DETERMINATION OF THE INFLUENCE OF VOLATILES EMITTED BY THE SEMIOCHEMICAL LURE, T.V. PHEROLURE® ON THE VOLATILE PROFILE OF A COMMERCIAL TOMATO FIELD

by

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Submitted in accordance with the requirements
for the application in the degree of

Masters of Environmental Science

in the

DEPARTMENT OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

at the

UNIVERSITY OF SOUTH AFRICA

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January 2021

Dedication

To my mother and father who never stopped believing in me and supported me every step of the way.

To my daughter, although small, for being the best motivator.

Declaration

I **Aletta Johanna van Tonder** hereby declare that the dissertation, which I hereby submit for the degree of **Master of Environmental Science** at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other institution. I declare that the dissertation does not contain any written work presented by other persons whether written, pictures, graphs or data or any other information without acknowledging the source.

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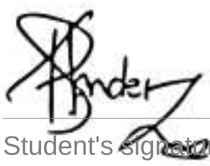

Student's signature

18 February 2021

Date

Acknowledgements

I would like to express my sincere gratitude to the following individuals and organisations for their assistance and support towards the completion of this study:

- I would like to thank God for putting this opportunity on my path and then giving me the strength, determination and support systems to complete it
- My family, for everyone's continuous support and understanding
- Dr Gerhard Nortjé, my study supervisor, and Prof Ben Botha, my co-supervisor, for believing in me and supporting me every step of the way
- Insect Science (Pty) Ltd, my employer and support system. Thank you for your encouragement and support, both financially and emotionally, for the duration of my studies
- Every single person that helped, motivated and supported me throughout my studies
- University of South Africa for funding
- Dr Divan van Greunen and Dr Wilma Augustyn for data interpretation and assistance in data analysis.

Abstract

The use of pheromone-based or semiochemical lures and devices for the detection of insect pest population and monitoring in agriculture is a common practice. In many countries the use of these devices is exempt from registration requirements based on regulatory thresholds set by the relevant authorities, however, not in South Africa. The question arises whether the pheromones or semiochemicals dispensed through such devices, influence the naturally occurring compounds observed and whether a concern of toxicity and ecotoxicity is justified. A tomato field was selected in a commercial growing area of South Africa and a novel five-component lure, T.V. PheroLure®, was identified from a local manufacturer, Insect Science (Pty) Ltd. The T.V. PheroLure® consists of a Volatile Organic Compound (VOC) blend which is placed in a polyethylene bulb. Tomato VOCs were collected before, during and after the application of the T.V. PheroLure® which was used in combination with a yellow bucket funnel trap. The VOCs were collected at different heights (0 cm, 30 cm and 60 cm) of the tomato plants, from planting until harvest (22 weeks) and surrounding tomato fields without the T.V. PheroLure®. The results obtained indicated that: (i) the T.V. PheroLure® had no significant influence on the natural VOCs observed in the tomato field and (ii) that the height of sampling had no influence on VOCs observed. This study also indicated that apart from a slight increased contribution of limonene, there was no significant influence observed from the T.V. PheroLure® compounds on the natural background VOCs found in the tomato field. Therefore, it could be argued that the natural phenology of the plant has a greater influence on the VOCs observed than T.V. PheroLure® and that the concern of toxicity and ecotoxicity is unjustified when using these devices for monitoring purposes only.

Keywords: Tomato, South Africa, volatile organic compound, semiochemical, monitoring, T.V. PheroLure®

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List of Abbreviations

Abbreviation	Designation
ABW	African Bollworm
CRD	Completely Randomised Design
DAFF	Department of Agriculture, Forestry and Fisheries
Df	Error Degrees of Feedom
GC	Gas Chromatography
GHS	Globally Harmonised System of Classification and Labelling Chemicals
GRAS	Generally Recognised as Safe
IPM	Integrated Pest Management
LD_{50}	Lethal dose to 50% of the test population
LII	Legal Information Institute
MS	Mass Spectrometry
MVA	Multivariate Statistics Analysis
NOEL	No Observed Effect Level
OECD	Organisation for Economic Co-operation and Development
OPLS-DA	Orthogonal Projections to Latent Structures-Discriminant Analysis
PCA	Principal Component Analysis
SCLP	Straight Chain Lepidopteran Pheromone
SIMCA	Soft Independent Modelling of Class Analogy
T.V.	Texas Volatile
TD	Thermal Desorber / Desorption
TDU	Thermal Desorption Unit
TSL	Tomato Semi-Looper
USA	United States of America
VOC	Volatile Organic Compound
YBFT	Yellow Bucket Funnel Trap

List of Definitions

Term	Definition
Allelochemical	- mostly so-called 'secondary metabolite' produced by organisms such as plants, animals or microorganisms, and which are not needed for basic (primary) metabolism.
Agricultural remedy	- any chemical substance or biological remedy or any mixture or combination of any substance or remedy intended or offered to be used: • for the destruction, control, repelling, attraction or prevention of any undesired microbe, alga, nematode, fungus, insect, plant, vertebrate, invertebrate or any product thereof, but excluding any chemical substance, biological remedy or other remedy in so far as it is controlled under the Medicines and Related Substances Control Act, 1965 (Act 101 of 1965), or the Hazardous Substances Act, 1973 (Act 15 of 1973); or • as plant growth regulator, defoliant, desiccant or legume inoculant, and • anything else which the Minister has by notice in the Gazette declared an agricultural remedy for the purposes of this Act.
Ecotoxicology	- is the study of the effects of toxic chemicals on biological organisms, especially at the population, community, ecosystem and biosphere levels.
GRAS substances	- substances considered, among qualified experts, to have been adequately proven safe under the conditions of its intended use.
Pheromone	- a chemical released in minute amounts by one organism which is detected and acts as a signal to another member of the same species; examples are the volatile sexual attractants released by some female insects, which can attract males from a distance; some pheromones act as alarm signals.
Semiochemical	- is an organic compound used by insects to convey specific chemical messages that modify behaviour or physiology.
Straight Chain Lepidopteran Pheromone	- a group of pheromones consisting of unbranched aliphatics having a chain of nine to eighteen carbons, containing up to three double bonds, ending in an alcohol, acetate or aldehyde functional group.
Toxicology	- the study of poisons and their effects on living organisms.
Volatile Organic Compound	- VOCs are molecules that contain at least one carbon and one hydrogen atom (i.e., organic compounds) that vaporise easily at room temperature (i.e., volatile).

CHAPTER 1
INTRODUCTION

1.1 General introduction

The use of pheromone-based or semiochemical lures and devices for detection of insect pest population and monitoring in agriculture is a common practice (Witzgall *et al.,* 2010). In order to sell and use these lures for monitoring purposes, it is required by South African law to register these agricultural remedies under Act No. 36 of 1947, of the Department of Agriculture, Forestry and Fisheries (DAFF) (DAFF, 2015a; DAFF 2015b).

The Organisation for Economic Co-operation and Development (OECD) defines semiochemicals as "chemicals emitted by plants, animals, and other organisms - and synthetic analogues of such substances - that evoke a behavioural or physiological response in individuals of the same or other species" (OECD, 2002; 2017), which also includes pheromones and allelochemicals. Allelochemicals are semiochemicals which have an interspecific (between different species) effect, whereas pheromones have an intraspecific (between the same species) effect (OECD, 2003; 2017).

Pheromone-based lures are usually very species specific and mostly affect only the males of the specific species (Witzgall *et al.,* 2010). Other semiochemicals, such as those emitted by botanicals, or Volatile Organic Compounds (VOCs), may be argued to not be as species specific, but has the ability to affect both the males and females of a species. However, the specific mixture and ratios used in a VOC blend can make the blend more pest specific (Frérot *et al.*, 2017). The VOCs can be used separately or in combination with pheromones to aid in monitoring and control of pests to a great advantage in the implementation of Integrated Pest Management (IPM) programmes (Witzgall *et al.,* 2010). However, determining the most effective blend ratios and combinations can be time consuming as not all VOCs have synergistic effects when combined with pheromones. Sometimes the specific blend has been found to be a lot less effective than when VOCs and pheromones are used separately (Booysen, pers. comm., 2018; Hoffmann *et al*., 2020).

Pheromones commonly used in the Agricultural industry for the monitoring and control of insect pests in the order Lepidoptera are classified as Straight Chain Lepidopteran Pheromones (SCLPs) (OECD, 2003). The OECD (2002) defines SCLPs as 'a group of pheromones consisting of unbranched aliphatics having a chain of nine to eighteen carbons, containing up to three double bonds, ending in an alcohol, acetate or aldehyde functional group' (OECD, 2017). These SCLPs are naturally occurring substances produced by insects in the order Lepidoptera, which includes butterflies and moths (OECD, 2003; European Food Safety Authority, 2014). Extensive research has been done on these pheromones and it has been determined that current methods being used does not hold any concern to human or environmental health (OECD, 2003; European Food Safety Authority, 2014).

Semiochemicals used in agricultural devices are generally expected not to exceed naturally occurring concentrations in the environment (OECD, 2003; 2017). These devices are usually seen as products with non-toxic, target-specific modes of action, and active ingredients that occur naturally (OECD, 2003; 2017).

Part of the requirements which is needed for the registration of an agricultural remedy in South Africa, is that toxicity and ecotoxicity studies need to be conducted (DAFF, 2015b), which is a challenge for semiochemical-based agricultural remedy registrations. Most VOCs used in lures are complex and, in most cases, considered to be of no concern to human and environmental health due to the method of application and because they are not found to exceed naturally occurring concentrations (OECD, 2003; 2017).

Several VOCs are categorised as Generally Recognised as Safe (GRAS) substances by the Federal Food, Drug, and Cosmetic Act of the United States of America (USA) (Burdock and Carabin, 2004; Gad and Sullivan, 2014). These substances are considered, among qualified experts, to have been adequately proven safe under the conditions of its intended use. Food substances may be deemed as GRAS through scientific procedures or through experience, based on common use in food before 1958 (Burdock and Carabin, 2004; Gad and Sullivan, 2014). If a VOC has GRAS status, the risk assessment data generated through the classification process can be used to aid in the registration process. Alternatively, the expert judgement used to exempt certain VOCs from toxicological and ecotoxicological requirements can be used as motivation to exempt the same VOCs from data requirements in the South African registration process (DAFF, 2015b). It is clear from literature that in some cases, the VOC concentrations used in food is far higher than the concentrations used in agricultural remedies such as lures and mating disruption devices (OECD, 2002, 2003, 2017; European Food Safety Authority, 2014; Insect Science, 2018).

Tomatoes (*Solanum lycopersicum*) are one of the most commonly produced crops in the world and South Africa's annual tomato production is estimated around 600 000 tonnes (Post Harvest Innovation, n.d.). African Bollworm (ABW) [*Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)], seen as a major pest on this economically important crop, could be considered one of the world's most destructive crop pests (Ravi *et al.*, 2005; Prinsloo and Uys, 2015; Pinto *et al.*, 2017). This pest is the main pest which is attracted to the Texas Volatile (T.V.) PheroLure®. Tomatoes are also amongst others a main host for the Tomato Semi-Looper (TSL) (*Chrysodeixis acuta* [Walker] [Lepidoptera: Noctuidae]) (Prinsloo and Uys, 2015). Therefore, the decision was made to focus mainly on tomatoes for the purpose of this study.

The T.V. PheroLure®, is a polyethylene bulb containing a blend of five VOCs to attract and monitor both male and female moths of ABW and TSL, which is used in combination with the Yellow Bucket Funnel Trap (YBFT). The T.V. PheroLure® is currently not commercially available in South Africa due to legislation, but it has been used with great success in countries, such as Zambia and Zimbabwe, where monitoring products using VOCs are exempt from registration requirements (ZEMA, 2017; Booysen, pers. comm., 2018). Products available for the monitoring of ABW consists of two sex pheromones namely, (Z)-11-hexadecenal and (Z)-9-hexadecenal, which is known to only attract the male of the species (Chempac, 2020; Fite *et al*., 2020). Therefore, a lure that attracts both the males and females of this species could be used to a great advantage in monitoring and control programmes (Insect Science, 2018).

The T.V. PheroLure®, is a novel product in South Africa and as of yet no research has been done in South Africa other than the research done by the Insect Science in-house research and development programme (Insect Science, 2018). Although the compounds used in the T.V. blend is also known to be found in other crops and plant species, inter alia, kiwifruit, citrus fruits and Darwin's Orchids (MacLeod and Ames, 1990; WHO, 1998; Etschmann *et al.*, 2002; Matich *et al.*, 2003; Adams *et al.*, 2005a; Adams *et al.*, 2005b), specific work on this blend on tomatoes has not been conducted.

1.2 Research problem

South African law requires agricultural remedies to be registered under Act No. 36 of 1947 in order to use and sell these products (DAFF, 2015a; DAFF 2015b). Pheromone- or semiochemical-based lures used for detection and population monitoring of insects in agriculture, are classified as agricultural remedies and are therefore regulated by the Pesticides Act of 1947 (DAFF, 2015b).

Most VOCs used in lures are complex and, in most cases, considered to be of no concern to human and environmental health due to the method of use. The nature of agricultural remedies underwent a drastic metamorphosis since 1947, but despite these innovations, agrochemical companies are still mandated by South African law to follow this outdated procedure. VOCs and pheromones are exempt from toxicological and eco-toxicological requirements or registrations in various countries due to this fact. Nevertheless, South African law still require that toxicological and eco-toxicological studies be generated for the registration of agricultural remedies (DAFF, 2015a).

If this research proves that the VOCs put into the field by agricultural devices do not significantly influence the naturally occurring background VOCs, the throughput of the registration process may be significantly improved. However, it can be argued that until the latter is proven, the toxicological and ecotoxicological requirements set by South African authorities can delay and may even prevent the registration of new devices using VOCs as active ingredients. This delay has significant impacts on the agricultural industry. One of the major consequences' producers may face, is losing their export markets due to the lack of biological products, which prevent them from applying these products without the risk of residues. Globally, industries are moving towards a more environmentally friendly approach and if South Africa does not follow this trend, the potential of losing international markets may be a reality. One of two scenarios are likely to take place (i) producers will lose international markets (ii) producers will start using unregistered agricultural remedies, which is against the South African law. Unfortunately, the latter will most probably be the case (Marais, pers. comm., 2020).

1.3 Motivation of the study

The world is moving ever increasingly towards environmentally friendly approaches when it comes to crop protection products (Booysen, pers. comm., 2019; Vurro *et al.,* 2019). Using semiochemicals for monitoring, suppression and control of insect pests will become even more prevalent in the near future. For South Africa to remain relevant as an export country, it is essential that research concerning semiochemical-based products be conducted where necessary. South Africa currently has stringent regulations concerning the registration of semiochemical-based agricultural remedies. Even more stringent than some other countries, including the European Union and the USA in some regards (DAFF, 2015a; DAFF, 2015b; OECD, 2017; LII, 2004).

The current timeline set for the registration of agricultural remedies, is 418 to 627 working days (DAFF, 2015a), which is between one and a half to three years. If semiochemical-based agricultural remedies are exempt from toxicological and eco-toxicological requirements, the rate of the registration process could increase significantly. Therefore, although the aim of this study is only limited to one specific product and crop, the results of this study could have a significant impact on the South African agricultural industry.

1.4 Aim

The aim of this study was to determine the influence on VOCs in tomato fields when VOCs were introduced with the T.V. PheroLure®, a novel five-component lure, under commercial tomato production conditions. Specifically, to prove whether the influence of plant volatiles released into the field is significant or not. Therefore, if this is proven, one can motivate that there exists no concern for human and environmental health and that T.V. PheroLure® and other similar products should be exempt from toxicological and eco-toxicological requirements for registration as agricultural remedies.

1.5 Objectives

Four objectives were set to achieve the aim of this study:

1. Determine the natural background VOCs present in the tomato field utilising Gas Chromatography (GC) - Mass Spectrometry (MS);
2. Determine the VOCs emitted into the atmosphere by T.V. PheroLure® utilising GC-MS;
3. Compare the difference in VOCs present in the presence of T.V. PheroLure® versus naturally occurring background VOCs released by the tomato plant;
4. Compare the VOCs present in a tomato field before and during harvest.

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CHAPTER 2
LITERATURE REVIEW

2.1 Volatile organic compounds

Plant volatiles or Volatile Organic Compounds (VOCs) play an important role in plant-plant interactions, plant-pest interactions and has a major impact on atmospheric chemistry (Tholl *et al.,* 2006; Baldwin, 2010; Holopainen and Blande, 2012). VOCs can impact plant-pest interactions in various ways, inter alia, attracting or repelling a pest or influencing the behaviour of specific pests (Baldwin, 2010; Witzgall *et al.,* 2010; Holopainen and Blande, 2012; Frérot *et al.*, 2017). Utilised correctly within a semiochemical-based product, VOCs can play a vital role in Integrated Pest Management (IPM) programmes (Baldwin, 2010; Witzgall *et al.,* 2010; Holopainen and Blande, 2012).

The African Bollworm (ABW) (*Helicoverpa armigera*) and Tomato Semi-Looper (TSL) (*Chrysodeixis acuta*) are important known pests on tomatoes (*Solanum lycopersicum*) (Ravi *et al.*, 2005; Prinsloo and Uys, 2015; Insect Science, 2017; Pinto *et al.*, 2017). Internal research done by Insect Science (Pty) Ltd has shown that Texas Volatile (T.V.) attracts both the males and females of the aforementioned pests (Insect Science, 2018). The T.V. PheroLure® contains a blend of plant volatiles that attracts both ABW and TSL for up to 20 weeks (Insect Science, 2017). The blend consists of the following five components (Insect Science, 2018) (Figs. 2.1 to 2.6): (±)-Limonene ((S)-(-)-limonene and (R)-(+)-limonene) (Sigma-Aldrich, 2018a; 2018f; 2018g), methyl 2-methoxybenzoate (Sigma-Aldrich, 2018b), phenethyl alcohol (Sigma-Aldrich, 2018d), methyl salicylate (Sigma-Aldrich, 2018c) and phenylacetaldehyde (Sigma-Aldrich, 2018e). The only research done thus far on this specific T.V. blend, was conducted by Mullan (2003) on cabbages, using a ready-to-use grease formulation for the suppression of Cabbage Looper [*Trichoplusia Ni* (Hübner)] in Canada (Mullan, 2003).

The natural presence of these compounds has been identified in literature for several crops and plants, such as kiwifruit, citrus fruits and Darwin's Orchids (MacLeod and Ames, 1990; Awano *et al.*, 1997; Etschmann *et al.*, 2002; Matich *et al.*, 2003;

Adams *et al.*, 2005a; Adams *et al.*, 2005b). Research done elsewhere on tomatoes, has identified limonene, phenethyl alcohol, phenyl-acetaldehyde and methyl salicylate, four of the five components used in the T.V. PheroLure®, as tomato volatiles (Pyne and Wick, 1965; Dalal *et al.*, 1967; Viani *et al.*, 1969; Buttery *et al.*, 1971; Petró-Turza, 1986; Buttery *et al.,* 1987; Baldwin *et al.*, 2000; Tikunov *et al.*, 2005; Beltran *et al.*, 2006; Mayer *et al.*, 2008). Literature pertaining to the presence of methyl 2-methoxybenzoate specifically as a VOC in tomatoes, is currently lacking.

Researchers have reported more than 400 VOCs in the ripening of the tomato fruit (Wang *et al.,* 2016a; Wang *et al.*, 2018). Of these identified VOCs, it has been found that less than 10% play a significant role in the aromas of the tomatoes (Du *et al.,* 2015; Wang *et al.,* 2016a; Wang *et al.,* 2018). These compounds include, *cis*-3-hexenal, hexanal, 3-methylbutanal, *trans*-2-hexenal, *trans*-2-heptenal, phenyl acetaldehyde, *β*-ionone, 1-penten-3-one, *β*-damascenone, 6-methyl-5-hepten-2-one, *cis*-3-hexenol, phenethyl alcohol, 3-methylbutanol, 1-nitro-2-phenylethane, 2-isobutylthiazole, and methyl salicylate (Du *et al.,* 2015; Wang *et al.,* 2016a; Wang *et al.,* 2018).

2.1.1 Limonene

Limonene concentrations (Figs. 2.1 and 2.2) have been reported to range from 0.4 to 2.5 mg/g dry leaf weight per hour, for different plant species in the Central Valley of California (WHO, 1998). The presence of limonene has also been reported when profiling the scent emissions of Darwin's Orchids, (*Angraecum sesquipedale*) at a relative percentage of about 0.18% (Nielsen and Møller, 2015). Studies have also been done on the VOC emissions of whole fruit, pericarp tissue and greenhouse conditions of tomatoes (Wang *et al.*, 2016b; Catola *et al.*, 2018; Wang *et al.,* 2018). Wang *et al.* (2016b) reported limonene concentrations for two tomato fruit cultivars, red FL 47 at 0.061 mg/L and 0.125 mg/L for the locular gel and pericarp whereas Tasti-Lee® concentrations were reported at 0.049 mg/L and 0.100 mg/L for the locular gel and pericarp. Under greenhouse conditions, limonene emissions of 114.5 nmol/m^2 have been reported for tomato fruits (Catola *et al.*, 2018), whereas traces have also been reported in the fruit and flowers of kiwifruit (*Actinidia arguta*) (Matich *et al.*, 2003).

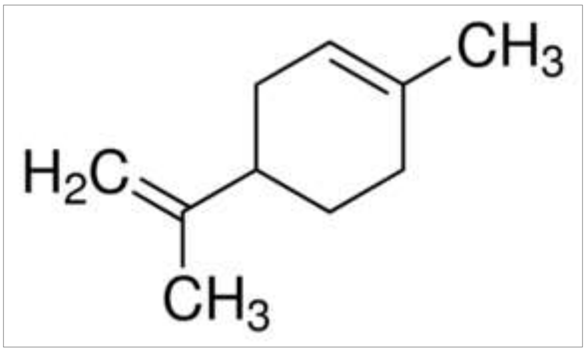

Figure 2.1: Chemical structure of (±)-limonene (Racemic CAS: 138-86-3)

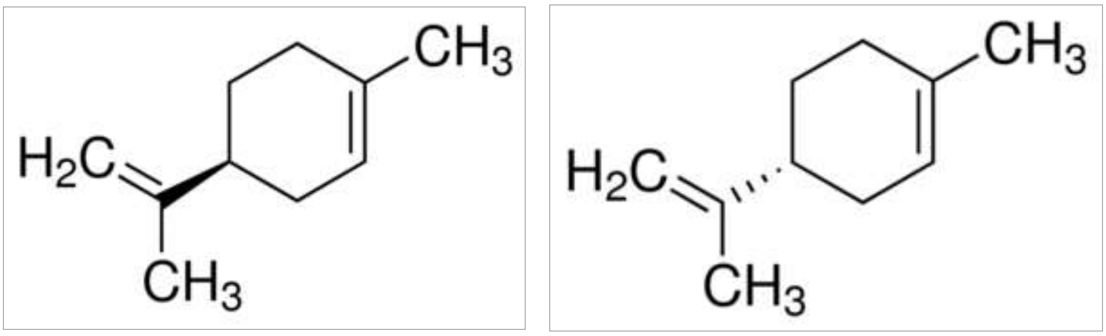

Figure 2.2: Chemical structure of (S)-(-)-limonene (CAS: 5989-54-8) and (R)-(+)-limonene (CAS: 5989-27-5)

Furthermore, Awano *et al*. (1997) identified limonene as a VOC in two Christmas orchid (*Calanthe)* species at concentrations of 0.1% and 0.6% for *C. izu-insu* and *C. sieboldii*, respectively. Similar to phenethyl alcohol, phenylacetaldehyde and methyl salicylate, research identifying limonene as tomato VOC has been conducted elsewhere, but not in South Africa (Buttery *et al.*, 1971; Petró-Turza, 1986; Buttery *et al.,* 1987; Tikunov *et al.*, 2005; Beltran *et al.*, 2006). Buttery *et al.* (1987) reported limonene concentrations of 4 mg/kg for the whole tomato leaf.

Human and environmental health issues associated with limonene mainly include possible skin irritation or sensitisation and acute and chronic aquatic toxicity (ECHA, 2017). However, literature indicated a low risk of acute toxic effects on terrestrial organisms and aquatic life based on the measured environmental concentrations (WHO, 1998). The major human health concern is the effects on the liver and a No Observed Effect Level (NOEL) of 10 mg/kg per day has been established, estimating a tolerable human intake of 0.1 mg/kg per day (WHO, 1998).

2.1.2 Methyl 2-methoxybenzoate

Not much research has been done on the natural presence of methyl 2-methoxybenzoate (Fig. 2.3), although, Adams *et al.* (2005a) reported naturally occurring concentrations of methyl 2-methoxybenzoate in food for the entire United States of America (USA) as 25 kg annually in 2005. Small amounts of about 0.03% have been reported in starfruit (*Auerrhoa carambala L*) from Selangor, Malaysia (MacLeod and Ames, 1990). When investigating volatile components in *Calanthe* species, Awano *et al.* (1997) reported methyl 2-methoxybenzoate as a VOC in *C. sieboldii.* However, research on the specific presence of methyl 2-methoxybenzoate as a VOC in tomatoes is still to be conducted. Lethal dose to 50% of the test population (LD_{50}) oral values for rats and rabbits has been reported as 3 800 mg/kg and >5 000 mg/kg respectively (DrugFuture, 2017). These values are not classified as acutely toxic according to the Globally Harmonised System of Classification and Labelling Chemicals (GHS) (UNECE, 2017) and therefore not considered a concern to human health.

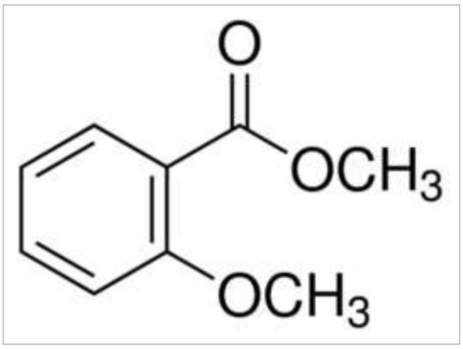

Figure 2.3: Chemical structure of methyl 2-methoxybenzoate (CAS: 606-45-1)

2.1.3 Phenethyl alcohol

Natural phenethyl alcohol (Fig. 2.4) production was estimated at 0,5 ton/year in 2002 (Etschmann *et al.*, 2002) and this amount increased in 2005 when Adams *et al.* (2005b) reported an annual amount of more than 695 tons naturally occurring in food for the USA. This increase could be contributed to a number of factors, inter alia, development of more sensitive detection methods or merely under reported totals in previous years. Similar to methyl 2-methoxybenzoate, phenethyl alcohol is also reported as a VOC in *C. sieboldii* (Awano *et al.*, 1997). Phenethyl alcohol was reported as a VOC in tomato plants (Viani *et al.*, 1969; Buttery *et al.*, 1971; Petró-Turza, 1986; Buttery *et al.,* 1987; Baldwin *et al.*, 2000; Tikunov *et al.*, 2005; Beltran *et al.*, 2006; Mayer *et al.*, 2008; Wang *et al.,* 2018). Baldwin *et al.* (2000) determined the phenethyl alcohol concentration in fresh tomatoes as 1 900 nL/L whereas Mayer *et al.* (2008) reported a range of 0.39 – 2.3 mg/kg for five different fresh tomato cultivars. Viani *et al.* (1969) reported 14% VOCs of fresh tomatoes to be phenethyl alcohol whereas Buttery *et al.* (1987) determined 0.04 mg/kg per whole tomato leaf to be phenethyl alcohol. Current data available for tomato VOCs, have been collected in other countries, whereas specific data pertaining to phenethyl alcohol concentrations in tomato fields in South Africa still needs to be collected. Phenethyl alcohol has been reported to cause skin and eye irritation at 100 mg/24 hours and 12g/10 minutes, respectively (DrugFuture, 2014). LD_{50} oral values have been reported as 1 790 mg/kg in rats and 2 540 mg/kg in mice (DrugFuture, 2014) which gives it an Acute Oral Category 4 GHS classification: harmful if swallowed (UNECE, 2017).

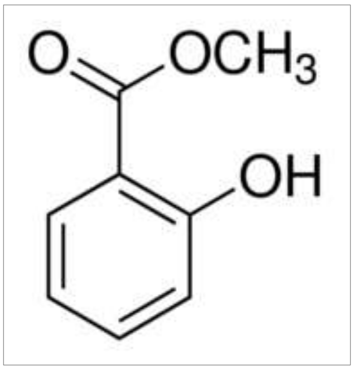

Figure 2.4: Chemical structure of phenethyl alcohol (CAS: 60-12-8)

2.1.4 Methyl salicylate

The amount of methyl salicylate (Fig. 2.5) occurring naturally in food for the USA has been reported as more than 2 tons annually (Adams *et al.*, 2005a). Awano *et al.* (1997) also identified methyl salicylate as a VOC in *C. sieboldii* similar to methyl 2-methoxybenzoate. As discussed previously, except for methyl 2-methoxybenzoate, methyl salicylate was reported as a VOC of tomato plants in research done elsewhere (Pyne and Wick, 1965; Dalal *et al.*, 1967; Viani *et al.*, 1969; Buttery *et al.*, 1971; Petró-Turza, 1986; Buttery *et al.,* 1987; Baldwin *et al.*, 2000; Tikunov *et al.*, 2005; Beltran *et al.*, 2006; Mayer *et al.*, 2008). Dalal *et al.*

(1967) determined concentrations of 1.1 mg/kg, 0.20 mg/kg and 0.82 mg/kg methyl salicylate for field grown, greenhouse grown and artificially ripened tomatoes, respectively.

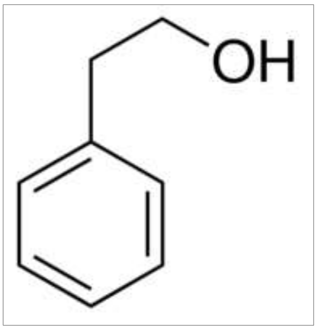

Figure 2.5: Chemical structure of methyl salicylate (CAS: 119-36-8)

Viani *et al.* (1969) determined that 18% of fresh tomato VOCs were methyl salicylate whereas Baldwin *et al.* (2000) stated that fresh tomatoes had a methyl salicylate concentration of 48 nL/L. Buttery *et al.* (1987) determined a methyl salicylate concentration of 0.006 mg/kg per whole tomato leaf. Local data pertaining to methyl salicylate in tomatoes is still lacking. As per limonene and phenethyl alcohol discussed previously, methyl salicylate is also considered a skin and eye irritant (WHO, 1998; DrugFuture, 2014; 2019a). Published oral LD_{50} values are 887 mg/kg for rats, 1 110 mg/kg for mice and 2 100 mg/kg for dogs (DrugFuture, 2019a). The GHS classification is: Acute Oral Category 4: Harmful if swallowed (UNECE, 2017).

2.1.5 Phenylacetaldehyde

Global production of phenylacetaldehyde (Fig. 2.6) has been reported by Adams *et al.* (2005a), as more than 10 tons/year. Research done elsewhere reported phenylacetaldehyde as a VOC in tomato plants (Viani *et al.*, 1969; Buttery *et al.*, 1971; Baldwin *et al.*, 2000; Tikunov *et al.*, 2005; Beltran *et al.*, 2006; Mayer *et al.*, 2008; Du *et al.,* 2015; Wang *et al.,* 2018). Fresh tomato phenylacetaldehyde concentrations were determined to be more or less 15 nL/L by Baldwin *et al.* (2000), whereas Mayer *et al.* (2008) reported a concentration range of 0.11-0.86 mg/kg phenylacetaldehyde for five different fresh tomato cultivars. Between 10% and 14% of fresh tomato VOCs were reported to be phenyl acetaldehyde by Viani *et al.* (1969), when using two chromatographic columns, DEG 1 and Apiezon, respectively. Tomato VOC studies, specifically on phenylacetaldehyde in South Africa has not been conducted up to date. Literature provides LD_{50} oral values of 1 150 mg/kg for rats, 3 890 mg/kg for mice and guinea pigs (DrugFuture, 2019b), therefore, phenylacetaldehyde is classified as an Acute oral Category 4 similar to methyl salicylate and phenethyl alcohol (DrugFuture, 2014, 2019a, 2019b; UNECE, 2017).

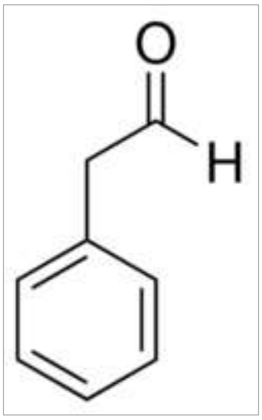

Figure 2.6: Chemical structure of phenylacetaldehyde (CAS: 122-78-1)

2.2 Regulatory aspects

As discussed previously, studies have been done on the VOC content of tomatoes (Pyne and Wick, 1965; Dalal *et al.*, 1967; Viani *et al.*, 1969; Buttery *et al.*, 1971; Petró-Turza, 1986; Buttery *et al.,* 1987; Baldwin *et al.*, 2000; Tikunov *et al.*, 2005; Beltran *et al.*, 2006; Mayer *et al.*, 2008; Du *et al.,* 2015; Wang *et al*., 2016a; Wang *et al.,* 2018), specifically looking at the T.V. PheroLure® compounds. However, in South Africa studies specifically on these VOCs in tomatoes has not been done. Identification of background concentrations for specific VOCs are very difficult to determine and therefore natural background data is usually not available (OECD, 2017). The Organisation for Economic Co-operation and Development (OECD) has therefore developed formulas to estimate the release of semiochemicals and pheromones from a high population of the source (OECD, 2017). These formulas can be used if some information pertaining to the source involved is available (OECD, 2017). For VOCs, this may be more complex as several factors contribute to the emission of VOCs. Factors that influence VOC emissions may include, but are not limited to weather conditions, maintenance to the plant and spray programmes (Botha, pers. comm., 2018; OECD, 2017).

According to South African law, agrochemical companies are required to register all agricultural remedies before any commercial sales can be made (DAFF, 2015a). These remedies include biological products used to attract insects for monitoring purposes (DAFF, 2015b). One of the concerns from the South African DAFF is that the emittance of these semiochemical products will influence the natural background VOC presence. The T.V. PheroLure® consists of five specific components shown in Figs. 2.1 to 2.6. Research is therefore required in order to determine whether this concern from DAFF is justified. Based on the toxicological data available, as well as the GHS classifications for the different VOCs, it may be a good assumption that the T.V. PheroLure® may be of low risk to human and environmental health (WHO, 1998; DrugFuture, 2014, 2017, 2019a, 2019b; ECHA, 2017; UNECE, 2017). However, the influence of these devices on the natural environment will first need to be determined before any valid conclusions can be made.

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CHAPTER 3
METHODOLOGY

3.1 Study area

The study was conducted on a ZZ2® commercial tomato farm, Jachtpad, Mooketsi in the Limpopo province, Republic of South Africa (RSA). Jachtpad is situated in the Mopani District Municipality, within the Greater Letaba Local Municipality, approximately 20 km northwest of Modjadjiskloof (Fig. 3.1). Figure 3.2 indicates the ZZ2® commercial tomato block Rivierland 4B (GPS: 23°32'29.2"S 30°14'13.8"E), Jachtpad, Mooketsi where the trial was conducted.

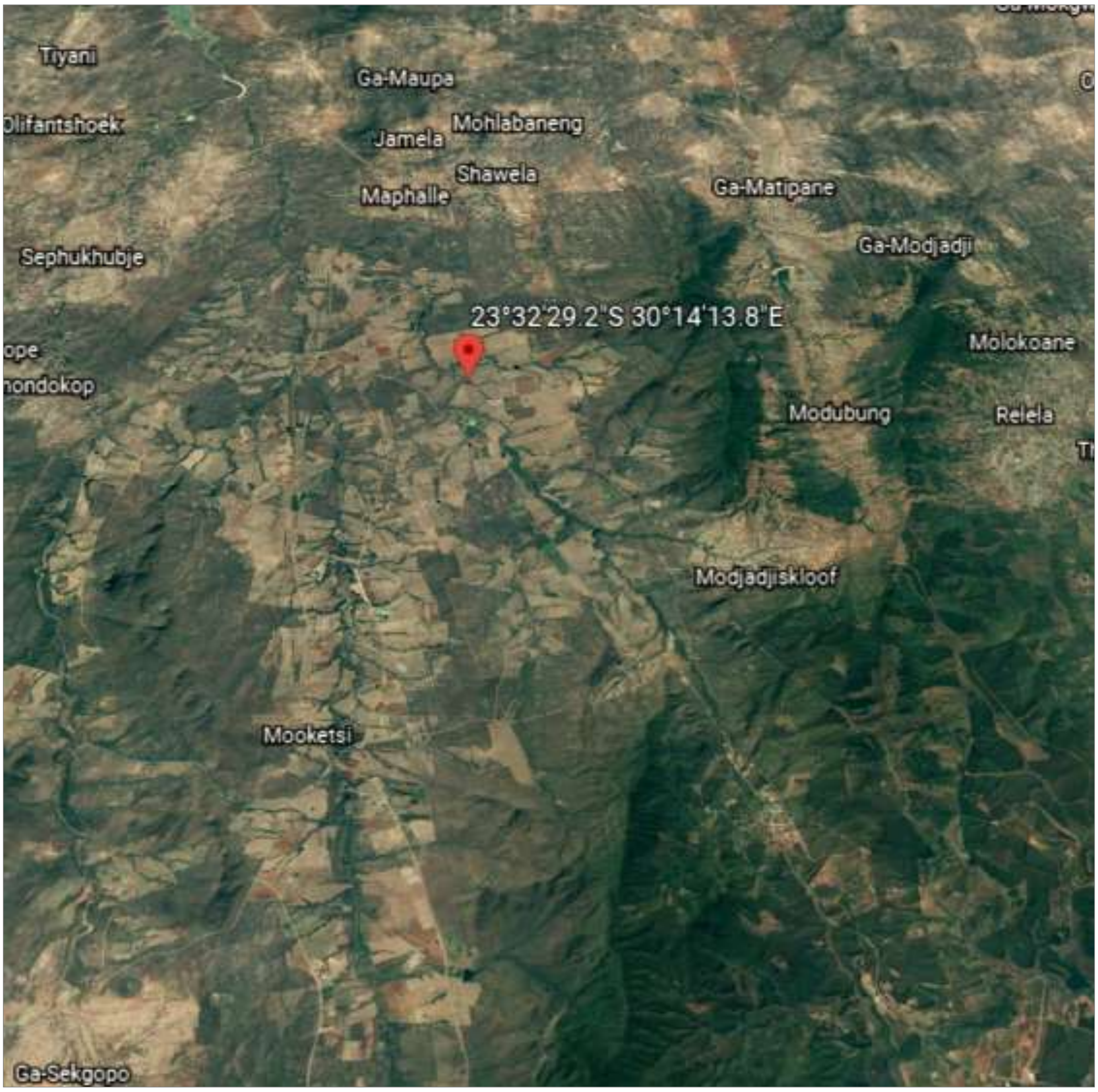

Figure 3.1: Location map of the block Rivierland 4B (Google Earth Pro, 2020)

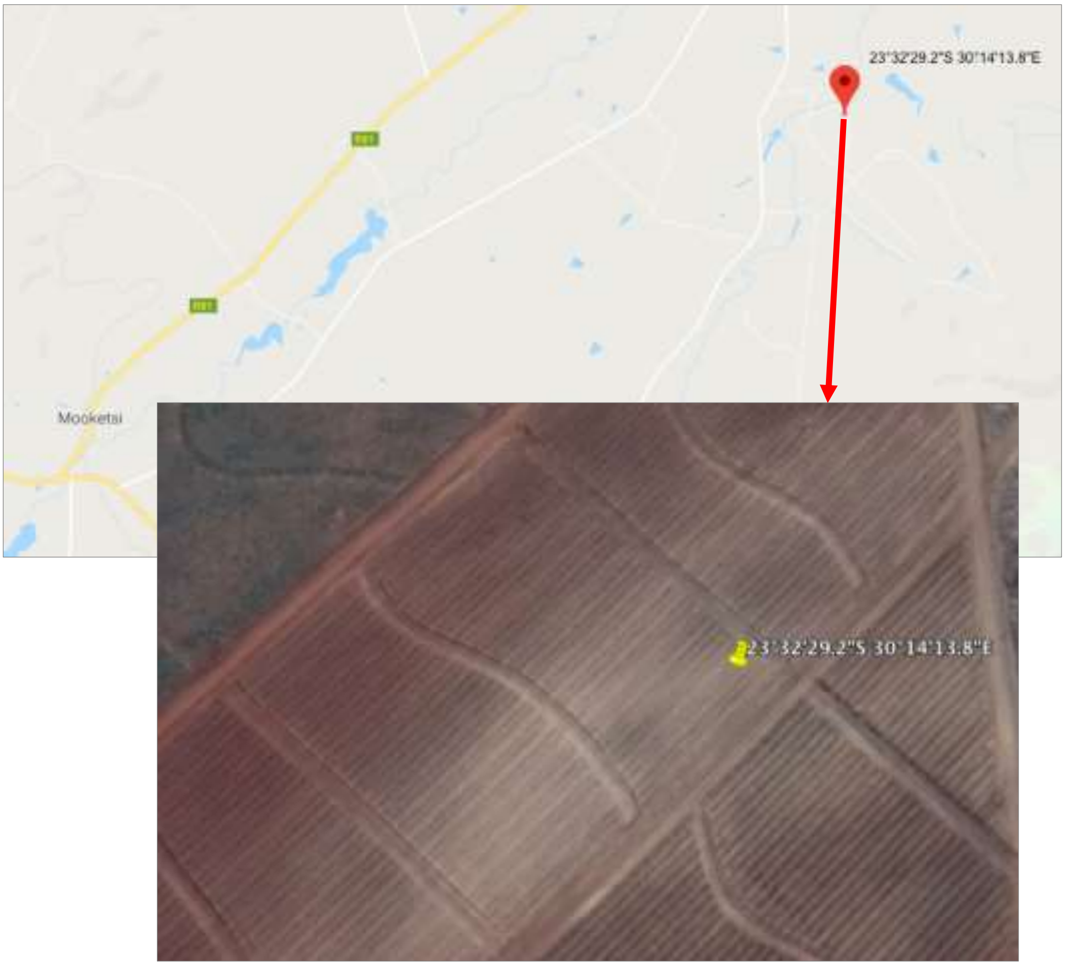

Figure 3.2: ZZ2 tomato farm block Rivierland 4B (Google Maps, 2018)

Mooketsi falls within the subtropical fruit production area of the RSA with a long-term average annual rainfall ranging from 600 to 1000 mm and is situated in a frost-free area. Figures 3.3 and 3.4 indicates the annual averages for the maximum and minimum temperatures, average temperature, average monthly rainfall and average humidity for Jachtpad from 2008 to 2019 (du Toit, pers. comm., 2019). From 2008 to 2019, the average maximum temperature was 29.8 °C and the average minimum temperature was 15.8 °C. The green line indicates the average temperature for each specific year.

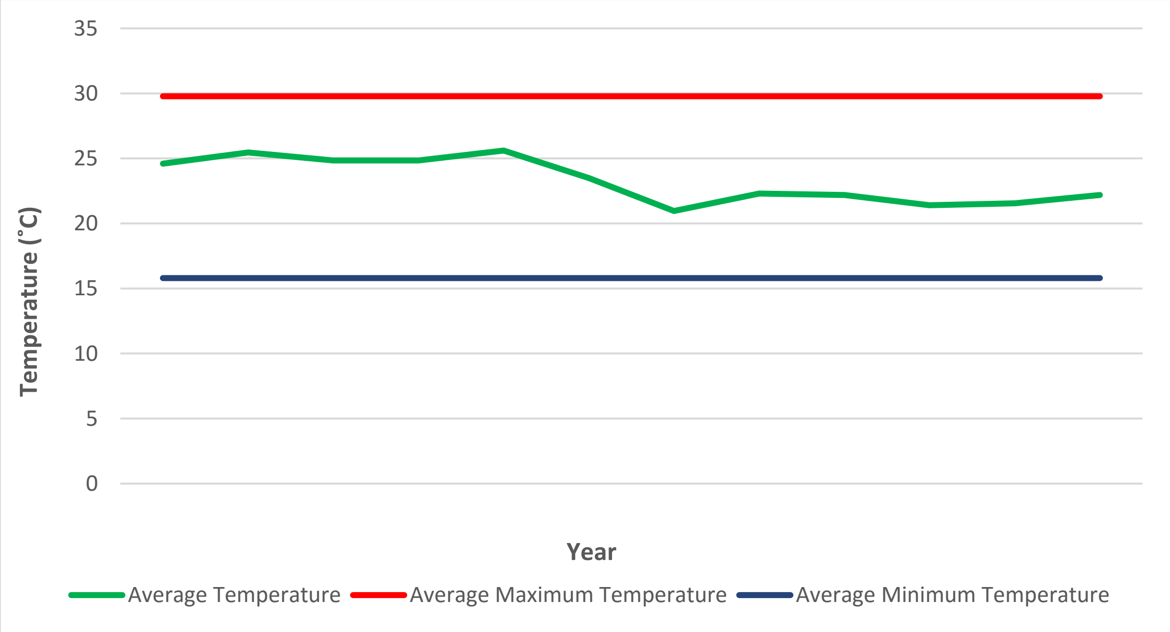

Figure 3.3: Jachtpad average annual temperatures (2008 to 2019)

From 2008-2019, the average monthly rainfall per year (Fig. 3.4) for Jachtpad was 43.2 mm and the annual average humidity for this region was 63.7%. The maximum montly rainfall for Jachtpad was recorded in 2013 with an average of 65.4 mm and the minimum in 2018 with an average of 23.8 mm, whereas the maximum humidity was recorded in 2019 with an average of 72.0% and the minimum in 2012 with an average of 54.9%.

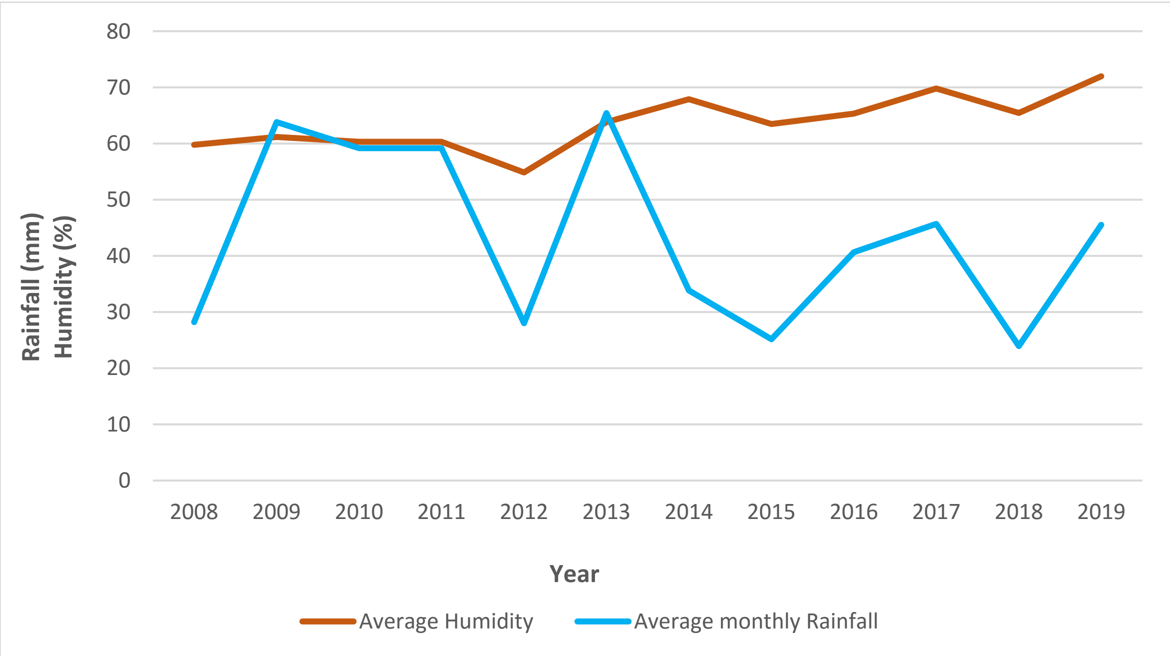

Figure 3.4: Jachtpad average monthly rainfall and average humidity (2008 to 2019)

Major pest and diseases found in commercial tomato fields around the world are listed in Table 3.1 (Ravi *et al.*, 2005; Prinsloo and Uys, 2015; Insect Science, 2017; Pinto *et al.*, 2017; Booysen, 2018) and Table 3.2 (Brits, 2006; Prinsloo and Uys, 2015; Insect Science, 2017; Booysen, 2018), diseases commonly associated with tomatoes are either fungal, bacterial or viral diseases (Brits, 2006).

Table 3.1: Major tomato pests

Major tomato pests		
African Bollworm (ABW) (*Helicoverpa armigera* [Hubner] [Lepidoptera: Noctuidae])	Tomato Semi-Looper (TSL) (*Chrysodeixis acuta* [Walker] [Lepidoptera: Noctuidae])	Potato Tuber Moth (*Phthorimaea operculella* [Zeller] [Lepidoptera: Gelechiidae])
Whitefly (*Bemisia tabaci* [Gennadius] [Hemiptera: Aleyrodidae])	Leaf Miner (*Liriomyza operculella* [Zeller] [Diptera: Agromyzidae])	Tomato Leaf Miner (*Tuta absoluta* [Meyrick] [Lepidoptera: Gelechiidae])
Cutworms (*Agrotis spp*. [Lepidoptera: Noctuidae])	Western Flower Thrips (*Frankliniella schultzei* [Trybom] [Thysanoptera: Thripidae])	Aphids (*Macrosiphum euphorbiae* [Thomas] [Hemiptera: Aphididae])
Stinkbugs (*Nezara viridula* [Linnaeus] [Hemiptera: Pentatomidae])	Leafhopper (*Circulifer tenellus* [Baker] [Homoptera: Cicadellidae])	Red Spider Mites (*Tetranychus evansi* [Baker and Pritchard] [Acari: Tetranychidae])

Table 3.2: Diseases associated with tomatoes

Diseases associated with tomatoes		
Bacterial Speck (*Pseudomonas syringae*)	Early Blight (*Alternaria solani*)	Late Blight (*Phytophthora infestans*)
Gray Mould (*Botrytis cinerea*)	Powdery Mildew (*Leveillula taurica*)	Tomato Yellow Leaf Curl virus transmitted by Whitefly
Tomato Spotted Wilt Virus transmitted by Thrips	Tomato Mosaic Virus transmitted by Thrips	Mosaic Virus diseases caused by Cucumoviruses (cucumber mosaic virus) Potyvirus (e.g. potato Y virus) transmitted by Aphids.

3.2 Research design and methodology

A qualitative research design was used to achieve the research aim by determining the background Volatile Organic Compound (VOC) presence in a commercial tomato field and the influence of the VOCs emitted by the T.V. PheroLure® thereon.

Sampling of VOCs in this study has been done alongside with an agricultural remedy registration trial over and above the chemical spray programme used in the field. An agricultural remedy registration trial needs to be done according to the relevant Department of Agriculture, Forestry and Fisheries (DAFF) guidelines (DAFF, 2015a; 2015b). It is required that these trials should have at least four replicates in order to have a meaningful statistical difference, and that the Error Degrees of Freedom (Df) should be twelve or more (DAFF, 2015b). $Df = (t-1)(r-1) \geq 12$ where t, is the number of treatments, and r is the number of replicates. If similar products are already available on the market, it is required that the new agricultural remedy must be compared to the commercially available remedy or remedies (DAFF, 2015a; 2015b). Therefore, the trial in this study consisted of five different treatments (Table 3.3) with six replicates on a one-hectare site (one treatment every 20 meters and every third row) following a Completely Randomised Design (CRD) (Insect Science, 2018). The treatments in each replicate were then rotated on a weekly basis. Pheromone traps are mobile traps and the usual spray applications are not used. Therefore, the traps are rotated weekly to eliminate any possible bias.

This study only focused on compounds of one product, namely, T.V. PheroLure® from Insect Science (Pty) Ltd, in a single crop namely tomatoes (*Solanum lycopersicum*).

Table 3.3: Five different treatments used in the registration trial

Treatment	Pheromone combinations
1	T.V. PheroLure® (2000 mg) - VOC lure
2	ABW PheroLure® (1 mg) - Pheromone lure
3	ABW PheroLure® with T.V. PheroLure® (1 mg + 2000 mg) - VOC and pheromone lure combined
4	ABW lure (Chempac (Pty) Ltd) (3.5 mg) - Pheromone lure
5	Control - no lure

Within this CRD for the agricultural remedy registration trial, five random sites were selected for sampling the volatile content in the air as shown in Fig. 3.5.

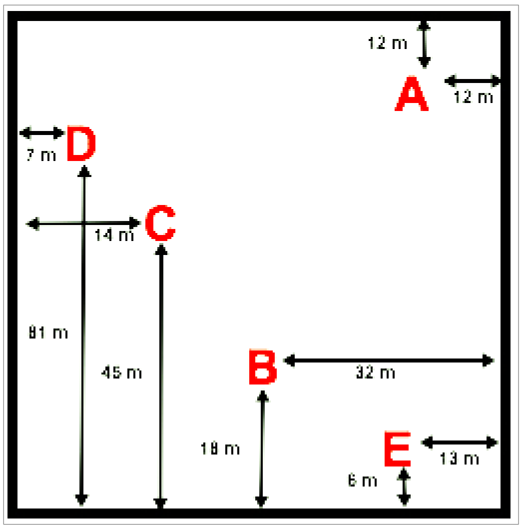

Figure 3.5: Site selection for sampling of volatiles

Figure 3.6 indicates the trial layout as well as the random sampling site selection. The trials commenced after the necessary permissions and ethical clearance (as per Section 3.7) was obtained and after planting was completed during week 34 of 2019.

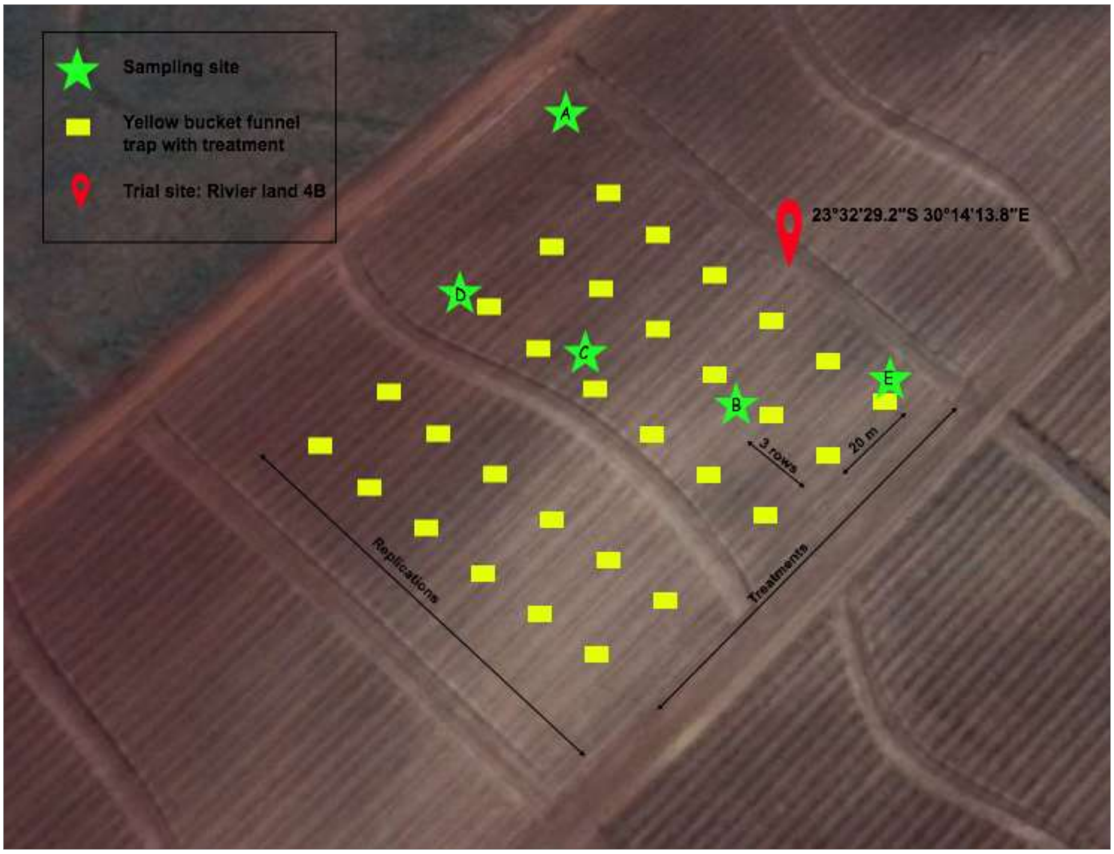

Figure 3.6: Trial layout (Google Maps, 2018)

3.3 Sampling technique

Sampling at the random five sampling points (Fig. 3.6) was done by the adsorption of the compounds on sorbent tubes (Tenax® TA) which was later thermally desorbed, with a Thermal Desorber (TD) and analysed using Gas Chromatography-Mass Spectrometry (GC–MS). Tenax® TA (Fig. 3.7) stainless steel tubes (dimensions 89 mm x 4.5 mm inner diameter x 6.5 mm outer diameter and packed with 197 mg of a sorbent) (Joubert, 2011; Markes International, 2020) were used for the VOC collection. Tenax® TA has a compound range of n-$C_{6/7}$ to ~n-C_{30} with a high volatility range of 100°C up to 450°C, making it thermally stable (Joubert, 2011; Markes International, 2014). Other reasons why the Tenax® TA tube was selected for the analysis was because the tubes are hydrophobic, making it more suitable for very humid conditions and the material can be conditioned to give very low background signals, smaller than 1 ng/compound (Joubert, 2011; Markes International, 2020). The above-mentioned properties allowed for the sampling of volatile and semi-volatile compounds (Joubert, 2011). Tenax® TA tubes were received conditioned and capped from Chemetrix (Pty) Ltd.

Figure 3.7: Tenax® TA tubes

The VOCs were adsorbed on the Tenax® TA tubes by using a BioVOC Breath Sampler (Markes International in South Africa) (Fig. 3.8), which is a kind of syringe (displacement volume of 0,130 litres), hereafter referred to as the syringe. VOCs were adsorbed on the tube when the screw-in plunger was pulled out to fill the syringe. Once the syringe had been filled, the tube was removed, and the screw-in plunger was used to discharge the remaining air in the syringe.

Figure 3.8: BioVOC breath sampler

Sampling was done by adsorbing the air content sampled with the syringe onto stainless steel Tenax® TA tubes. Thermal desorption was then utilised, and the desorbed VOCs were transferred to the GC-MS column for analyses using MSD ChemStation D.03.00.611 software (Agilent Technology) and the NIST05 mass spectral library was used for identification of the compounds.

This analysis was initially expected to be used to determine the natural background VOCs in the tomato field. However, the five specific compounds of T.V. PheroLure® were not detected in the fifteen syringe samples. Therefore, the number of syringes used per sampling was increased to thirty and later ninety syringe samples. Ninety syringes ensured that the specific VOCs could be detected in low levels and that the sorbent material was not saturated, which ensured no chromatographic errors. This was done to determine the natural background VOCs in the tomato field using the TD-GC-MS and the data was used to determine the release of the specific five compounds of the T.V. PheroLure®. This was done at each sampling point of the tomato plants at ground level (0 cm), 30 cm above the ground and 60 cm above the ground. Thus, fifteen samples in total were taken per round of sampling, as well as random samples throughout the trial in the surrounding fields (blank samples).

3.4 Analyses of samples

3.4.1 Thermal desorption

A Thermal Desorption Unit (TDU), feeding into the injection port of the GC, was utilised for the TD of the sampled VOCs retained on the packing material in the sample tube. The TDU was controlled by unity thermal desorption system control software, version 2.0. The TD was pre-purged with helium for 2 minutes after which time the VOCs were desorbed from the tubes at 280 °C for 10 min at a flow rate of 30 mL.min^{-1} splitlessly. Thereafter it was cryo-focused on a general-purpose hydrophobic cold trap ($C_{4/5}$ to $C_{30/32}$) at -10°C, with a split ratio of 4.6:1. The trap was heated to 300°C at a rate of 100°C.s^{-1} and kept at the maximum temperature for 3 minutes. The desorbed volatiles were transferred to the GC column through a heated fused silica line at 190°C.

3.4.2 GC-MS analysis

GC-MS analysis was performed on the 6890 series GC system, equipped with a 5973 MS detector coupled to the TDU. The respective peaks were recorded and integrated using MSD ChemStation D.03.00.611 software (Agilent Technology). Volatilised compounds were separated with a Zebron ZB-5 fused silica capillary column (5% phenyl-dimethylpolysiloxane, 30 m x 0.25 mm inner diameter x 0.25 µm film thickness) (Phenomenex) and detected with the MS detector set at 300°C. Helium was used as the carrier gas and the column flow was measured as 4.2 mL.min^{-1}, at 40°C at a constant pressure of 103,42 kPa. The oven temperature was programmed to start at 40°C and increased to 70°C at a rate of 2.5°C.min^{-1} and was held at this temperature for 5 minutes. A second ramp consisted of an increase to 154°C at a rate of 6 °C.min^{-1}, where it was held for 1 minute, followed by an increase to 250°C at 10°C.min^{-1}, held for 2 minutes. Finally, the oven temperature was increased to 300°C at 10°C.min^{-1} and held for 5 minutes.

3.5 Objectives

Four objectives were set to achieve the aim of this study:

1. Determine the natural background VOCs present in the tomato field utilising Gas Chromatography (GC) - Mass Spectrometry (MS);
2. Determine the VOCs emitted into the atmosphere by T.V. PheroLure® utilising GC-MS;
3. Compare the difference in VOCs present in the presence of T.V. PheroLure® versus naturally occurring background VOCs released by the tomato plant;
4. Compare the VOCs present in a tomato field before and during harvest.

Objective 1: samples of the natural air were taken in the tomato field before any loaded traps were placed in the tomato fields, as well as throughout the trial.

Samples were taken at ground level (0 cm), 30 cm above the ground and finally 60 cm above the ground at the randomly selected sites within the one-hectare block (Figs. 3.5 and 3.6). The aforementioned samples were used as the initial control samples in this experiment. The pre-determined ninety syringe samples were taken per Tenax® TA tube at a sampling site for the initial assessment.

Objective 2: VOC sampling of the selected tomato field with the loaded traps were done following Objective 1. The VOCs were sampled by adsorbing ninety syringes of air from the designated sampling sites on the tomato field onto stainless steel Tenax® TA tubes (Joubert, 2011). Samples were collected in the field by sampling ninety syringes of air at the opening in the T.V. PheroLure® loaded Yellow Bucket Funnel Trap® (YBFT) followed by the thermal desorption of the Tenax® TA tubes after which time the desorbed VOCs was transferred to the GC-MS column for analyses.

This procedure was repeated once a week on the same day for the first seven weeks of the trial. Thereafter monitoring was done each fortnight up to week 15. The final three samples were taken at week 20, week 21 (one week after loaded traps was removed) and week 22 at harvest time, before the tomato fields were reworked. Table 3.4 shows the sampling schedule of the VOCs in the tomato field for the duration of the trial.

Table 3.4: Sampling schedule of the VOCs in the tomato field

Week	Sample	
0	1	control sample: before traps are put in the field
1	2	
2	3	
3	4	
4	5	
5	6	
6	7	
7	8	
9	9	
11	10	
13	11	
15	12	
20	13	
21	14	one week after loaded traps have been removed
22	15	harvest of crop: before land is reworked

Objective 3: following the conclusion of the trial, all data was summarised and statistically analysed in which the VOCs emitted by the tomato plants was compared against the VOCs emitted by the loaded traps over the 20-week lifespan of the T.V. PheroLure®. These results were also compared to the samples taken at week 21 when all the loaded traps were removed from the field.

Objective 4: the final analysis was done to compare the VOCs present before (samples 1 to 10) and during harvest (samples 11 to 15). At sample 15 the tomato fruit had been harvested (see Table 3.4).

3.6 Statistical analysis

The collected VOC data was analysed using Soft Independent Modelling of Class Analogy (SIMCA), a Multivariate Statistics Analysis (MVA). SIMCA was used to perform a Principal Component Analysis (PCA) to determine significant differences between the different data profiles and an Orthogonal Projection to Latent Structures-Discriminant Analysis (OPLS-DA) to determine differences between two specific groups. These analyses are described in more detail in Chapter 4.

3.7 Ethical and other considerations

The necessary ethical clearance (Appendix B) was obtained from the University of South Africa as well as the necessary permission from ZZ2® (Appendix C); an open line of communication was kept with the farm manager for the duration of the trial. TUT gave permission for the use of their chemical laboratory (Appendix D).

Changes in the environment of the tomato field may cause more VOCs to be emitted during certain times. It was therefore essential to have a good line of communication with the relevant stakeholders in order to determine the appropriate sampling time. Changes in the natural environment of the tomato field was recorded and unnecessary changes avoided. It was important to take the chemical spray programme into account when collecting the samples.

Sampling of the VOCs was done in an ethical manner and scientific misconduct did not take place when collecting the samples or processing the data. The tomato fields were left in the same condition that it was found in. Climatic variables such as heavy rainfall or drought or any other adverse events that could influence the VOC emittance were reported in results.

3.8 References

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CHAPTER 4
RESULTS AND DISCUSSION

4.1 General overview

This chapter outlines the results obtained during this study and only focuses on compounds of one product, namely, T.V. PheroLure® from Insect Science (Pty) Ltd, in a single crop namely, tomatoes (*Solanum lycopersicum*).

A total of fifteen sets [five sampling sites (A-E) on the tomato field (Fig. 4.1) with samples at a height of 0 cm, 30 cm and 60 cm of the tomato plant] of samples were collected for the duration of the trial of 22 weeks. More than 250 Volatile Organic Compounds (VOC) were identified when performing a Thermal Desorption (TD) coupled with Gas Chromatography-Mass Spectrometry (TD-GC-MS) analysis using MSD ChemStation D.03.00.611 software (Agilent Technology) and the NIST05 mass spectral library.

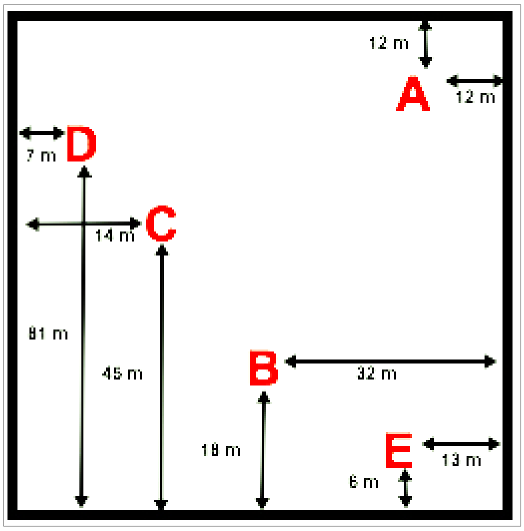

Figure 4.1: Selected sampling sites A to E

Resulting chromatographic data was filtered by eliminating irrelevant compounds based on the following criteria: (i) Identification using the NIST05 library; (ii) with a certainty of less than 60 %; (iii) a retention time (RT) less than 5 min to eliminate volatiles associated with laboratory gasses (< 5 minutes); (iv) chemicals associated with the chemical spray programme the farmer used and; (v) compounds associated with the tar poles used in the tomato fields. The final result of the elimination process concluded that 72 VOCs remained for interpretation and analyses.

The filtered chromatographic data was then exported to a Microsoft Excel spreadsheet and analysed using Soft Independent Modelling of Class Analogy (SIMCA) which is a Multivariate Statistics Analysis (MVA). A Principal Component Analysis (PCA) model was initially constructed from the chromatographic data, to enable the identification of clusters, groups and outliers (Sandasi, *et al.*, 2011). The PCA model displayed a very small variation between collected data (R^2_{cum} = 0.151 and Q^2_{cum} = 0.022 for a two principal component model). The scores scatter plot for the first two principal components (Fig. 4.2) demonstrates that the data points are loosely clustered together with a few outliers identified. This grouping and separation of data points indicate that there are chemical compositional differences between these groups.

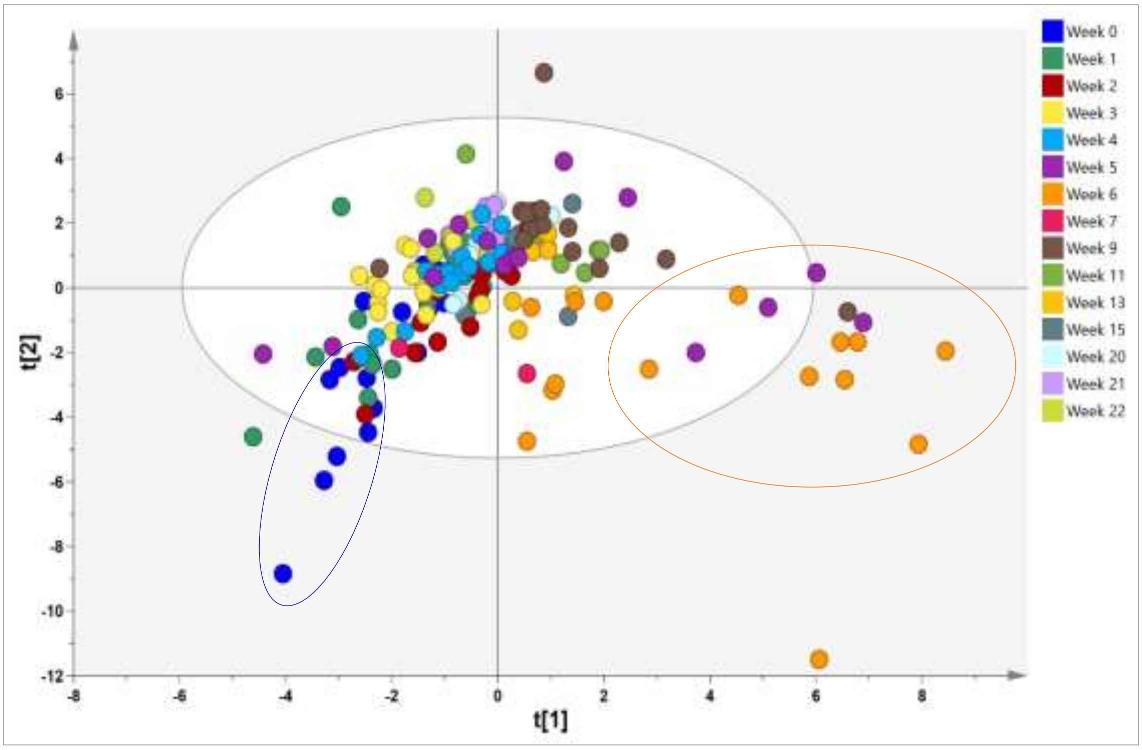

Figure 4.2: PCA scores scatter plot with VOC data collected weekly for the duration of the trial, indicating VOC profile differences observed at week 0 (blue circled) and weeks 5 and 6 (orange circled)

Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) is a regression technique that improves the separation obtained by rotating PCA-components (Wishart, 2008). The OPLS-DA can also be used to identify variables that are responsible for class discrimination (Bylesjö *et al.*, 2006; Mehl *et al.*, 2014).

The PCA scores plot of all filtered chromatographic data obtained indicated that the data clustered together with only a separation occurring at two instances in the tomato field at, Week 0 (blue circled) and weeks 5 and 6 (orange circled) (Fig. 4.2). Possible explanations for these observations include the physiology of the plant and environmental change. These explanations are discussed in detail in Section 4.2.3.

All filtered chromatographic data were separated and divided into two groups, namely, group 1 (all blank samples) and group 2 (remaining data) in order to create an OPLS-DA model. The OPLS scores plot was colour coded according to height of sampling (Fig. 4.3), which indicated that sampling heights did not contribute to separation observed on the plot. There was also no separation between sampling heights of 0 cm (green), 30 cm (red) and 60cm (blue) observed. Similar to Fig. 4.2, the only differences in chromatographic data observed as shown in Fig. 4.3, were during week 0 (blue circled) and weeks 5 and 6 (orange circled), which indicated that the sampling height had no significant influence.

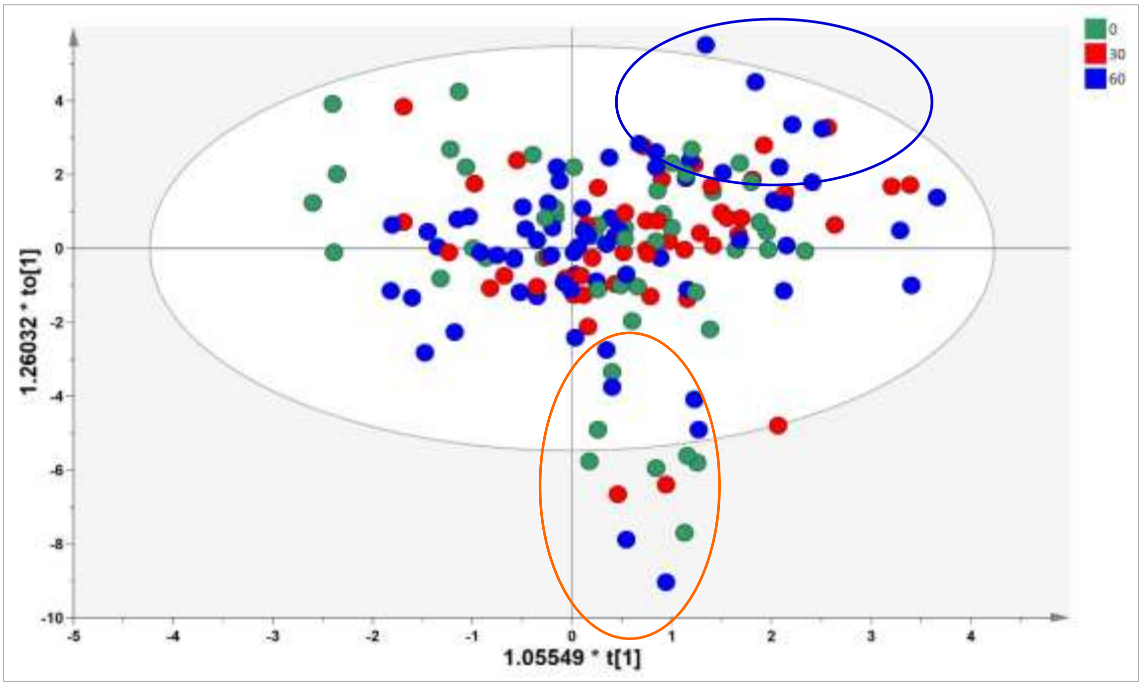

Figure 4.3: OPLS-DA scores plot comparing chromatographic data of VOCs colour coded to present sampling at different heights (0 cm, 30 cm and 60 cm)

4.2 Objectives achieved

4.2.1 Objective 1

Determination of the natural background VOCs present in the tomato field utilising GC-MS. The natural VOCs present in a tomato field were determined by taking samples at Week 0, before traps were put in the field, as well as throughout the trial in the surrounding field (blank samples). Table 4.1 indicates the VOCs identified by the NIST05 library as the natural VOCs present in a commercial tomato field (Rivierland 4B) at a NIST05 library certainty of more than 60 %. The specific T.V. PheroLure® VOCs are marked in bold.

Table 4.1: 72 natural background VOCs identified in the tomato field

72 natural background VOCs identified in the tomato field			
1	alpha-Caryophyllene	37	Heptadecane
2	alpha-Phellandrene	38	Heptadecane, 9-octyl-
3	alpha-Pinene	39	Heptanal
4	beta-Phellandrene	40	Hexacosane
5	(+)-4-Carene	41	Hexadecanal
6	1-Docosene	42	Hexadecane
7	1-Hexadecanol	43	Hexanoic acid
8	1-Hexadecene	44	Hexatriacontane
9	1-Hexanol, 2-ethyl-	45	Isopropyl myristate
10	1-Nonadecanol	**46**	**Limonene**
11	1-Nonadecene	**47**	**Methyl 2-methoxybenzoate**
12	1-Octadecanol	**48**	**Methyl salicylate**
13	1-Octadecene	49	n-Decanoic acid
14	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, l-	50	n-Hexadecanoic acid
15	2-Octanone	51	Nonadecane
16	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-*E*)-	52	Nonanal
17	4,8,12-Tetradecatrienal, 5,9,13-trimethyl- (Farnesyl acetaldehyde)	53	Nonanoic acid
18	5,9-Undecadien-2-one, 6,10-dimethyl-	54	Octacosane
19	Acetophenone	55	Octadecane
20	Benzaldehyde	56	Octadecanoic acid
21	Benzene, 1-methyl-2-(1-methylethyl)-	57	Octanal
22	Benzene, 1-methyl-4-(1-methylethyl)-	58	Oleic acid
23	Benzoic acid, 2-hydroxy-, 3-methylbutyl ester	59	Pentadecanoic acid
24	Benzophenone	**60**	**Phenethyl alcohol**

72 natural background VOCs identified in the tomato field			
25	Butanoic acid, butyl ester	61	Phenol
26	Caryophyllene	**62**	**Phenylacetaldehyde**
27	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	63	Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester
28	Cyclotetradecane	64	Squalene
29	Decanal	65	Tetracosane
30	Docosane	66	Tetradecanal
31	Dotriacontane	67	Tetradecanoic acid
32	*E*-14-Hexadecenal	68	Triacontane
33	Eicosane	69	Tricosane
34	Eucalyptol	70	Tridecane
35	Heneicosane	71	Tritetracontane
36	Heptacosane	72	Undecane

* **Bold** indicates five specific VOC compounds of T.V. PheroLure®

The loadings plots (Fig. 4.4) derived from the OPLS-DA model display the differences in the Y-variables (orthogonal), in this case the separated volatile compounds, in relation to each other. This type of plot identifies compounds with similar information in relation to the X-variables (predictive), sampling dates and sites. The compounds represented in the loadings plot are arranged in ascending order. The compounds displayed on the left side of the plot are associated with week 0 samples and the compounds on the right are associated with blank samples. The red highlighted columns indicate the specific T.V. PheroLure® compounds.

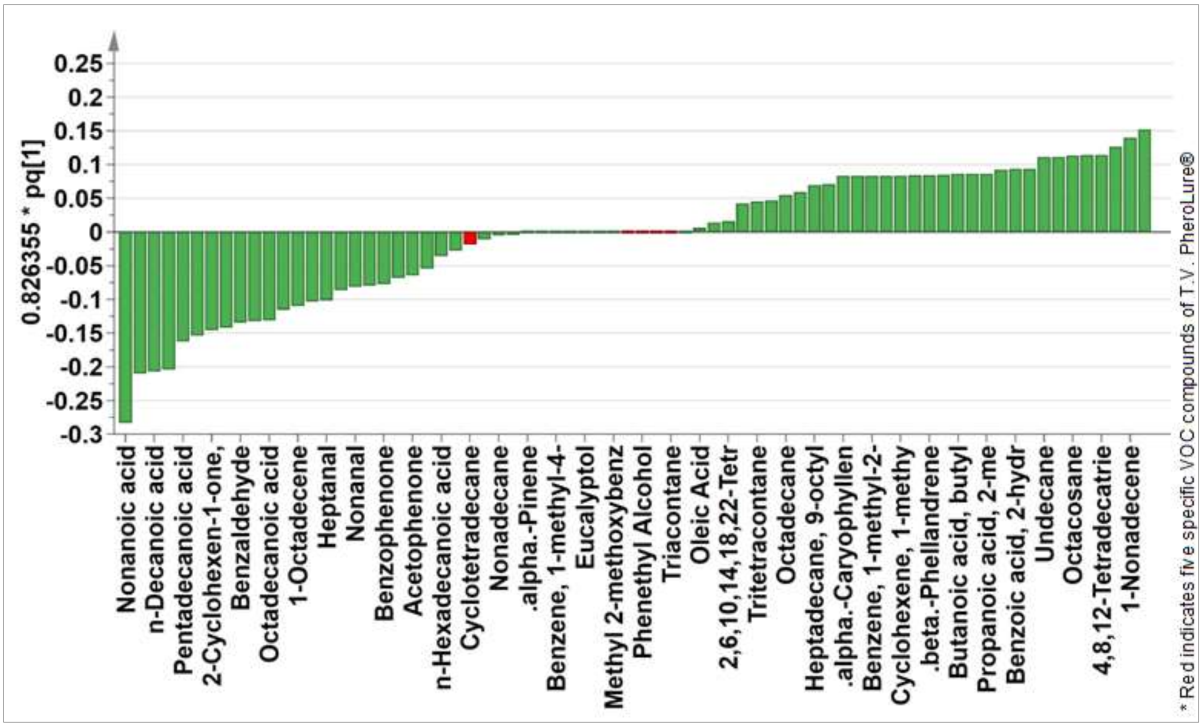

Figure 4.4: OPLS-DA loadings plot for chromatographic data of week 0 compared to blank samples

As shown in Figs. 4.5 and 4.6, the plots indicate that the background VOCs that are associated with week 0 samples include: nonanoic acid, 2-octanone, n-decanoic acid and phenol. The compounds associated with blank samples include: isopropyl myristate, 1-nonadecene, 1-hexadecanol and farnesyl acetaldehyde. Similar results were also reported by two independent research groups (Wang *et al.,* 2016a; Wang *et al*., 2018). From Fig. 4.4 it can be seen that the contribution of these compounds, to the separation based on total volatiles, are very small and negligible.

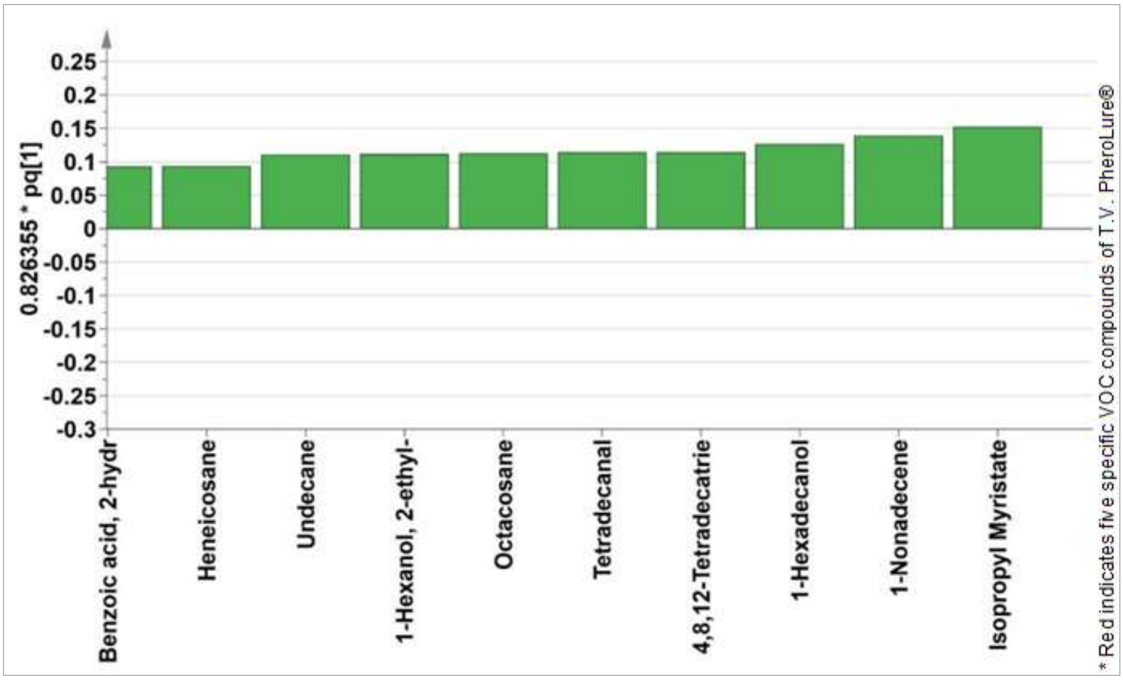

Figure 4.5: Enlargement of ten compounds on left (week 0 variables) of OPLS-DA loadings plot for chromatographic data of Week 0 compared to blank samples

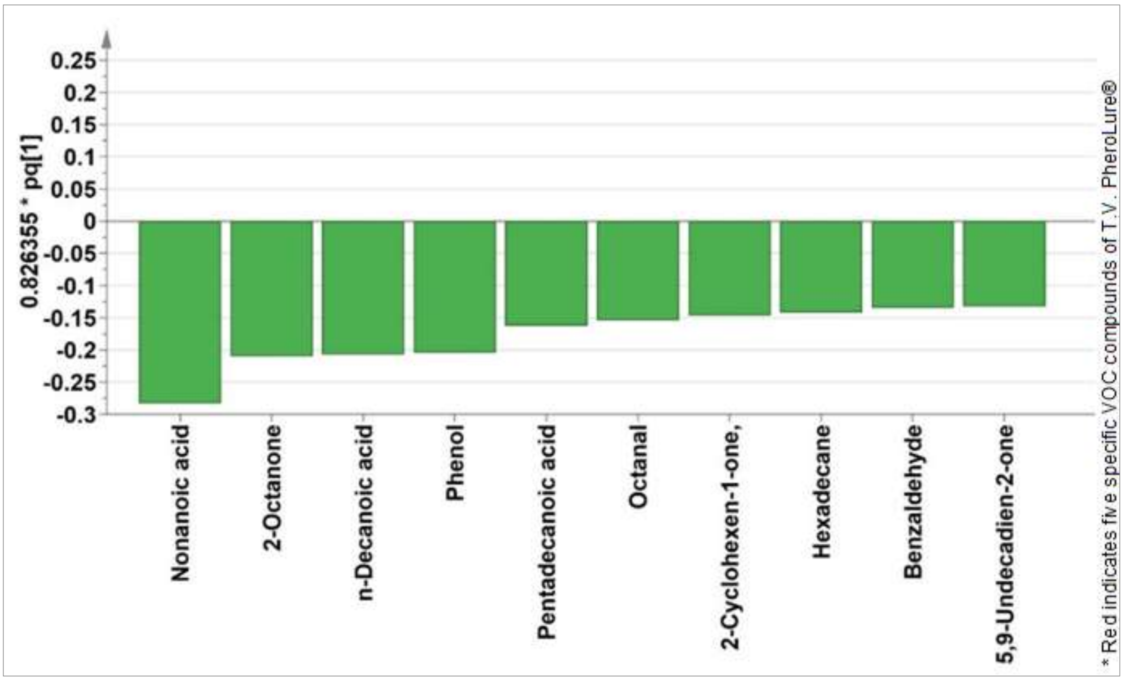

Figure 4.6: Enlargement of ten compounds (blank variables) of OPLS-DA loadings plot for chromatographic data of Week 0 compared to blank samples

4.2.2 Objective 2

Determination of the VOCs emitted into the atmosphere by T.V. PheroLure® utilising GC-MS. T.V. PheroLure® consists of five specific VOCs (limonene, methyl 2-methoxybenzoate, phenethyl alcohol, phenylacetaldehyde and methyl salicylate). When the T.V. PheroLure® loaded Yellow Bucket Funnel Traps® (YBFT) (Insect Science, 2017) were sampled, only methyl salicylate was detected at levels greater than 1% of the total sample volume. Therefore, the VOCs released from the T.V. PheroLure® occurs in such low concentrations that the method used in this study, could not detect them. The T.V. PheroLure® loaded YBFT data was collected in the field by sampling ninety syringes of air at the opening of the loaded YBFT (Fig. 4.7). The VOCs detected from the selected sampling sites (A to E, Fig. 4.1), corresponded with the natural background VOCs reported in Table 4.1, for which similar results were also reported in other studies (Wang *et al.,* 2016a; Wang *et al.,* 2018).

Figure 4.7: Yellow bucket funnel trap® loaded with T.V. PheroLure®

The VOCs detected and identified with the NIST05 library (certainty of > 60 %) at levels greater than 1% of total sample volume when sampling T.V. PheroLure® loaded YBFT are shown in Table 4.2.

Table 4.2: VOCs detected in T.V. PheroLure®

VOCs detected in T.V. PheroLure®	
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-*E*)-	
Decanal	Nonanoic acid
Isopropyl myristate	Octadecanoic acid
Methyl salicylate	Octanal
Hexadecanoic acid	Tetradecanoic acid
Nonanal	

* **Bold** indicates specific VOC compound of T.V. PheroLure®

4.2.3 Objective 3

Comparing of the differences in VOCs present in the presence of T.V. PheroLure® compared to naturally occurring background VOCs released by the tomato plants. Sampling was done at the five selected sampling sites (A to E, Fig. 4.1) for the duration of the trial (22 weeks) at the three different heights as described in sections 3.3 and 3.4. The resulting chromatographic data obtained was grouped into two groups: Group 1 (samples without T.V. PheroLure®) and Group 2 (samples with T.V. PheroLure®). An OPLS-DA model was created, but unfortunately a poor model was obtained ($R^2Y = 0.238$ and $Q^2 = -0.231$) with no separation observed between the groups, indicating that the two groups had similar compounds at comparable concentration levels present as shown in Fig. 4.8. As previously indicated, the samples taken in week 0 (B) and weeks 5 and 6 (A) (Fig. 4.8) differed from the rest of the samples. In both instances, in week 0 (B) and weeks 5 and 6 (A), one would expect the VOCs present to differ from each other.

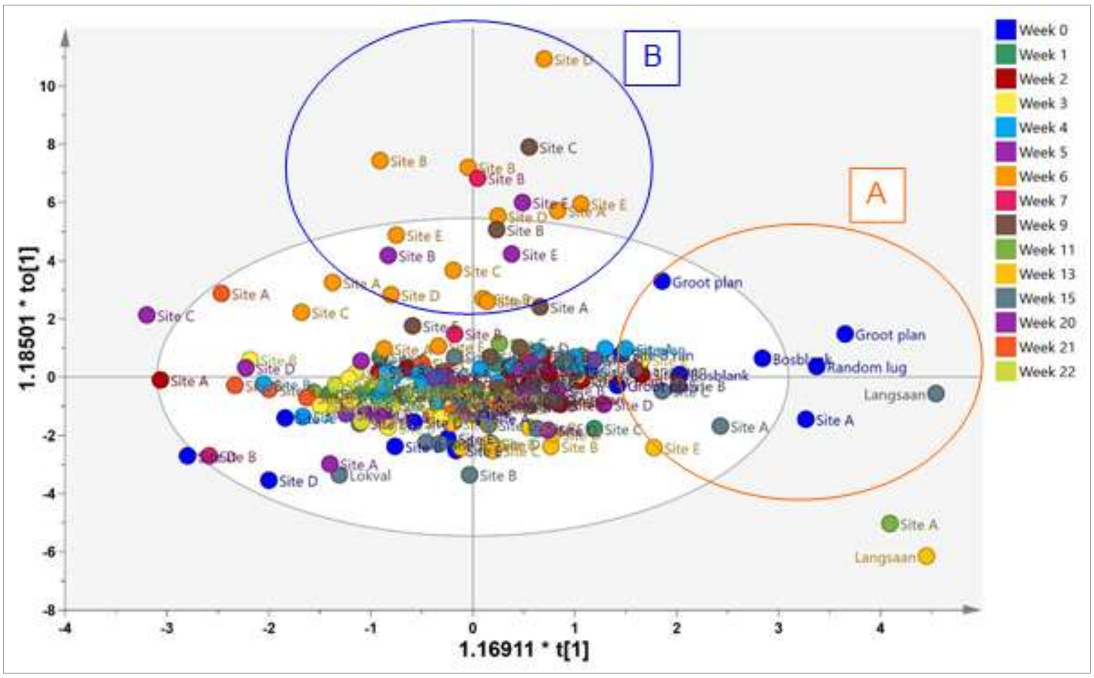

Figure 4.8: OPLS-DA score plot for VOCs present with and without T.V. PheroLure® present; A: Week 0; B: Week 5 and 6

Samples encircled and marked A, on Fig. 4.8 refers to all the samples taken on the very first day of sampling. The tomato plants were very young and planted just the day before, as shown by Fig. 4.9.

Figure 4.9: Tomato plants at Week 0 (section A on Fig. 4.8)

One would therefore, expect that the volume of VOCs associated with the plant be negligible in this phenological stage (Fig. 4.9). Samples circled and labelled B refers to the samples taken during the flowering stage of the plants (Fig. 4.10). This indicates that there is a higher incidence of VOCs present in the natural atmosphere during the flowering stage of the tomato plants possibly due to volatile aromas released by the flowers.

Figure 4.10: Tomato plant during flowering weeks 5 and 6 (section B on Fig. 4.8)

It is expected that the VOCs present in the tomato field during the flowering stage would differ from the natural VOC profile of the plant in the other phenological stages.

4.2.4 Objective 4

Comparison of the VOCs present in a tomato field before and during harvest. Harvesting of the tomato fruit commenced in week 13 (sample 11). The resulting chromatographic data was therefore divided into two groups to create an OPLS-DA model for further analysis. Group 1 (blue: weeks 0 to 11) and Group 2 (green: weeks 13 to 22). Figure 4.11 is the OPLS-DA scores plot, displaying clear separation between the two groups.

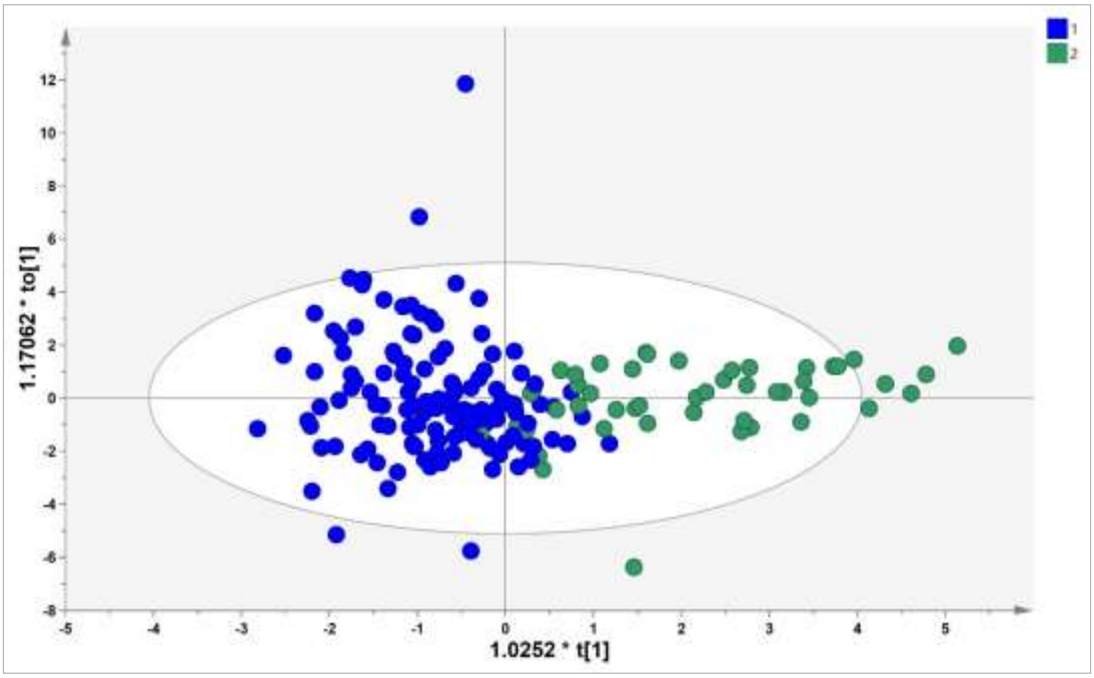

Figure 4.11: OPLS-DA scores plot comparing VOC emitted before (group 1: blue, weeks 0 to 11) and during harvest (group 2: green, weeks 13 to 22)

The OPLS-DA loadings plot (Fig. 4.12) indicates that the compounds decanal; nonanal; 5,9-undecadien-2-one; 6,10-dimethyl-2,6,10,14,18,22-tetracosahexaene, contributed to the separation according to the samples taken before harvest, whereas compounds that contributed to the separation during harvest include, butanoic acid butyl ester, undecane, acetophenone and isopropyl myristate.

The separation observed can be contributed to many factors of which one possibility may be the absence of fruit and flowers on the plants. Other factors which can also influence the VOC emissions are changes in the environment, such as humidity, excessive rainfall or drought, which may lead to increased volatile emissions (Holopainen and Gershenzon, 2010).

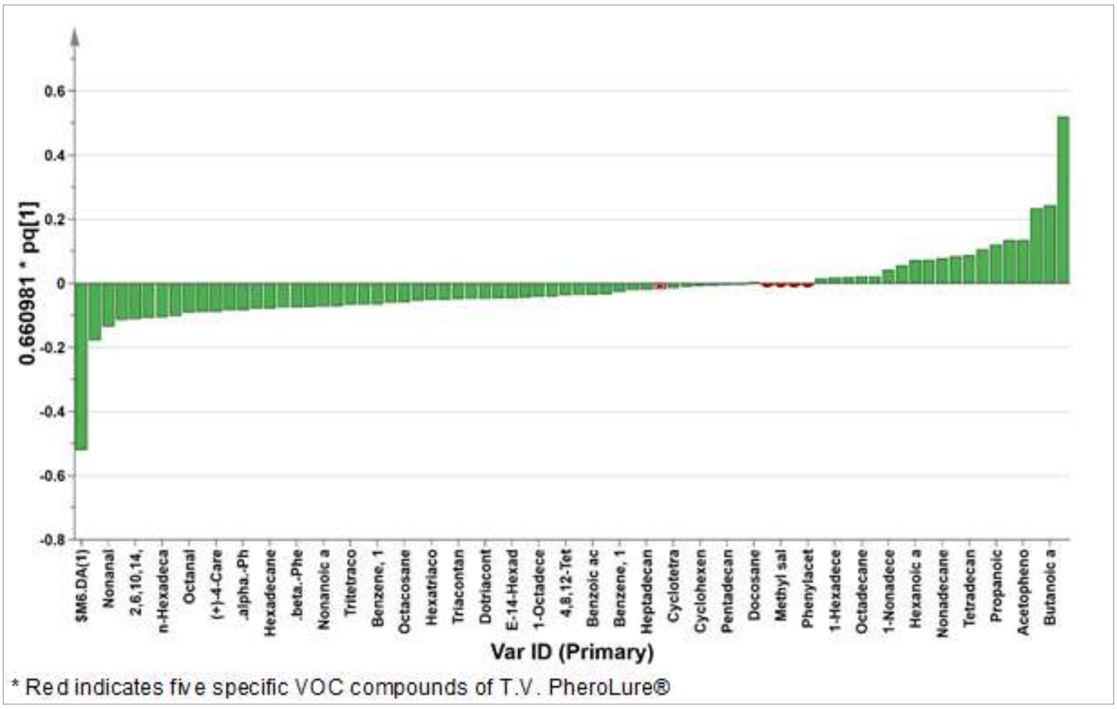

Figure 4.12: OPLS-DA loadings plot indicating the contribution of the variables to separation of VOCs before and during harvest

The results demonstrate that differences in volatile compounds are most prominent in Weeks 0 and 5 and 6 (Fig. 4.2) as well as before and during harvest of the tomato fruit (Fig. 4.11). These changes may be contributed to changes in the physiological stages of the plant or environmental changes rather than the use of semiochemical-based products like T.V. PheroLure®. Furthermore, Fig. 4.3 indicated that the height of sampling had no significant impact on the VOCs observed. VOCs reported in the loadings plots (Figs. 4.4 to 4.6) indicated that apart from a slight increased contribution of limonene, there was no significant influence observed from the specific T.V. PheroLure® compounds on the natural background VOCs found in the tomato field. This is confirmed in Fig. 4.8 indicating differences only for weeks 0 and 5 and 6.

4.3 References

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CHAPTER 5
CONCLUSIONS AND FUTURE RESEARCH

5.1 Dissertation summary

Volatile Organic Compounds (VOC) in a commercial tomato field were sampled to determine the influence semiochemical-based lures and devices such as T.V. PheroLure® could have on the natural background VOCs found in this tomato field. Samples taken in the field were analysed using a Thermal Desorber (TD) coupled to a Gas Chromatography-Mass Spectrometry (TD-GC-MS) system to determine the presence of VOCs present with and without the T.V. PheroLure® loaded Yellow Bucket Funnel Traps® (YBFT). Furthermore, VOCs collected were used to determine the influence height (0 cm, 30 cm and 60 cm) had on the VOCs present in the samples and whether there was a difference in VOC presence before and during harvest.

It was shown in Chapter 4 that apart from a slight increased contribution of limonene, there was no significant influence observed from the T.V. PheroLure® compounds on the natural background VOCs found in the tomato field (Chapter 4; Figs. 4.4 and 4.10). There were differences observed between Week 0 and Week 5 and Week 6 samples (Figs. 4.2 and 4.6) and Fig. 4.3 indicated that the height of sampling had no significant impact on the VOCs observed. The differences in VOC presence observed in weeks 0 and 5 and 6 of sampling could possibly be contributed to the physiology of the plant (plant age and flowering) rather than the use of the T.V. PheroLure®. Furthermore, the changes in VOCs observed before and during harvest may be contributed to the change in the natural environment of the tomato plant.

5.2 Objectives answered

More than 400 VOCs have been identified in the literature pertaining to tomato fruit. In this study 72 VOCs were identified as natural background VOCs in a commercial tomato field. When sampling the VOCs released by the T.V. PheroLure®, only one VOC (methyl salicylate) was detected at a level greater than 1% of the sampling volume. There were no significant differences observed when comparing the VOCs present with and without T.V. PheroLure® in the tomato field. However, differences were observed during weeks 0 and 5 and 6 of sampling. Harvesting started at week 13 and when comparing the VOCs present before (weeks 0 to 11) and during harvest (weeks 13 to 22), with the exception of a slight increased contribution of limonene before harvest there were no significant differences observed pertaining to the specific VOCs found in the T.V. PheroLure®.

5.3 Contribution of the study

In most parts of the world, semiochemical-based lures are considered to be of no environmental or human health concern. In South Africa the regulations are more stringent and the law requires that toxicity and environmental toxicity studies be generated for the registration of agricultural remedies. Results obtained in this study indicated that the influence of semiochemical-based lures on the natural background VOCs is negligible. Therefore, it can be argued that the concern for environmental and human health is unjustified, although further studies should be done to confirm this.

Furthermore, this study can be used to motivate less stringent South African regulations for the governing of semiochemical-based lures to enable fast-tracking of the registration process. This in turn will contribute to a decrease in the use of unregistered products in the agricultural industry as well as moving towards a more sustainable and environmentally friendly approach of pest management.

5.4 Future research

Further research should include:

- VOC determination and quantification in different crops at various crop phenological stages to have a more holistic idea of each crop;
- Methods should be developed and validated to quantify the VOCs detected naturally compared to the VOCs introduced;
- Correlation of VOCs present in different crops and host plants associated with the same pest species;
- Correlation between trap catches and VOC concentrations in an agricultural setting.

APPENDICES

Appendix A: Turnitin digital receipt

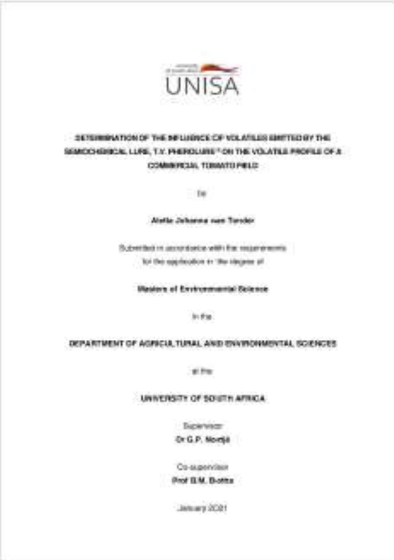
turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Aj Van Tonder
Assignment title:	DES Submissions 2021
Submission title:	MSc Environmental Science disserta..
File name:	Hanna-let_Dissertation.pdf
File size:	1.93M
Page count:	67
Word count:	15,234
Character count:	81,294
Submission date:	16-Feb-2021 03:21PM (UTC+0200)
Submission ID:	1510696527


UNISA

DEPARTMENT OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF SOUTH AFRICA

Supervisor

Co-supervisor

January 2021

Appendix B: Ethical clearance

UNISA university of south africa

CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 08/04/2019

NHREC Registration # : REC-170616-051
REC Reference # : 2019/CAES/083
Name : Ms AJ Rautenbach
Student #: 50823809

Dear Ms Rautenbach

Decision: Ethics Approval from 04/04/2019 to 31/03/2020

Researcher(s): Ms AJ Rautenbach
50823809@mylife.unisa.ac.za

Supervisor (s): Dr GP Nortje
nortjcp@unisa.ac.za; 011-471-2286

Prof EM Botha
bothabm@tut.ac.za; 012-382-6289

Working title of research:
Quantification of natural volatile organic compound (VOC) concentrations versus cocentrations dispensed by the agricultural device, T.V. PheroLure in a commercial tomato field

Qualification: MSc Environmental Science

Thank you for the application for research ethics clearance by the CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for a one-year period, **subject to submission of the relevant permission letter**. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

Due date for progress report: 31 March 2020

Please note the points below for further action:

1. The permission from ZZ2 is outstanding. The researcher is cautioned that field trials may not commence until this has been obtained and submitted to the committee.
2. More detail is required on how data analysis will be done. What is the motivation for the use of ANOVA? How will it be implemented?

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*The **minimal risk application** was **reviewed** by the CAES Health Research Ethics Committee on 04 April 2019 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
2. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
5. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
6. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
7. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

*The reference number **2019/CAES/083** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

URERC 25.04.17 - Decision template (V2) - Approve

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Appendix C: Letter of consent ZZ2

Bertie van Zyl (Edms) Bpk (Pty) Ltd
Reg. No. 65/03114/07

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SOUTH AFRICA / SUID-AFRIKA
☎ (015) 395-2040/9
Fax (015) 395-2042

To Whom It May Concern

This letter confirms that A.J. Rautenbach (50823809) has obtained the necessary permission to conduct the trial work for her research towards a MSc. at the University of South Africa at ZZ2, Mooketsi; Farm Jachtpad.

Project title: Quantification of Natural Volatile Organic Compound (VOC) Concentrations versus Concentrations dispensed by the Agricultural device, T.V. PheroLure® in a commercial tomato field.

I hope you find this in order.

Yours sincerely

Open Rubric

Appendix D: Letter of consent TUT laboratory

Tshwane University of Technology

We empower people

Department of Chemistry
Faculty of Science

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BothaBM@tut.ac.za

18 September 2018

TO WHOM IT MAY CONCERN

I hereby confirm that I am the co-supervisor of Ms Rautenbach and that she may use the laboratory at TUT (Tshwane University of Technology) to analyse the volatile components required for her project. The laboratory is situated at the Arcadia Campus of TUT (175 Nelson Mandela Drive ARCADIA) and it is approved by the university.

Thank you.

BM BOTHA (PROFESSOR)