensity between 1,000-2,000 (μmol m^{-2} s^{-1}), whereas values of 500-1,000 (μmol m^{-2} s^{-1}) were reported by Tambunan *et al*. (2006) in the tropical region of Okinawa (Japan). The average PAR in this study was 1,600 μmol m^{-2} s^{-1} and did not vary much as the plants were measured between 10 am and 3 pm. In this study β-pinene was the only compound that was

correlated with light intensity. It appears that the effect of light intensity is usually mediated through other controlling factors for example temperature, fertilization status and water stress, via a variety of mechanisms.

- High temperatures damage photosynthetic enzymes which leads to photoinhibition (Hickler *et al*. 2008);
- Water stress and stomata closure reduce the CO_2 assimilation rate (Sharkey, 1990; Chaves, 1991; Ort *et al*. 1994; Cornic and Massacci, 1996; Castrillo and Calcagno, 1989; Medrano *et al*. 1997);
- Fertilization improves Photosystem I and II by increasing the number of soluble proteins;
- High light intensity provides energy and can limit the amount of CO_2 fixed as a result of stomatal closure (Magel *et al*. 2007).

In tropical conditions, light intensity is seldom limiting but it can result in stressful conditions in water limited and high temperature environments. High light intensity stress is a more frequent occurrence in temperate environments. Plants can modify their leaf structure and metabolism (for example, leaf thickness, cuticle thickness, phloem loading and unloading rates and high metabolic rates) to help alleviate the stress (Kerstiens, 1996; Bonal and Guehl, 2001). Studies on evergreen Mediterranean oak species, growing under high light intensity, show high rates of monoterpene synthesis and emissions. Under these conditions monoterpenes are not stored in the tissue and emissions result from recently fixed carbon (Staudt *et al*. 1993; Seufert *et al*. 1995, Loreto *et al*. 1996 a; b). Sharkey *et al*. 1991b, Loreto and Sharkey, 1993; Harley *et al*. 1994; Litwak *et al*. 1996; Monson *et al*. 1995 suggested that evergreens species, growing at high light intensities, have high emission capacities. However, monoterpene emissions are generally regarded as light independent, with storage taking place in resin ducts or glands (Monson *et al*. 1995; Lerdau and Keller, 1997). These species usually exhibit large storage pools compared to the emission rates (Schindler and Kotzias, 1989; Juttner, 1991).

Isoprene and monoterpenes have been observed to be emitted from plants growing at high light intensity which may be related to the light dependent production of DMAPP from photosynthetic intermediates, and increased fluxes of intermediates through the plastidial MEP pathway leading to increased stomatal DMAPP concentrations. The enzyme which catalyzes the conversion of DMAPP to isoprenoids, appears to be light-activated (Silver and Fall, 1991).

5.4.4 Soil water content, texture and pH of soils

Soil moisture reflects the differences between inputs (precipitation) and outputs (evapotranspiration, runoff, drainage) from the system (Small *et al*. 2009). Soil water content and soil water storage (SWS) are extremely important for crop growth and yield. There was no relationship between BVOC emissions and soil water content in this study which is not surprising because soil water status was not studied in sufficient detail to give a comprehensive picture. The sampling and gravimetric measurements that were conducted at the five periods over the season give only a snap shot of what the soil water content was at that time and in no way allow for an interpretation of how water status effects emission rates. The interpretation is also confounded by the fact that Mangoes, Bananas and Avocados are irrigated. Soils collected under Avocados and Banana species were clayey and wetter when compared with soils having a loamy or sandy texture under the other plant species.

However, the data do show a very interesting pattern which may be linked to water status. All plant species showed emissions in September, immediately after the onset of the spring rains. It is also well known that very high soil biogenic emissions of nitric oxide also occur at the time of the spring rains. The co-occurrence of BVOCs and NO_x in the atmosphere is expected to impact on the chemistry of the atmosphere. In this study the highest emission rates occurred in the wettest sampling months (February and April) which could imply that soil water content indirectly affects the BVOCs emissions. .

The proportion of assimilated carbon lost as BVOCs increases dramatically as water stress progresses (~250 % when photosynthesis was almost zero (Brilli *et*

al. 2007). In contrast, other authors found that emissions rapidly decreased with water deficit, but following re-watering, isoprene emission capacity sometimes exceeds the capacity before the stress (Tingey *et al*. 1981; Loreto and Sharkey, 1993; Fang *et al*. 1996; Funk *et al*. 2004; Pegoraro *et al*. 2005; Monson *et al*. 2007). Emissions were lower in the dry hot months but dramatically increased after the rainfall in February and April. Brilli, (2007) found that isoprene is inversely related to PEP carboxylase activity. Thus, when stomata are closed due to water stress and available CO_2 is limited it would imply a concomitant inhibition of respiration and increase PEP availability for DMAPP formation.

In this study soil pH in no way influenced the BVOCs emission rates Soils under plantations typically become more acidic (Morris and Lowery, 1988), the effect usually being ascribed to the uptake of basic cations into the forest biomass (Reuss and Johnson, 1989). Pine needle litter contains acidic organic compounds that are released into the soil during decomposition (Fey, 2001) although the contribution of this process to overall acidification is not clear. Soils sampled from the plantation sites (Pine and Eucalyptus) were more acidic that those sampled from crop sites.

5.4.5 Leaf nitrogen content

Effect of leaf nitrogen content on BVOC emissions is not well understood. Previous work demonstrated both positive (Monson, 1994, 1995; Harley *et al*. 1994; Lerdau *et al*. 1995; Litvak *et al*. 1996) and negative correlations (Harley, 1994; Ekberg *at al*. 2009) between BVOC emissions and leaf nitrogen. Results from this study demonstrated a negative correlation between LNC and emissions for isoprene, α-pinene and linalool. The availability of soil nitrogen, either as nitrate or ammonium will impact leaf nitrogen concentrations. In this study, the leaf nitrogen concentration ranged from 0.95 % to 2.84 %, with Pines having the lowest and Banana the highest concentration. The application of fertilizer to the crop species would have increased the foliar nitrogen concentration and this is particularly evident in Bananas. The effect of leaf nitrogen content on the rate of emissions is complex and similar to the those

factors discussed under the section on PAR, with soil water status and stomatal conductance all playing a role (Shangguan *et al*. 2000).

Leaf nitrogen content is correlated with photosynthetic activity of the plant. Plants that are growing under non light limiting conditions will have large photosynthetic pools (Mooney *et al*. 1981; Field, 1983; Field and Mooney, 1983, 1986; Evans, 1989) together with soluble proteins and enzymes (Schmitt and Edwards, 1981; Osmond *et al*. 1982) to drive the process of photosynthesis. This will result in high specific leaf areas and high potential relative growth rates (Hunt and Cornelssen, 1997). The impact on emissions is not clear. The LNC is effected by season and plant phenology, evergreen trees in temperature regions have been shown to have low leaf nitrogen content during the summer, but to increase their nitrogen content from autumn to winter (Givnish, 2002). Similar results were found in this study.

5.5 BVOC Emissions and Land Use

The province of Mpumalanga hosts commercial forestry, subtropical crops, temperate crops and savanna areas. The aim of this study was to provide information on BVOC emissions for a range of species that have not been characterized before in South Africa. In Mpumalanga, the total land area is 8.2 million hectares of which 28.5 % is conserved, 39.7 % is grazed, 21.6 % is arable, 6.3 % is forestry and 3.9 % is classified as other. The findings from this study showed that there are no differences in the isoprene emission rates from crop and plantation species but that the monoterpene emission rates are significantly higher (x2) from plantation species. Therefore, the BVOC emission rates are approximately double from the plantation species than those from the crops. This statement is true when one combines the plantation species and compares it with the combined crop species, however, the variability across the different species, allows for a more complex interpretation. Pines have a distinctly different time of maximum emission (September) and low emissions in February. The maximum rate in September for Pines is similar to the maximum rate for other species in February. The timing of release of the terpenes could have influences on the atmospheric chemistry at these different times of the year. If one was to attempt to

scale these data by plant functional type (crops vs plantations) and land area, some assumptions need to be made. Firstly, the canopy volumes and foliar densities are assumed to be higher in the plantation species and secondly, the emission rates measured are assumed to be similar from all positions in the canopy. The area planted to crops is approximately 30 % more than the area planted to forestry. Based on these two very simplistic assumptions and the associated planted areas it is suggested that plantations rather than the crops may be having the greatest impact on the atmospheric chemistry of the area. This suggestion obviously needs further analysis but it is useful. However, when one compared the findings from this study with the previous work conducted in the savanna areas, much higher emissions rates were measured than in this study and the area under savannas is significantly greater than the area under forestry and crops. Therefore at a provincial scale, emissions from savannas have the greatest impact on atmospheric chemistry. This finding has far reaching consequences for land use change.

CHAPTER 6: CONCLUSIONS AND FUTURE RESEARCH

Data on BVOC emissions from subtropical regions are very limited. Research into BVOC emissions over the years has improved the understanding of the processes leading to the production of BVOCs, the factors controlling these emissions and regional and global estimates. This study improves the knowledge of BVOC emissions in the subtropical part of South Africa and it also raises some points which require further investigation. This section discusses these points and suggests possible future directions for research of BVOC emissions in South Africa. Data from this study of five selected species will contribute towards the database of existing published isoprene and monoterpene emission rates. These data will be useful for building BVOC emission inventories in the Mpumalanga area, as well as for other regions where these plants grow.

The range of emission rates for the crop species measured were 0.03 to 1.02 mg C m^{-2} hr^{-1} for isoprene and 0 to 0.3 mg C m^{-2} $hr^{-1}$for monoterpenes; for the plantation species these numbers were 0.3 to 0.5 mg C m^{-2} hr^{-1} for isoprene and 0.002 to 0.9 mg C m^{-2} $hr^{-1}$for monoterpenes. In summary, plantation species had higher emission rates than crop species, with respect to monoterpenes but this was not true for isoprene emissions. This statement is true when one combines the plantation species and compares it with the combined crop species, however, the variability across the different species, allows for a more complex interpretation. For the monoterpenes, Pines have a distinctly different time of maximum emission (September) and low emissions in February. The maximum rate in September for Pines is similar to the maximum rate for other species in February. The timing of release of the terpenes could have influences on the atmospheric chemistry at these different times of the year. There were seasonal patterns, most strongly linked to the onset of the spring rains and high temperatures. These findings were particularly evident for the plantation species which were not irrigated, however, the responses of the crop species to seasonal rainfall is more complex because of the interaction with the irrigation. The emissions declined

significantly in the winter months. There was some suggestion that there was a negative correlation between leaf nitrogen content and emission rates.

The emission rates obtained in this study needed to be scaled up in order to provide a regional estimate. In order to achieve this, a much more in depth seasonal study needs to be carried out in which the system water balance needs emphasis. Water availability and high temperatures are the two abiotic factors which most markedly influence rates in this region. A regional energy and water balance model, by land use, would be essential for providing a regional emission budget. The region is biologically diverse, the methods developed in this study are rigorous and could be used to carry out a rapid screening of many hundreds of species in order to assess whether one would require a species composition component of the model or whether one could elegantly use a functional plant type approach, Whole canopy analysis and leaf area indices will also be needed.

Land use change is taking place very rapidly in subtropical and semiarid regions of the world. The majority of the published data on BVOC emissions is from temperate, species poor areas. This study has provided some insight into BVOC emissions from a subtropical region which is biologically diverse.

CHAPTER 7: REFERENCES

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