

The Diversity and Ecology of Mites (Acari) in Vineyards

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ABSTRACT

The common grapevine (*Vitis vinifera* L.) is the main species used for wine making, with South Africa being one of the top wine exporting countries. Grapevine is vulnerable to a range of pests, one of these being mites. Plant-parasitic mites are extremely damaging pests with a rapid generation time, high fecundity and a tendency to over-exploit their hosts. Disconcertingly, the diversity of mites in vineyards in South Africa is virtually unknown. Surveys have been done with predatory mites and phytophagous mites being recorded, but no recent studies focussing on their ecology, pest status and seasonal cycles have been collected. The aim of this study was to survey phytophagous and predatory mite diversity and to investigate pest status of the plant feeding mites of South African grapevine, including the recently introduced, invasive *Brevipalpus lewisi*. Sampling was done over a two-year period and included four conventional farms and one organic farm found in the Winelands region of the south Western Cape, South Africa. Each conventional farm contained a motherblock, nursery and commercial vineyard while the organic vineyard only consisted of a commercial vineyard. At each site vine branches were collected on a regular basis from November 2016 to April 2018. During the winter months weed and cover crop samples were also collected at the conventional farms. Mites were collected from vine leaves with a mite brushing machine. Weeds and cover crops were inspected with a microscope and mites were collected from them with a fine brush. Mites were slide mounted and identified. The predatory mite diversity from plant samples was much higher than expected. *Euseius addoensis* and *Typhlodromus praeacutus* were the most abundant predatory mites found in the commercial vineyards and nursery material with *T. praeacutus* and *Neoseiulus barkeri* the most common in motherblocks. *Brevipalpus* species were the abundant phytophagous mites, with *Tetranychidae* being less abundant. *Brevipalpus lewisi* was the most dominant species. It did not cause any visual symptoms of damage on the vine. *Brevipalpus lewisi* did not seem to have natural enemies that were at sufficient densities to affect any control. The seasonal cycles for the predatory and phytophagous mites were established over a period of two seasons; from November 2016 to May 2017 and from November 2017 to April 2018. In commercial vineyards *E. addoensis* and *T. praeacutus* were the only predatory mites that were present throughout the entire season. The other

predators were present for one or two months. Motherblocks and nurseries had sporadic occurrences of predators. In all three vineyard blocks *B. lewisi* was dominant throughout the seasons. The organic vineyard survey showed a high diversity of predatory mites and an absence of plant-feeding mites. The dominant predators were also *E. addoensis* and *Typhlodromus saevus*. In this study it was found that the main grapevine mites did not migrate to alternate hosts like the cover crops and weeds during winter. Mites that were found on both ground cover and vines were *Tydeus grabouwi* and *Tetranychus ludeni*. The findings of this study forms baseline data to develop management strategies to be used in the wine industry. Understanding the diversity and seasonal cycles of the mites occurring on grapevine will make for better decision making in pest control.

OPSOMMING

Die wingerdplant (*Vitis vinefera* L) is die vernaamste plantspesies wat by wynproduksie in Suid-Afrika, een van die voorste wynuitvoerlande, betrokke is. Die wingerdplant is vatbaar vir 'n reeks plae, waarvan myte 'n belangrike een is. Plantparasitiese myte kan groot skade aanrig weens hul hoë voortplantingstempo, hoë vrugbaarheid en hul geneigdheid om hul gashere uit te buit. Ongelukkig is die diversiteit van myte wat in wingerde voorkom, feitlik onbekend. Voorlopige opnames is al van plantvretende myte en roofmyte gemaak, maar geen navorsing is al oor hul ekologie, plaagstatus en seisoenale siklusse gedoen nie. Die doel van hierdie studie was om 'n opname te maak van die plantvretende en roofmyte, en om die plaagstatus te bepaal van die plantvretende myte wat in Suid-Afrikaanse wingerde voorkom. Die status van *Brevipalpus lewisi* wat onlangs bekendgestel is, is ook ondersoek. Monsters is oor 'n tydperk van twee jaar op vier konvensionele plase en een organiese plaas in die Wynlandstreek van die Suidwes-Kaap in die provinsie Wes-Kaap in Suid-Afrika versamel. Op elke konvensionele plaas was daar 'n moederblok, 'n kwekery en 'n kommersiële wingerd. Die organiese plaas het slegs 'n kommersiële wingerd gehad. Wingertakke is op elk van hierdie plase op 'n gereelde tydperk van November 2016 tot April 2018 versamel. Gedurende die wintermaande is onkruid en dekgewasse op die vier konvensionele plase ook versamel. Myte is met die hulp van 'n mytborselmasjien van wingerdblare versamel. Die onkruid en dekgewasse is met 'n mikroskoop ondersoek en die myte is met 'n fyn kwas verwyder. Al die myte is op skyfies gemonteer en geïdentifiseer. Die diversiteit van roofmyte in die wingerde was hoër as wat verwag is. *Eueseius addoensis* en *Typhlodromus praeacutus* was die volopste roofmyte in die kommersiële wingerde en kwekerye met *T. praeacutus* en *Neoseiulus barkeri* die volopste in die moederblokke. *Brevipalpus*-spesies was die dominante plantvretende myte terwyl *Tetranychidae* skaarser was. *Brevipalpus lewisi* was die vernaamste plantvretende spesies. Hierdie spesies het geen natuurlike vyande nie, en geen fisieke simptome van skade is op die wingerdblare opgemerk nie. Die seisoenale siklusse vir die roofmyte en plantvretende myte was vasgestel oor 'n tydperk van twee seisoene; van November 2016 tot Mei 2017 en van November 2017 tot April 2018. In die kommersiële wingerde was *E. addoensis* and *T. praeacutus* die enigste roofmyte wat gedurende die hele seisoen teenwoordig was. Al die

ander roofmyte was vir slegs een of twee maande teenwoordig. Die roofmyte in die moederblokke en kwekerie het sporadies voorgekom. By al drie wingerdblokke was *B. lewisi* regdeur die seisoen dominant. Die organiese studie het 'n hoë diversiteit roofmyte getoon en 'n afwesigheid van plantvretende myte. Die vernaamste roofmyte was *E. addoensis* en *Typhlodromus saevus*. In die studie is gevind dat wingerdmyte nie na alternatiewe gasheerplante soos onkruid en dekgewasse migreer nie. Die myte wat wel op dekgewase sowel as wingerde aangetref is, was *Tydeus grabouwi* en *Tetranychus ludeni*. Die bevindings van hierdie navorsingstudie vorm die grondslag waarop pesbestuurstrategieë ontwikkel kan word om die wynbedryf en myt-ekologie te bevorder. Danksy begrip van die diversiteit en seisoenale siklusse van myte wat op wingerdblare voorkom, kan beter besluite geneem word vir die bestuur van peste.

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CHAPTER 1

GENERAL INTRODUCTION

Mites belong within the lineage Arthropoda which contains two ancient lineages; Mandibulata and Chelicerata. Mites are the most successful and diverse of the chelicerates (Walter & Proctor, 2013). Mites are extremely small in size, contain mostly four pairs of legs, lack wings and antennae and belong to the class Arachnida. Mites and ticks belong to the sub class Acari. What makes a mite different to other Arachnida, is that its mouthparts are situated as a separate structure at the front of the body called the gnathosoma. The rest of the body is fused to form the idiosoma (Evans, 1992). Thus, it does not consist of a head, thorax and abdomen.

Mites have evolved to feed on plants, fungi and bacteria, to being predators, saprophytes, parasites and symbionts (Krantz, 2009). With this, they have managed to occupy a wider range of habitats than any other arthropod group (Krantz, 2009). Their small body size allows them to easily disperse through air and wind currents, and also to be transported by larger animals, a process called phoresis (Krantz, 2009).

Seeing that mites occur in all habitats, they play an important role in ecology, but they also are a valuable component in human developments such as agriculture. Mites can be beneficial by preying on agricultural and ornamental crop pests (Gerson, et al. 2003). Some have also been established as effective weed control agents (Gerson, et al. 2003). Non-predatory mites are effective nutrient cyclers. Many are also highly detrimental as disease transmitters to plants and animals and serious ornamental and crop pests (Krantz, 2009). These crops include tropical fruit, deciduous fruit, citrus, vegetables, tea, nuts and berries.

1.1. THE WINE INDUSTRY

Grapevine has been growing wild for millions of years before the Greeks and Romans became responsible for the expansion of vines across Europe. Although there may have already been vines growing before the Roman Empire, the establishment of cultivated vineyards is largely credited to the Romans (Iland, et al. 1968). Gradually the culture of vine growing and winemaking progressed to other continents and countries around the world including North and South America, Australia, New Zealand and South Africa (Iland, et al. 1968).

Vitis vinifera L. is the main species used for winemaking, and hybrids are primarily used as rootstocks for *V. vinifera* cions. A season consists of a vegetative cycle and a reproductive cycle. The vegetative cycle entails the growth of the roots, shoots, trunks and arms, while the reproductive cycle involves the start and completion of inflorescence, leading to fruit set, berry formation and ripening. In the Southern Hemisphere a season starts during spring (September – November) until winter (June – August). Harvest takes place during autumn (March – May) (Iland, et al. 1968).

If you put all the cultivated grapevines from all over the world together, it would cover 10.5×10^6 ha (Helle & Sabelis, 1985; Vincent, et al. 2012). A greater variety of clones, rootstocks and developments in vineyard practices have led to an increase in wine quality across most regions of the wine world (Iland, et al. 1968). These improvements lead to each country having their own hybrids, contributing to a successful industry. Different rootstocks may influence the growth of the cion, thus affecting budburst, the length of budburst to harvest and the timing of ripening and picking (Iland, et al. 1968). For this reason, most countries all have their own hybrids that are specially adapted for their surroundings.

Grapevines are vulnerable to many diseases and pests (Vincent, et al. 2012). A rapid shift in climate, can lead to a pest or disease outbreak. Fungal and bacterial diseases thrive under humid conditions, whereas in the arid areas like Mediterranean regions, insects and mites are considered the main threat to grapevines (Helle& Sabelis, 1985).

1.2. MITES IN AGRICULTURE

Mites have a worldwide distribution. They occur in all habitats imaginable, varying from parasites, to vectors, predators or saprophytes and they can cause serious damage to livestock, agricultural crops, ornamental plants and stored products (Smith & Craemer, 1999). In agricultural systems, plant-parasitic mites are extremely damaging pests with a rapid generation time, high fecundity and a tendency to over-exploit their hosts (Walter & Proctor, 2013). Some mites can even transmit diseases to humans (Smith & Craemer, 1999). Although they are known as a pest, many are beneficial to man. Mites occur in close relation to humans and therefore play an important role in their surroundings (Smith & Craemer, 1999). Mites are very interactive and intuitive with their environment, and this makes them strong indicators of disturbance in terrestrial as well as aquatic systems and leading components of biological diversity (Walter & Proctor, 2013).

1.3. MITES ON GRAPEVINE

The greatest threat to grapevine plants are diseases, insects and mites. Phytophagous mites belong to Acariformes and mainly to the order Trombidiformes (Krantz & Walter, 2009). All the phytophagous mites in Prostigmata feed only on fluids (Walter & Proctor, 2013). Predatory Prostigmata have chelate chelicerae which they use to crush their prey to extract their fluids (Walter & Proctor, 2013). The majority of plant feeding Prostigmata have stylet-like mouthparts, ideal for puncturing hostplant (Lindquist, 1998) and sucking out plant fluids.

Patterns at family and genus levels show only a few lineages have made the transition to a life on plants (Walter & Proctor, 2013). The surface of a leaf is a challenging habitat for any small creature, because they are more exposed to the dehydrating effects of wind and the likelihood of being washed away by rain (Walter, 2004). The leaf epidermis was the main evolutionary obstacle mites had to overcome. Most plant-feeding mites puncture the plant cells and suck out the contents. This way the chemical defences of the plant are also avoided (Walter & Proctor, 2013). Plant parasitism has evolved many times into different lineages of mites so that today the majority of

monocotyledons, dicotyledons, coniferophyta and vascular plants are invaded by one or more species of mite (Jeppson, et al., 1975; Helle & Sabelis, 1985a; Helle & Sabelis, 1985b; Lindquist, et al. 1996). What follows is a description of the various mite groups that are relevant to this study, and excludes the superfamilies Eriopyoidea and Tarsonemoidea and the order Sarcoptiformes.

1.3.1. Trombidiformes

The superfamilies and families that form Trombidiformes all have different food preferences. Due to them all eating various food types, the chelicerae are an important identification trait, as it shows great variety (McDaniel, 1979).

Amongst the phytophagous mites, the most important are those belonging to the families Eriophyidae, Tarsonemidae, Tenuipalpidae and Tetranychidae, since they frequently reach a damaging level in vineyards (Klock, et al. 2011). Early detection of specialist and generalist mites are crucial to develop further mite management strategies in vineyards (Klock, et al. 2011).

1.3.1.1. Tetranychidae

Spider mites form part of the superfamily Tetranychoidae that consist of five families, all united by having a pair of elongate, extrusible cheliceral stylets inserted in an eversible stylophore formed from the fused cheliceral bases (Hislop & Jeppson, 1976). The other families are Tenuipalpidae (Flat mites), Tuckerellidae (Peacock mites), Linotetraniidae and Allochaetophoridae.

Spider mites (Tetranychidae) are a phytophagous pest in many crops around the world, including grapevine (*Vitis vinifera* L.) (Smith Meyer & Craemer, 1999; Mani, et al., 2014). Spider mites have been adapted to feed on plant cells. They absorb leaf cell contents and with it decrease the plant's abilities to photosynthesise (Flaherty & Wilson, 1999). Tetranychidae are divided into two subfamilies; Bryobiinae and Tetranychinae (Helle & Sabelis. 1985a). Bryobiinae do not produce

webbing whereas Tetranychinae produce silk and webbing (Helle & Sabelis, 1985a). One thousand two hundred Spider mite species belong to more than 70 genera in the world (Migeon & Dorkeld, 2006).

Tetranychus urticae Koch (Two-spotted spider mite) is a polyphagous spider mite that feeds off parenchyma plant cells. *Tetranychus urticae* have an extensive host range of over 200 host plants. It is a major pest due to being easily adaptable and is problematic in field crops, glasshouse crops, horticultural crops, ornamentals and fruit trees (van den Boom, et al. 2003; Agrawal, 2000; Gribic, et al. 2011; Magalhaes, et al. 2009). *Tetranychus urticae* causes leaf damage that ultimately affects plant growth, vigor and physiology (Pringle, et al. 1986; Walter, et al. 2009).

1.3.1.2. Tenuipalpidae

Mites belonging to the family Tenuipalpidae are called flat mites or false spider mites, because they do not spin webbing. Tenuipalpidae also belong to the superfamily Tetranychoidae. Tenuipalpidae differ from the other families in the superfamily by having a simple palpus that lacks a claw on the penultimate segment (Smith Meyer, 1979). The segmentation on the palpus is often reduced (Smith Meyer, 1979). *Brevipalpus* is the largest genus in the Tenuipalpidae family with more than 280 species worldwide (Hao, et al. 2016). *Brevipalpus* contain many species that are of economic importance. Genera *Brevipalpus*, *Tenuipalpus* and *Dolichotetranychus* are particularly important as plant pests (Hatzinikolis, 1986).

These species occur world-wide and have a wide host plant range (Hatzinikolis, 1986). Most specialised species form plant galls (Walter, et al. 2009). Tenuipalps feed on stems, fruit or leaf surfaces, but tend to occur on the lower leaf surfaces near the midrib and veins (Walter, et al. 2009). Their build is perfectly adapted to lie flat against plant surfaces. Tenuipalps are dorsoventrally flattened. They damage plants by feeding and injecting toxic saliva on bud tissues, the epidermal cells of the stems, leaves and fruits and act as a vector for plant viruses (Hao, et al. 2016; Childers, et al. 2003a). *Brevipalpus obovatus* Donnadieu, *Brevipalpus lewisi* McGregor,

Brevipalpus californicus (Banks) and *Brevipalpus phoenicis* (Geijskes) can easily reach economically damaging levels (Hao, et al. 2016). Species within *Brevipalpus* are considered highly important economic pests within the family Tenuipalpidae and especially *B. californicus*, *B. obovatus* and *B. phoenicis* as all three are vectors of rhabdoviruses (Ochoa, et al. 1994; Childers & Derrick, 2003, Childers, et al. 2003b; Gerson, 2008; Kitajima, et al. 2010; Rodrigues & Childers, 2013; Alberti & Kitajima, 2014).

1.3.1.3. Tydeoidea

The superfamily Tydeoidea has a worldwide distribution and defined by families Triophytydeidae, Ereyneidae, Iolinidae and Tydeidae (Walter, et al. 2009). These families include omnivorous species that feed on pollen, fungi and leaf tissues; predatory species that feed on arthropod eggs, mites, nematodes and specialised hematophagous parasites (Walter, et al. 2009).

Tydeidae is a large family of weakly sclerotized, non-sclerotized and heavily sclerotized striate or reticulate mites (Walter, et al. 2009; Baker, 1965). Tydeidae consist of about 30 genera and 340 known species (Walter, et al. 2009). Tydeids contain predators, fungivores, pollen and plant feeders and scavengers. They occur in soil, moss, straw, leaf litter, bird nests, fungi, stored food products and on plants (Marshall, 1970; Kazmierski, 1998; Baker, 1965). Some tydeids are resistant to desiccation, which allow them to survive in deserts. This contributes to the superfamily being capable of occurring in the arctic tundra and Antarctic maritime (Thor, 1933; Andre, 1980; Usher & Edwards, 1986). Not much is known on how tydeids interact with their environment.

1.3.2. Mesostigmata

1.3.3.1. Phytoseiidae

Phytoseiids belong to the order Mesostigmata. This assemblage contains mites with a large variety of lifestyles and habitats. The majority are free-living predators with the remaining consisting of symbionts of mammals, birds, reptiles or arthropods (Strandmann & Wharton, 1958; Yunker, 1973; Treat, 1975; Walter & Proctor, 1999). Most mesostigmatids possess prominent sclerotized shields on the idiosomatic dorsum and venter which convey their characteristic incremental development, from larval instar up to adult molt (Lindquist, et al. 2009).

Phytoseiids are large, fast and proactive predators feeding mostly on mites but also small insects, nematodes and fungi. Some also feed on plants, pollen and extrafloral exudates. They are divided into three subfamilies; Amblyseiinae, Typhlodrominae and Phytoseiinae (Chant & McMurtry, 1994). Due to the diversity of their feeding patterns and life history traits, phytoseiids can be placed into four groups correlating with the lengths of certain dorsal setae (McMurtry, et al. 2013). Type I contain specialized mite predators with three subdivisions according to prey specificity. Type Ia consist of phytoseiids that have adapted to preying on spider mites that have a more complicated web (CW-U life type of Saito, 1985). It has been shown by Saito (1985) that not only *Tetranychus* species create that web, but also some *Eotetranychus* species, but contain mainly *Phytoseiulus* species. Type Ib contain mite predators of web-nest producing mites (Tetranychidae). These mites have adapted to prey on *Schizotetranychus*, *Stigmaeopsis* and some *Oligonychus* species (McMurtry, et al. 2013). Type Ic are specialized predators of Tyeoidea. These predators consist of *Paraseiulus* and *Typhlodromina* (Duso, pers. Comm with JAM, 2009) and possibly *Proprioseiopsis* species (Momen, 2011). Type II are selective predators of tetranychid mites. These predators are often associated with spider mites that create dense webbing. These species include *Neoseiulus* and *Galendromus* (McMurtry, et al. 2013). Type III contains generalists that feed on mites from Astigmata, Prostigmata and small insects and nematodes. Type IIIa contain generalist predators living on pubescent leaves. Species of *Paraphytoseius*, *Phytoseius* and some *Kampimodromus*, *Typhlodromus* and *Typhlodromus* species are frequent on pubescent leaves. The morphological traits of these mites allow them to colonise microhabitats not occupied by larger phytoseiids, thus avoiding competition and escaping predation from larger phytoseiids (Seelman, et al. 2007). Type IIIb are generalist predators living on glabrous leaves. This subgroup

is likely to be the most diverse. It contains most of the species from Neoseiulus and Amblyseius and some species from Amblydromalus. Type IIIc are generalist predators living in confined spaces on dicotyledonous plants. These mites often prey on eriophyids and spider mites. Type III d are generalist predators living in confined spaces on monocotyledonous plants. Type III e are generalist predators from soil and/or litter habitats (McMurtry, et al. 2013). Type IV contain pollen feeding generalist predators. These are phytoseiids where pollen form an important element of their diet. It includes genera *Euseius*, *Iphiseius* and *Iphiseiodes* (Reis & Alves, 1997); Villanueva & Childers, 2007).

1.3.3. Phytoseiidae as biocontrol agents

Phytoseiids are the best studied group of predatory mites due to their success in controlling mites, whiteflies and thrips (Thysanoptera) (Gerson, et al. 2003). Phytoseiids have been established as an effective biocontrol agent for mites in many crops including vineyards (McMurtry & Croft, 1997; Croft, et al. 1998; Greco, et al. 2005; Escudero & Farragut, 2005; Fraulo & Liburd, 2007). Specialist phytoseiid species assemble in response to pest kairomones and plant volatiles caused by herbivory (Sabelis & Dicke, 1985; McMurty & Croft, 1997). They have the ability to quickly increase their population as a response to the infestations (McMurty & Croft; Croft, et al. 2004). Generalist phytoseiids are considered a more sustainable approach (McMurty, 1992; James & Whitney, 1993), due to specialists' tendency to over-populate and over-exploit the pest abundance, leading to emigration and starvation, thereby contributing to unstable prey-predator dynamics (McMurty, 1992; Nyrop, et al. 1998; Jung & Croft, 2001). Generalists can move to an alternate food source when pests are absent (McMurty, 1992), instead of migrating. However, generalist phytoseiids are susceptible to pesticides (James, 1990). Phytoseiids are also efficient at controlling eriophyids, because they are able to detect them from a distance via the volatiles emitted by infested plants (Dicke, 1988; Dicke, et al. 1988; Aratchige, et al. 2004).

Predatory mites are considered an effective method in limiting mite outbreaks (Sentenac, et al. 1993). Predatory mites are a natural source of control that should be utilised and encouraged.

Pesticides that kill off predators should only be considered as the last resort (Smith Meyer, 1996). Mite pests that are not effectively controlled by their natural enemies, should still allow the predators as a control method by combining them with pesticides (Smith Meyer, 1996).

A major factor that lead to the use of phytoseiids as biocontrol agents in integrated pest management (IPM) and integrated mite control (IMC) programmes, is the spider mites' ability to develop resistance to toxicants (McMurty, 1982; Gribic, et al. 2011).

For the sustainable and efficient control of mites, it is crucial to positively identify each pest species, recognise the damage it causes, know its biology and life history and understand the seasonal occurrence and basic strategy required for its control (Smith Meyer, 1996).

1.4. AIM AND OBJECTIVES

There is ample information about mite taxonomy, but not much is known about their natural history in South Africa. One area that is lacking knowledge is pertaining to mite interactions with their surroundings and each other (intra- and interspecific relationships). Little is known about the natural history of mites in vineyards in South Africa, in particular. South Africa has a successful wine industry, yet the the potential threats and opportunities these minute creatures hold are largely unknown. The aim of this study was to investigate the mite diversity and pest status of phytophagous mites in vineyards in South Africa, with the intention of providing baseline data from which to develop pest management systems, with a focus on biological control potential.

This study included surveys of motherblocks, nursery material and commercial vineyards to determine the mite diversity at each vineyard growth phase. The first stage takes place in motherblocks, where roots are grown. This includes rootstock motherblocks and cion

motherblocks. The roots from the motherblocks are planted as stems in nurseries, which can also be rootstock or cion. The plant material is kept in nurseries where they grow for six to eight months before they are sold or planted for commercial use (Fig. 1.1).



Motherblock



Nursery



Commercial vineyard

Figure 1.1: The three main growth stages of vineyards used for wine production; motherblocks, nurseries and commercial vineyards.

There are three data chapters, following the general introduction:

The focus of Chapter two is to assess the diversity of mites in each vineyard planting. The objectives entailed establishing the predatory and phytophagous mite assemblage structure in nurseries, motherblocks and commercial vineyards, in order to determine potential quarantine risks, identifying potential pest species as well as assessing their pest status to inform IPM programmes. A comparative survey of mites in an organic vineyard was performed to ensure sampling mites that may be more sensitive to rigorous pesticide usage.

Chapter three entailed monitoring the vineyards by collecting samples every two weeks as to determine the seasonal population trends of predatory and phytophagous mites for each vineyard planting. This would benefit management plans as one can determine when would be the best time to implement spray programmes without killing beneficial predators and to target pests during the time before they become abundant.

Chapter four looked at determining the effect of cover crops and weeds growing around and between the vines during the winter months and the mites associated with these plants. The objective was to determine alternate host plants to target for management purposes. If pest species occurring on the vines, utilize weeds as alternate refuges, these may need to be targeted for control to break the cycle.

Chapter five is the description of a new predatory phytoseiid mite that was discovered whilst collecting vine samples for this research study.

Each chapter is written as an individual publication and therefore some repetition may occur.

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CHAPTER 2

THE MITE DIVERSITY IN AN ORGANIC AND COMMERCIAL VINEYARD PLANTINGS AS WELL AS COMPARISON BETWEEN TWO COLLECTION TECHNIQUES

2.1 INTRODUCTION

The diversity of mites in vineyards in South Africa is virtually unknown. Surveys have been conducted with predatory and phytophagous mites being recorded, but none inspecting motherblocks, nurseries and commercial vineyards. There is especially a lack of knowledge regarding the composition of predatory mites in grapevine (de Villiers, et al. 2011). Despite knowing the damaging effects of mites in general, we are not aware of their economic importance in vineyards, especially in South Africa where there is a lack of capacity and published material.

Duso & Vettorazzo (1999) conducted a three-year study monitoring two vineyards in Italy, each containing two grape varieties. The aim was to look at the population dynamics on the different varieties. It was found that at each variety a different phytoseiid species dominated. *Amblyseius andersoni* Chant was persistent in the less pubescent leaf under-surface variety and *Phytoseius finitimus* Ribaga dominated the pubescent leaf under-surface variety. The effect of woody margins and wind on the dispersal rate of phytoseiid mites in vineyards in France was tested by Tixier, et al. (2000) over two years. Samples were collected from the vineyards and surrounding vegetation. During the two years the population density increased, with dispersal being affected by both wind and woody margins. A survey to establish the mite diversity associated with Merlot and Chardonnay cultivars was conducted in Brazil for 11 months (Klock, et al. 2011). By taking 20 monthly samples, these authors collected a total of 11 598 mites belonging to 14 families and 52 species, with Phytoseiidae showing the highest species richness.

It is of importance to look at the diversity, so that we can start having a better understanding of the various ecological processes involving mites, which enable us to manage these processes more effectively. This would mean managing damaging pest mites and protecting beneficial mites, which serve important ecosystem services, such as biological control. There is also an increasing threat of invasive mites occurring in nursery material (Saccaggi, et al. 2017). Thus, it is important to look at all these components, so that informed management plans can be developed.

The retrieval of mites in the field and in the laboratory tend to be a tedious process. There is also uncertainty as to which method should be the preferred method when doing survey sampling. Mites have the tendency to jump off when a plant is handled; so many specimens can be lost in the process. There is a range of mite collection methods that can be used. Direct counting is the most popular method. Leaf samples are collected in the field and directly studied under the microscope and all the mites are counted on each leaf (Smith Meyer, 1996). Other direct methods include sweeping and beating of potential host plants (McDaniel, 1979). One could also wipe mites off the host with a brush (McDaniel, 1979). For the paper-impression method, leaves are pressed between mimeograph paper or a similar type of absorbent paper. The mites leave imprints on the paper to ensure a semi-permanent record and it overcomes mite movement (Smith Meyer, 1996). One must know beforehand what species you are working with, because species identification will not be possible with this method. Using a mite-brushing machine entails passing leaves between two rotating brushes (Smith Meyer, 1996). Mites are brushed off and fall onto a disc bearing paper of a sticky coating marked with a grid. This method allows identification of species, but not all leaf types are suitable for the machine.

Morgan, et al (1955) compared the direct collection method with indirect methods to find the most effective manner of collecting mites. The direct method entails directly counting and removing mites from leaves samples with or without the help of a stereo microscope. The indirect techniques included the paper-impression method, removal with solutions, the mite brushing machine and beating with twigs and foliage. Macmillan & Costello (2015) tested the effectiveness of the mite brushing machine at estimating population densities of *Tetranychus urticae* Koch on grapevine

leaves. It was concluded that the machine gives constant higher counts than visual inspection of leaves. Harris, et al (2017) evaluated the mite brushing machine with Tullgren funnel, the direct method and ethanol washing (the wet method) as to find the best extraction method for *T. urticae* on apple and cherry leaves. The mite brushing machine proved most effective, given the leaf structure. Not all leaf types are compatible with the mite brushing machine.

The aim of this study was to investigate the mite diversity for phytophagous and predatory mites in motherblocks, nurseries and commercial vineyards found on wine farms in the Western Cape Winelands region. Thus, by sampling every phase used in vineyard development, more precise conclusions can be gathered pertaining to potential quarantine risks associated with grapevine plantings. In addition, an organic vineyard survey was included to qualitatively compare the diversity between two commercial vineyard types; one being organic and the other conventional. The majority of surveys take place in vineyards implementing pesticide programmes. Most of the vineyards in the study region are conventionally managed and consequently all of the surveys were done in these vineyards, with the exception of the survey in the organic vineyard.

A survey conducted in an organic vineyard could provide an indication of the natural mite composition in vineyards without the results being influenced by factors relating to the treatments applied in conventional farming. There were no organic motherblocks and nurseries available to include in the survey. The organic vineyard was also used to test the most effective method for collecting mites. The two methods that were compared in this case were a) collection of vine leaves by hand. Thus, cutting vine branches, placing them in a plastic bag and inspecting the leaves in the laboratory and b) collecting vine samples and immediately placing the vine branches in 70% ethanol for at least one minute (ethanol washing/ wet method) and inspecting the mites in ethanol in the laboratory.

Flat mites (Tenuipalpidae), especially the genus *Brevipalpus* is known for spreading viruses in plants. These viruses are collectively known as Brevipalpus-transmitted viruses (BTVs) (Navia, et al. 2013). These viruses can have a detrimental effect on crops. Although *B. lewisi* has not yet been

reported as a vector of BTVs, it can cause direct damage to the host plant and reach pest status (Childers, et al. 2003a). The presence of *Brevipalpus* in vineyards will affect trade, and could lead to quarantine. *Brevipalpus lewisi* is a quarantine pest, regulated in the international exchange or trade of fresh fruits and propagation material of their host plants (Navia, et al. 2006). A survey was done to find out if *Brevipalpus lewisi* McGregor is also present in vineyards in the Limpopo province. Apart from growing citrus, farmers also grow table grapes in Limpopo, as Wellington mostly grow grapes for wine and Limpopo only grow table grapes. Plant material is often exported to Limpopo from Wellington nurseries, therefore this practice was deemed a potential threat.

This study will strengthen the knowledge of mite diversity in vineyards by surveying an organic vineyard and conventional vineyards containing commercial vineyards, motherblocks and nurseries and to ultimately provide baseline data from which to develop pest management systems and assess quarantine risks.

2.2. MATERIALS AND METHODS

2.2.1. Site description

The four conventional farms were all situated in Wellington (33.6405° 19.0097° E), Western Cape province. Each farm in Wellington had a commercial vineyard, motherblock and nursery that was sampled every second week from November 2016 until May 2017 and again from November 2017 until April 2018. The weed and cover crop samples were also collected at these sites from July until October 2017 (Chapter 4) (Fig. 2.1).

One organic farm was sampled once a month in Stellenbosch from November 2017 until April 2018 (Fig 2.1). A survey was done on table grape vineyards in Limpopo. Samples were collected at farms in Globlersdal (25.1674°S, 29.3987°E), Roedtan (24.5973°S, 29.0787°E), Marble Hall (24.9651°S, 29.2815°E) and Mookgophong (24.5165°S, 28.7174°E).

Table 2.1: The five study sites and their coordinates. Sites 1 to 4 had their own commercial vineyard, motherblock and nursery. Site 5 only had one organic vineyard.

SITE	DEGREES SOUTH	DEGREES EAST
1	33.6039	19.0133
2	33.6262	19.0244
3	33.6756	19.0219
4	33.6065	19.0181
5	33.9736	18.7822

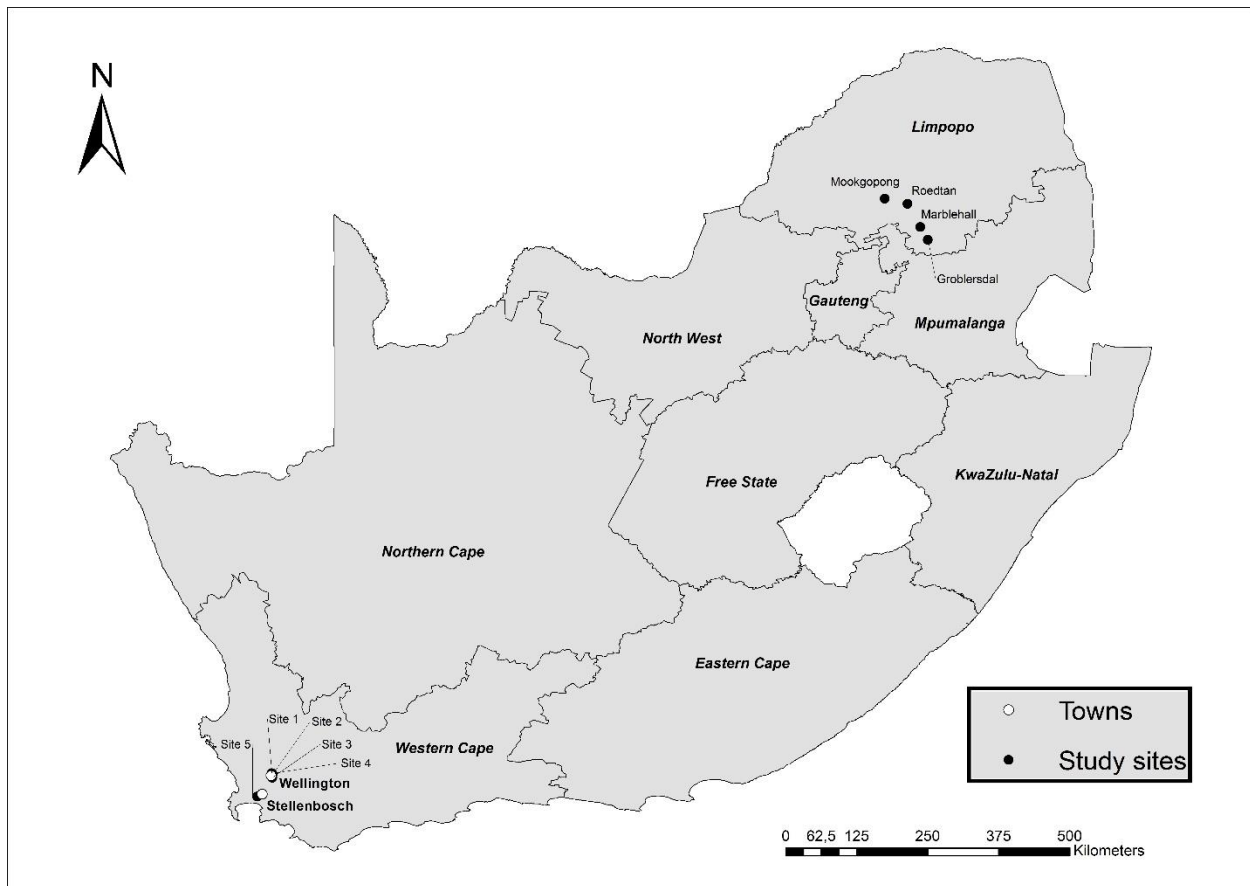


Figure 2.1: Map of the Western Cape indicating the all the study sites. Site 1 - 4 are farms in Wellington each containing a nursery, motherblock and commercial vineyard. Site 5 is situated in Stellenbosch and only contains a commercial vineyard. Field work was conducted at these sites from November 2016 until April 2018. A survey was done in March 2016 in four towns in Limpopo; Mookgopong, Roedtan, Groblersdal and Marble Hall.

2.2.2. Conventional vineyard survey

The fieldwork was conducted in vineyards in the Winelands region of the south Western Cape Province, South Africa. Sampling sites consisted of four conventional wine farms in Wellington each including a commercial vineyard, nursery and a motherblock. Nursery material is planted at the start of the season; October/November and removed at the end of the season, April/May. Motherblock material is pulled out, and replanted approximately every ten years. The commercial vineyards differed in age from 10 to 30 years old. Each farm had their own management approach with a treatment programme. Appendix A contains a summary of the treatments the farmers used on the vineyards during the time of the study; 2016 to 2018. The motherblocks and nurseries were used for cultivating a range of cultivars. Some of the farms also exported their nursery material. The commercial vineyards were an average of 5ha, the motherblocks 3ha and the nurseries 3ha.

2.2.1.2 Experimental Design

In Wellington samples were collected bi-monthly over a two-year period from November 2016 until April 2018. No samples were collected during winter and spring (June 2017 – October 2017). Ten vine branches and sub-branches were collected at each vineyard planting. Damaged vine leaves or leaves displaying odd symptoms were preferred. Vine branches containing vine leaves were cut with sterilised garden shears, wrapped in towelling paper and placed in zip-lock plastic bags. This was done to prevent the leaves from perspiring and wilting which allowed the leaves to stay fresh in the fridge for up to six weeks. A field day consisted of visiting the four farms, each containing a commercial block, nursery and motherblock, sampling ten samples at each block and thus collecting a total of 120 vine samples.

2.2.1.3. Sampling and laboratory work

All the vine samples were processed by running them through a Leedom engineered mite brushing machine (Fig 2.2). The machine has two bristles that comb the leaf on both sides, with the mites falling onto a Perspex plate. The mites were slide mounted with polyvinyl alcohol medium following the guidelines of Krantz & Walter (2009). Mites were identified with a Leica DM 2500 microscope with phase contrast using x1000 magnification with emersion oil. Identification to family level was done with the help of descriptive keys (Lindquist, et al. 2009; Walter, et al. 2009; Zhang, 2003) and identified to species with the guidance of acarologists Prof. E. Ueckermann and Davina Saccaggi.



Figure 2.2: The Leedom engineered mite brushing machine.

2.2.1.4. Data analysis

Rank abundance

The rank abundance plots were calculated for each vineyard planting using the mites that occurred at each vineyard planting. The total count at each site was calculated and divided by the individual

count for each species and used to create a ranking according to their relative abundance (Magurran, 2004). This was also done to calculate the overall abundance across all study sites for predatory and phytophagous mites.

Correspondence analysis

To compare the association between each site and their mite diversity a multiple correspondence analysis was used with the mite species as column variables and the vineyard plantings as supplementary variables. This analysis was conducted using Statistica 13.0 (TIBCO Software Inc., Palo Alto, USA).

General linear models

Where Levene's test for Homogeneity of Variance showed to be significant, indicating abundance data are not normally distributed, General Linear Models were used to illustrate the weighted means of mite occurrence of each vineyard planting. This was calculated using Statistica 13.0 (TIBCO Software Inc., Palo Alto, USA).

Diversity index

By using the total species found at each stage, the Shannon-Wiener and Simpson diversity index was calculated for each vineyard planting, namely motherblock, nursery and commercial vineyard.

2.2.3. Organic vineyard survey

Fieldwork was conducted on a commercial organic vineyard in Stellenbosch (33.9736° S, 18.7822° E). Samples were collected monthly from an organic 19-year-old Cabernet sauvignon 3ha block. Other vineyard cultivars on the farm included Merlot and Shiraz. The data from all four conventional farms was compared with the organic Cabernet sauvignon vineyard data.

2.2.2.1. Experimental design

The organic farm survey started in November 2017 until April 2018. Samples were collected once a month. Ten random samples (vine branches) were collected using the hand collection method, and another ten samples were collected via the wet method. The hand collection method entails cutting a vine branch with sterilised garden shears, wrapping it in towelling paper and placing it in a zip-lock plastic bag. Wet sampling entails cutting a vine branch, but immediately placing the branch in a plastic bag containing 70% ethanol. The bag is shaken and the vine sample is removed after a minute from the bag, after which the ethanol is poured over into a jar and sealed. Samples were randomly selected, but chosen so that both samples (hand collection and wet collection sample) came from the same trunk.

2.2.2.2. Sampling and laboratory work

All the hand collected vine samples were processed by running them through a Leedom engineered mite brushing machine. The branches were inspected by hand for mites. The plate was then taken to a microscope where the mites are studied. The ethanol used to wash the vines leaves were poured into Petri dishes and studied under the microscope. Krantz & Walter (2009) guidelines were used in slide mounting the mites with a polyvinyl alcohol mounting medium. Mites were identified with a Leica DM 2500 microscope with phase contrast using x1000 magnification with oil induction. Identification to family was done with the help of descriptive keys (Lindquist, et al. 2009; Walter, et al. 2009; Zhang, 2003) and identified to species with the guidance of acarologists Prof Eddie Ueckermann and Davina Saccaggi.

2.2.2.3. Data Analysis

Rank abundance

The relative abundance was calculated for the mites present in the organic vineyard as well as the mites present in the conventional commercial vineyards. The total count of each was calculated and divided by the individual count for each species and ranked and plotted according to their relative abundance.

2.2.4. Table grape survey in Limpopo province

2.2.4.1 Experimental design

The fieldwork was conducted on 30 March 2017. This period was suggested by technical advisors as being the most suitable for finding mites, and due to logistical constraints (distance) could only be conducted once-off. Samples were collected from four farms in Limpopo all containing table grape vineyards. Samples were collected in Globlersdal (25.1674°S, 29.3987°E), Roedtan (24.5973°S, 29.0787°E), Marble Hall (24.9651°S, 29.2815°E) and Mookgophong (24.5165°S, 28.7174°E). A minimum of ten random vine branches and sub-branches were collected at each farm. The samples were cut with sterilised garden shears, wrapped in tissue paper and placed in a zip-block plastic bag.

2.2.4.2 Sampling and laboratory work

Samples were placed in a cooler box and examined in the laboratory. All the mites were removed from the leaves by running the leaves through a mite brushing machine. The mites are picked off the plate and placed in a tube with 70% ethanol. The specimens are then slide mounted for identification. The slides were identified with a Leica DM 2500 microscope with phase contrast using x1000 magnification with oil immersion and descriptive keys (Lindquist, et al. 2009; Walter, et al. 2009; Zhang, 2003).

2.2.5. Identification key

An identification key containing all the mites collected during the entire two-year study in the Wellington Winelands region was constructed. The key was compiled using existing identification keys (Lindquist, et al. 2009; Walter, et al. 2009; Zhang, et al. 2003) and with the expertise of Acarologist Prof Eddie Ueckermann. The main distinguishing characters from each species were used to differentiate between the species in the key.

2.3. RESULTS AND DISCUSSION

2.3.1. Conventional vineyard survey

The commercial vineyard displayed the highest diversity with eight phytophagous species and nine predatory species (Table 2.2). Appendix D lists all the mite species found during the entire research study. Diversity indices were calculated for the predatory and phytophagous species diversity at each vineyard planting. The diversity indices both showed a stronger diversity for predatory mites at all three vineyard plantings with a low diversity and high unevenness for the phytophagous mites in commercial vineyards, motherblocks and nurseries. (Table 2.3.

Table 2.3: The diversity of phytophagous and predatory mites in the three different vineyard plantings as indicated by Shannon Wiener - and Simpson diversity indices.

Index	Mite	COMMERCIAL VINEYARD	MOTHERBLOCK	NURSERY
Shannon	Predatory	0.64	0.77	0.90
Wiener	Phytophagous	0.47	0.07	0.54
Simpson	Predatory	0.79	0.66	0.88
	Phytophagous	0.03	0.04	0.04

General Linear Models displayed the weighted means for the different vineyard blocks, which were shown to be significant { $F = (4.16) = 2.604$, $p = 0.02$, $ss = 202.9$ } with the highest species diversity found in commercial vineyards and the lowest in motherblocks. This is illustrated by rank-abundance plots (Fig 2.3). All three vineyard types have an uneven species distribution with one phytophagous species dominating, namely, *Brevipalpus lewisi* McGregor.

The rank-abundance plot comparing predatory and phytophagous mites (Fig 2.4.) found a higher relative abundance of the dominant phytophagous species and a lower general diversity overall. Predatory mites on the other hand displayed shared dominance and a more even distribution with a higher general diversity overall. Even though there is a much higher diversity (Fig 2.3) in predatory mites, *B. lewisi* is not successfully being controlled. Each farm uses its own pesticide programme accordingly. The main treatments the farmers used on their plantings are not miticides, but insecticides and are only applied during an outbreak. The products used during the different vineyard growth stages are listed in Appendix A. These consisted predominantly of fungicide applications.

Table 2.2: Predatory and phytophagous mite species found in each vineyard type in Wellington, South Africa, sampled from 2016 to 2018.

PREDATORY MITE SPECIES/GENUS/FAMILY	COMMERCIAL	MOTHERBLOCK	NURSERY
<i>Agistemus collyerae</i>	√	√	
<i>Anystis baccarum</i>	√		
<i>Balaustium</i> sp	√		√
Bdellidae	√		
Eupalopsellidae		√	
<i>Eusieus addoensis</i>	√		√
<i>Hemicheyletia</i> sp			√
Iolinidae		√	√
<i>Neoseiulus barkeri</i>		√	√
<i>Pronematus ubiquitusubiquitous</i>	√		√
<i>Tydeus grabouwi</i>	√		√
<i>Tydeus</i> sp	√		
<i>Typhlodromus praeacutus</i>	√	√	√
<i>Typhlodromus saevus</i>			√
PHYTOPHAGOUS MITE SPECIES/GENUS/FAMILY	COMMERCIAL	MOTHERBLOCK	NURSERY
<i>Brevipalpus lewisi</i>	√	√	√
<i>Brevipalpus obovatus</i>	√	√	√
<i>Brevipalpus phoenicis</i> complex	√	√	
<i>Oligonychus vitis</i>	√		
<i>Tetranychus</i> sp	√	√	
<i>Tetranychus ludeni</i>	√		√
<i>Tetranychus urticae</i>	√		

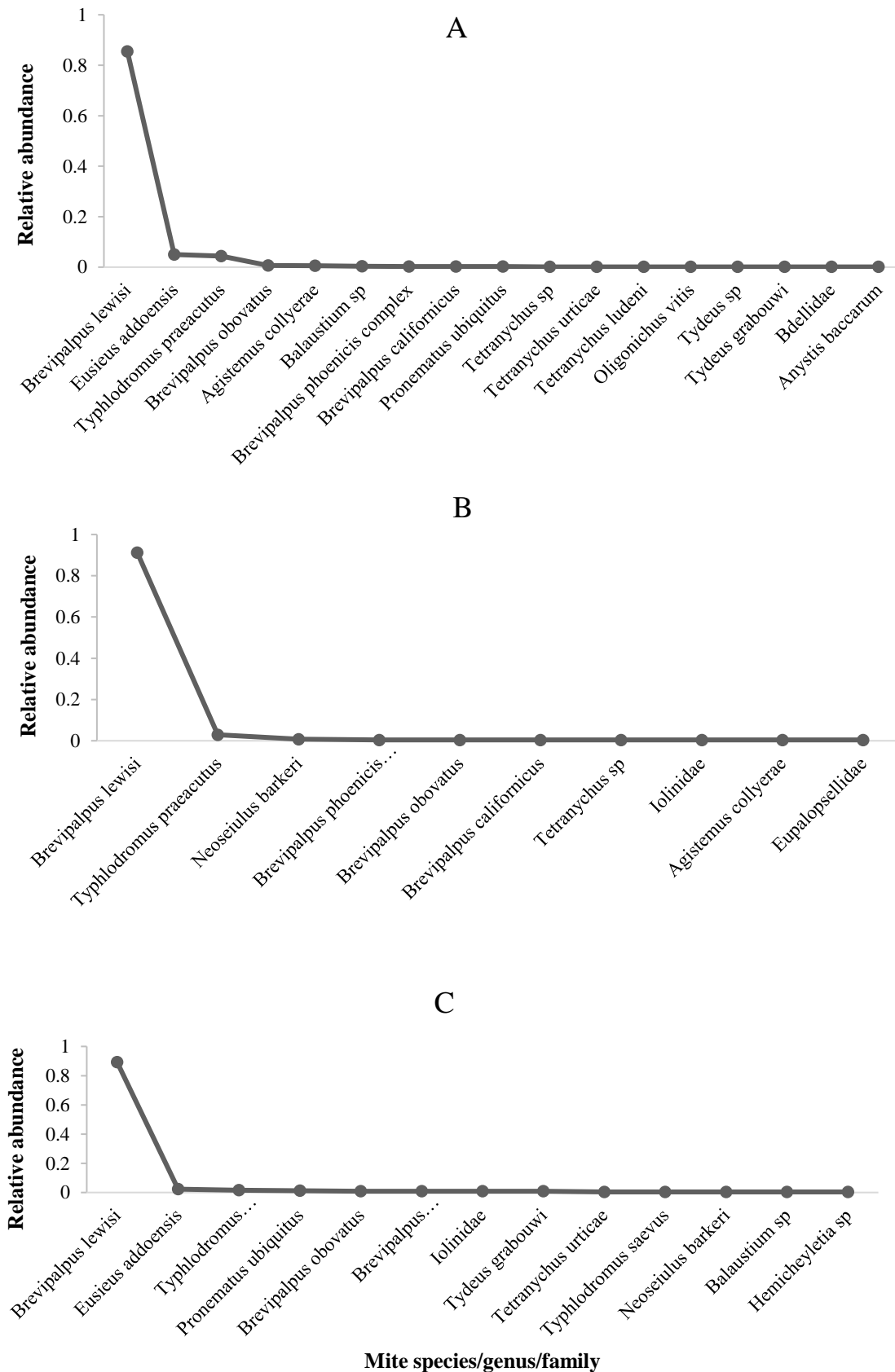


Figure 2.3 The rank-abundance plots of mites collected in the three different vineyard plantings sampled from 2016 to 2018; commercial vineyards (a), motherblocks (b) and nurseries (c).

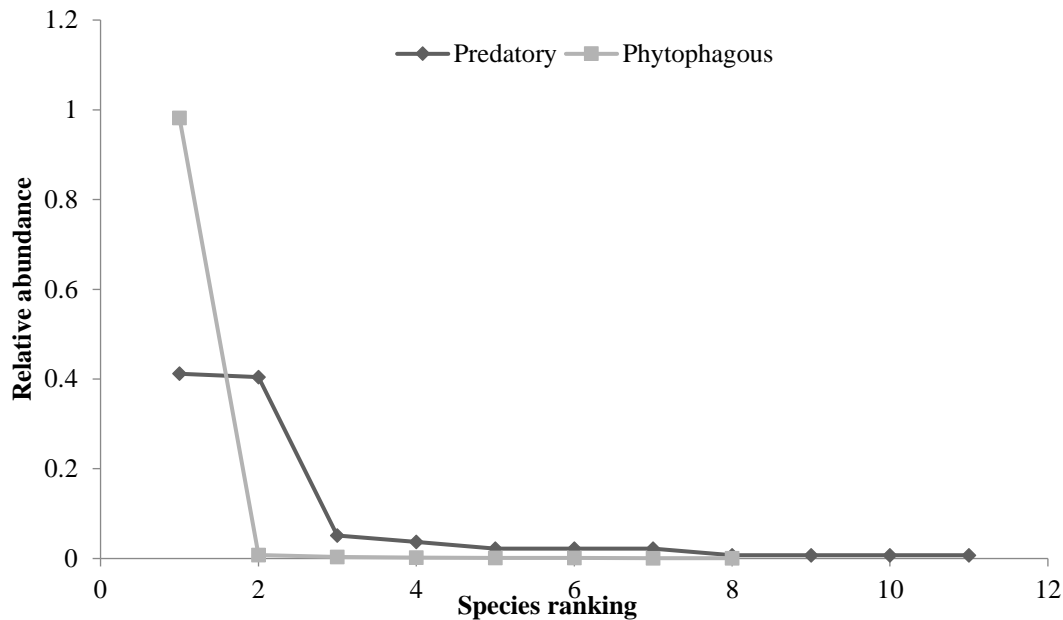


Figure 2.4: The rank-abundance between predatory and phytophagous mites collected at commercial vineyards, motherblocks and nurseries from 2016 to 2018.

The multiple correspondence analysis (Fig 2.5) showed the first dimension with inertia of 68.10% indicating a stronger relevance than the second dimension (31.90% inertia). Together dimension 1 and dimension 2 accounted for 100% of the variation. This indicates a strong association between the predators *Pronematus ubiquitous* McGregor and *Tydeus grabouwi* Meyer & Ryke with each other, to some extent in nurseries, based on the high order of magnitude on the graph of the first dimension. *Brevipalpus lewisi* was situated close to the origin point, therefore, at a lower order of magnitude, but closer to motherblocks and nurseries on the first dimension. *B. lewisi* was, however, prevalent at all three vineyard plantings (Fig 2.3). The predator *Neoseiulus barkeri* Hughs is an outlier, but it has a high order of magnitude on the positive side of the axis and therefore more associated with motherblocks. *Neoseiulus barkeri* was only present in nurseries and motherblocks (Fig 2.3).

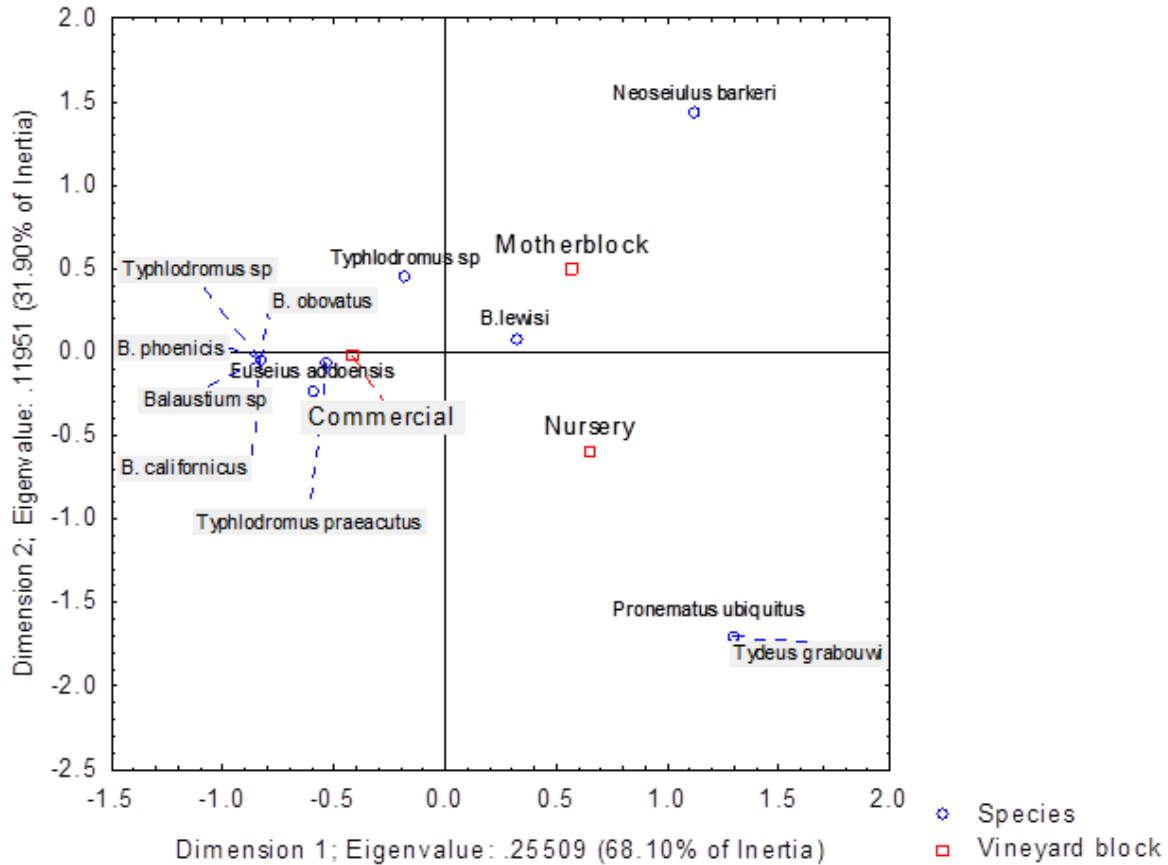
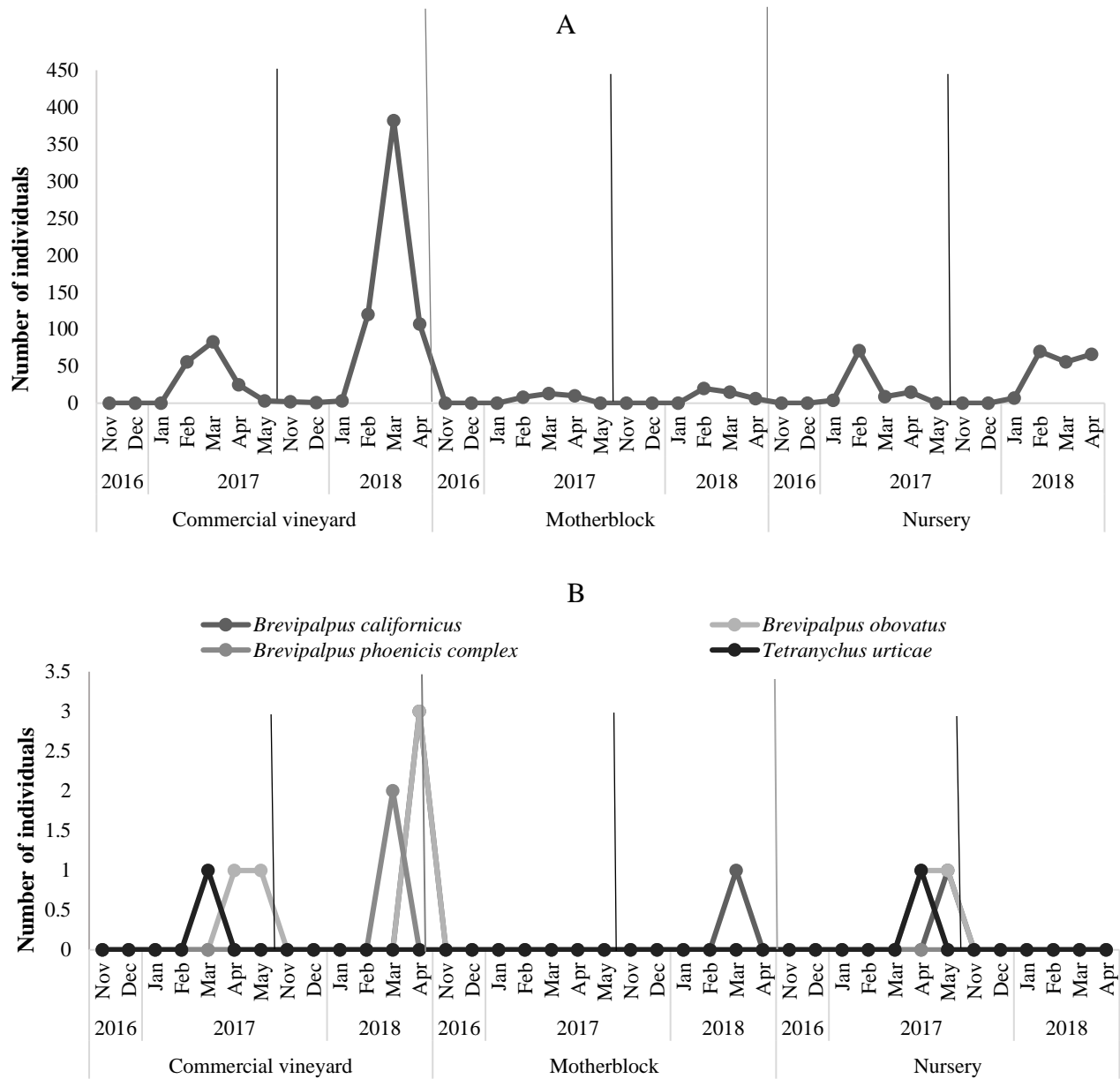


Figure 2.5: Multiple correspondence analysis indicating the mite diversity per vineyard block type; commercial vineyards, nurseries and motherblocks from November 2016 until April 2018.

The predatory mites *Typhlodromus* spp, *Balaustium* sp, *Euseius addoensis* (McMurtry) and *T. praeacutus* are closely associated with commercial vineyards as are all phytophagous mites; *Brevipalpus obovatus*, *B. californicus* and *B. phoenicis* complex (*B. phoenicis* has been redescribed as a complex of morphologically similar species (Beard, et al. 2015)). Commercial vineyards showed to have the strongest relationships with the most species.

Brevipalpus lewisi does not have any strong associations (Fig 2.5), yet it had the highest abundance of all the species (Fig 2.6 A). *Typhlodromus praeacutus* van der Merwe, *N. barkeri* and *B. lewisi* were the most frequent mites found in motherblocks (Fig 2.3). *Brevipalpus lewisi* is an invasive species, and the predatory mites seem to prefer the other *Brevipalpus* spp. (Fig 2.6), because the

majority of predatory mites occurred in association with *B. obovatus*, *B. californicus* and *B. phoenicis* complex as seen in the correspondence analysis (Fig 2.5). The low count in remaining phytophagous mites could also suggest that *Brevipalpus lewisi* is outcompeting *B. obovatus*, *B. californicus*, *B. phoenicis* complex, *Oligonychus* spp. and *Tetranychus* spp. (Fig. 2.6B). Spider mites *Oligonychus* spp and *Tetranychus* spp were the least abundant during the survey. It could also mean that the predatory mites show preference for particular prey species and that they would rather prey on spider mites than on *Brevipalpus* species. It could also be that *B. lewisi* is more resistant to the treatments being applied, but further detailed study would need to be done to determine the reason for this trend



The three vineyard plantings with the associated month and year samples were collected

Figure 2.6: The population fluctuations throughout the seasons for *Brevipalpus lewisi* (a) and the remaining phytophagous grapevine mites (b). Divisions between months of May and November indicate downtime in sampling.

Brevipalpus lewisi was detected for the first time in 2015 in South Africa on grapevine during a routine survey by the South African Department of Agriculture, Forestry and Fisheries (DAFF) (Saccaggi, et al. 2017). Surveys were then conducted in South Africa to confirm the localities of

B. lewisi in other provinces like Limpopo where table grape farming forms a large part of their farming industry. Currently *B. lewisi* occurs on grapevine in the Northern Cape and Western Cape provinces of South Africa (Saccaggi, et al. 2017). *Brevipalpus lewisi* tends to be more abundant in arid areas (Childers, et al. 2003a). Its distribution generally does not overlap with *B. obovatus*, *B. californicus* and *B. phoenicis* complex (Childers, et al. 2003a), yet here it is shown that they can co-occur. During April 2017 *B. lewisi* occurred with *B. obovatus* and *B. lewisi* occurred with *B. phoenicis* complex during March 2018 in commercial vineyards. There was one co-occurrence between *B. lewisi* and *B. californicus* during March 2018 in motherblocks, with no co-occurrences found in nurseries. (Fig 2.6).

The low abundance of species in the *B. phoenicis* complex, *B. obovatus* and *B. californicus* could mean that the predatory mites, which are generalists, prefer them above *B. lewisi*, or *B. lewisi* is out-competing the other *Brevipalpus* species. *Brevipalpus californicus*, *B. obovatus* and *B. phoenicis* complex are more widespread and are of higher economic importance due to being vectors for viruses such as citrus leprosis (Childers, et al. 2003b).

During the survey, no visible symptoms of damage were observed on the vine leaves whilst sampling. The discovery of *B. lewisi* in South Africa was by chance, and since then no mite-induced crop damage has been reported (Saccaggi, et al. 2017). *Brevipalpus* species can cause indirect damage by acting as a vector for crop viruses, particularly Citrus leprosis virus (Childers & Rodrigues, 2011; Rodrigues & Childers, 2013). Citrus leprosis virus is not present in South Africa (Lovisolo, 2001), which suggests *Brevipalpus spp.* are less of a threat to South African agriculture. Other diseases and symptoms associated with *Brevipalpus* species are “lepra explosive” (Vergani, 1942), leaf damage (Knorr, 1959) and twig lesions (Klotz, 1978). *Brevipalpus lewisi* is responsible for “bunch mite” symptoms on grapevine (Buchanan, et al. 1980). The other possibility is current management regimes for pest species are also successfully controlling *B. lewisi*. This is unlikely, seeing that high predatory mite diversity exists in vineyards, with a high abundance of *B. lewisi*. Thus, neither the pesticide plan nor the predatory mites are successfully suppressing *B. lewisi*. Saccaggi, et al (2018) suggest that the newly invasive predatory

mite *Agistemus collyerae* Gonzales-Rodriguez can control *Brevipalpus* species. *Agistemus collyerae* were found in the commercial vineyards and motherblock material of the current study, and was discovered with samples where *B. lewisi* was encountered (Saccaggi & Ueckermann, 2018).

The grapevine is a perennial plant, so the performance of a vine will be affected by what has happened during previous seasons (Iland, et al. 1968). The Western Cape experienced a drought from 2015 to 2017 due to a lack of rain (Araujo, et al. 2016). Mites tend to be sensitive to climatic changes (Duso & Vettorazzo, 1999). These hot and dry conditions, which result in vines being water stressed are ideal for *B. lewisi* (Childers, et al. 2003a), which could contribute to the population expansion seen in this mite (Fig 2.6A)). Without the awareness of mite presence and their environmental triggers, imbalances can occur creating new pest developments.

2.3.2. Organic vineyard survey

The rank-abundance between the organic and conventional farm both showed an uneven species distribution (Fig 2.7). The conventional farm had higher dominance and more diversity. There was a difference between the conventional vineyards' diversity and the diversity in the organic vineyard. This is largely due to the absence of phytophagous mites in the organic vineyard, whereas the conventional vineyards all had phytophagous mites present throughout the collection period. Appendix D also lists all the mite species found in both the organic vineyard and the conventionally-treated vineyards.

The conventional vineyard had eight phytophagous mite species present; *B. lewisi*, *B. obovatus*, *B. californicus*, *B. phoenicis* complex, *Tetranychus* sp, *Tetranychus ludeni* Zacher, *T. urticae* and *Oligonychus vitis* Zaher & Shehata (Table 2.3). Appendix B show the treatments that the organic vineyard used, which included sulphur and copper treatments, which could impact on mite abundance (Zhang, et al. 2012).

Euseius addoensis and *T. praeacutus* were the dominant predators in the conventional vineyard. *Euseius addoensis* was also the most abundant predator in the organic vineyard, with *T. saevus* and *P. ubiquitous* following thereafter (Table 2.3). Many unsprayed vineyards support a low, yet consistent predatory mite population (Karban, et al. 1997).

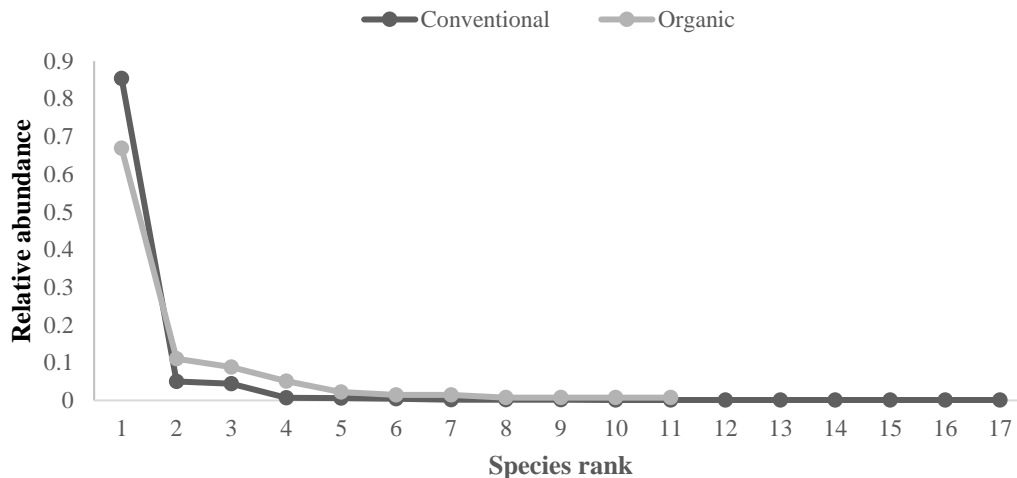


Figure 2.7: The rank-abundance of mite species found at an organic vineyard compared to conventional vineyards from November 2017 – April 2018.

The majority of phytoseiid mites present in both vineyards are generalists. There were also many of the same species occurring in both vineyards; *T. praeacutus*, *T. grabouwi*, *Tydeus* sp, *E. addoensis* and *P. ubiquitous*. Most *Typhlodromus* spp. are selective, but tend to enjoy a wider range of mite species and some species are complete generalists (McMurtry & Croft, 1997; McMurtry, et al. 2013).

Most generalist predators can reproduce on pollen, and many times just as effectively as on prey (Castagnoli & Simoni, 1990; Duso & Camporese, 1991; McMurtry & van de Vrie, 1973; Schausberger, 1992; Van Rijn & Van Houten, 1991). Generalists also do not tend to aggregate in prey colonies unlike specialised and selective predatory mites (Croft, et al. 1995; Lawson & Walde, 1993; McMurtry, 1992; Nyrop, 1988; Zhang, et al. 1992). Also, they are active foragers on both sides of the leaf (McMurtry & Croft, 1997).

Eusieus addoensis appears to persist under unsprayed conditions (Pringle, 1974) and is the most frequently encountered predatory species. It is also endemic to South Africa (Pringle, 1974). *Eusieus* spp. are generalists, with a great deal of their diet consisting of pollen; most of them have a higher reproductive rate on pollen (Abou-setta & Childers, 1987; Abou-setta & Childers, 1989; Ferragut, et al. 1987; McMurtry & Rodrigues, 1987; Osakabe, 1988). A population increase is often correlated with pollen fallout rather than an increase in prey species (McMurtry, 1992). The surveyed organic vineyard incorporates local flora around their vineyards. This not only attracts local fauna, but creates a consistent predator presence. There is a constant food source be it pollen or mites. Generalist phytoseiid mites are preferred above specialist phytoseiids as biocontrol method, especially on perennial plants (Prischmann, et al. 2006). Generalist phytoseiids can switch to an alternate food source when pests are absent and tend to be abundant in unsprayed vegetation and neighbouring plants (Boller, et al. 1988). Despite having a high reproductive capacity and thus a strong reproductive response when faced with high infestations, specialist phytoseiids tend to overexploit their pest food source leading to emigration or starvation causing unstable predator-prey dynamics (McMurtry, 1992; Nyrop, et al. 1998; Jung & Croft, 2001). Generalist phytoseiid mite species have been shown to effectively lower spider mite densities in Australian and European vineyards (Duso & Pasqualetto, 1993; James & Whitney, 1991; Kreiter, et al. 2001). Generalist and specialist phytoseiids also have the ability to co-exist in certain communities that lead to more effective pest control (Mori & Saito, 1979; Croft & MacRae 1992a, 1992b; Croft & Slone, 1997). This is due to the complimentary combination of density independent, early season impacts of generalists with the density dependent numerical responses of specialists later in the season as pest densities rise (Snyder & Ives, 2003).

2.3.3. Collection technique comparison

The wet sampling method has proved to be more successful in general, with a total of 117 specimens caught using the wet method and 19 specimens caught using the dry method (Fig 2.8). A greater diversity of predatory species (nine spp) were collected more frequently with the wet method. Only two predatory species were collected at low numbers with the hand method. This

indicates that mites do jump off when a plant is handled. Based on these results, both methods seem to be equally successful for diversity assessments, as the same diversity was found with both methods. The wet method shows it would be better suited for species richness and abundance surveys as more individuals were collected.

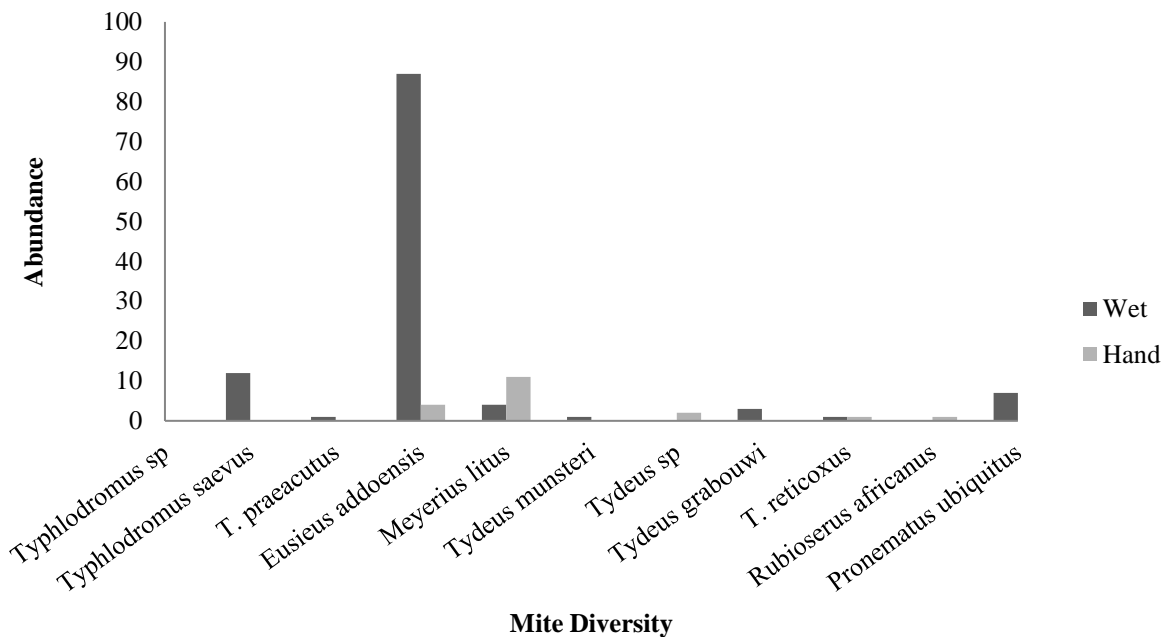


Figure 2.8: A comparison (Wet method SE= 7.71; Hand method SE=1.00) between two mite sampling techniques used in an organic vineyard. The wet method entails placing the vine samples in 70% ethanol and inspecting the ethanol thereafter whereas the hand method entails inspecting the leaf under the microscope. The organic farm was in Stellenbosch, Western Cape and sampled from November 2017 until April 2018.

2.3.4. Table grape survey in Limpopo province

Numerous Tetranychidae (spider mites) were found on all the cultivars, but no Tenuipalpidae (flat mites) were found. Specifically, no *Brevipalpus lewisi* was present. *Tetranychus waitei* Banks and *T. urticae* were the two most abundant Tetranychidae present.

2.3.5. Identification key

A taxonomic key referring to all the phytophagous and predatory mites on vineyards was compiled based on the entire two-year mite vineyard study (Appendix C). A whole range of identification characters were used in compiling the key as there is such a wide diversity in all mite families. An important trait is their chelicera as it can be fused, partially fused, completely separate and moveable or stylophore shaped. Other traits like presence or absence of certain setae and their length, the position of the stigmata and their external shield, the tarsi with solenidia, the opithosoma with varying amounts and varying of dorso-setae, the degree of reticulation of the prodorsum, the absence or presence of a thumb-claw process, the shape of palptarsus, the prodorsum with or without trichobothria and the size and shape of the spermatheca were used to distinguish between the mites found throughout all the research study in the vineyards.

2.4. CONCLUSION

In this study, the predatory and phytophagous mite diversity for all vineyard plantings were established. The predatory and phytophagous diversity differed at each vineyard planting with commercial vineyards having the highest diversity. The reason for this could be that commercial plantings provide a longer-term habitat for the mites to become established, as opposed to nurseries and motherblocks. Motherblocks showed to have the lowest diversity and abundance of species.

Brevipalpus lewisi was the dominant phytophagous mite in all vineyard plantings. It is an established vineyard pest in the Northern Cape and Western Cape. The presence of generalist Stigmaeidae like *A. collyerae* show potential in controlling *B. lewisi*, but further research should investigate this relationship in more detail and whether it has economic potential as an effective biological control mechanism. The presence of *B. lewisi* in motherblocks does pose a quarantine risk, as these plants are used to establish new nurseries and eventually commercial plantings all over the country. It is important to contain *B. lewisi* in its current distribution as it is a threat to other provinces like Mpumalanga province with its large citrus industry. The survey confirmed there were not any *Brevipalpus* spp. present in the Limpopo province at the time of the survey on the relevant farms surveyed. Regular *ad hoc* surveys should be done to monitor for the presence of *Brevipalpus* spp. All material being transferred between farms, but especially between

provinces should be clean of *B. lewisi* before trading. Without regular monitoring and checking new rootstocks, it will spread. Thus, any new material that gets delivered must be checked for *B. lewisi*. The Western Cape can not trade rootstocks across provinces without making sure it is free of *B. lewisi*.

The wet sampling method was considered more effective in abundance surveys as it is less time consuming, based on results of the current study. Inspecting the mites in the ethanol is more time efficient than inspecting each leaf or running each vine leaf through the mite brushing machine. With the wet method, a high predatory diversity was observed, primarily for *E. addoensis*, while the hand sampling method did not show high numbers of this species indicating some sampling bias between the two methods. Predatory mites are more active (McMurtry & Croft, 1997) than phytophagous mites and the ethanol washing gives them little opportunity to escape. The conventional vineyard showed both predatory and phytophagous mites occurring, while the organic vineyard did not have any phytophagous mites present. This could be an indication the sulphur treatments could have had an effect. Previous studies have shown sulphur to be an effective control method, with it successfully controlling pests and having no impact on predatory mites (Gent, et al. 2009). Yet, sulphur has also shown to disrupt predatory mite feeding behaviour (Beers, et al. 2009) and mite populations (Costello & Albers, 2003) in other studies. *Brevipalpus lewisi* is susceptible to sulphur dust and several non-organophosphorous pesticides (www.cabi.org). It is more likely that the sulphur and other natural treatments are controlling the phytophagous pests, while the surrounding local fauna is acting as a food source to the predatory mites.

There were similarities between the organic farm and conventional farms, with five predatory species that were present in both types, especially with the majority being generalists and *E. addoensis* being the dominant predator in both organic and conventional vineyards. Although the comparison between organic and conventional could not be statistically compared due to sample size differences, these are all indicators showing that the predatory mite diversity in local conventional vineyards are not severely being affected by pesticides. These predatory mites

present in conventional vineyards could be useful to the industry by acting as natural biological control agents of pests.

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CHAPTER 3

THE PHENOLOGY OF PREDATORY AND PHYTOPHAGOUS MITE POPULATIONS IN VINEYARD PLANTINGS

3.1. INTRODUCTION

Once a species diversity is established, it is beneficial to be able to determine the population cycles of species of interest. This baseline data is helpful in improving management strategies, as it provides information of when to focus management strategies (Duso & Vettorazzo, 1999). Regular monitoring of fruit crops decreases the chance of getting an unexpected pest outbreak. Although fruit farmers do regular pest control monitoring, mites tend to be overlooked as they are not easily spotted with the naked eye (Pringle & Heunis, 2017). Knowledge of the population cycles of phytophagous and predatory mites would both benefit control and monitoring. The predicted presence of a beneficial predatory mite can be incorporated into management strategies in combination with treatments or exclusively as a biocontrol agent. Knowing when phytophagous mites start increasing in number can determine when monitoring efforts should start.

By illustrating the population fluctuations, we can establish vineyard mite population peaks of both phytophagous and predatory mites, as well as dominant and co-occurring species. Population cycles for predatory and phytophagous mites have not been established in South African wine grape vineyards. In Europe, mites tend to only colonise the vine foliage during the summer season, and not before as previously assumed (Schruff, 1985).

The results of a two-year population study by Shibao, et al. (2004) in a vineyard in Osaka, Japan showed the phytoseiid mite *Euseius sojaensis* (Ehara) population peak coincided with population increases of the thrip species *Scirothothrips dorsalis* Hood (Thysanoptera: Thripidae). Through this, they could determine that *E. sojaensis* was the main predatory mite preying on *S. dorsalis* and

thus showed potential as a control method for *S. dorsalis*. de Villiers & Pringle (2007) conducted a study on the temporal occurrence of table grape pests in commercial vineyards in the Hex River Valley, South Africa with the aim of creating monitoring systems for the management of table grape pests. A benefit of establishing seasonal cycles, is being able to see if the predator and prey co-occur and at what densities they occur. Timing is essential to introducing a predatory mite for control (Stavrinides, et al. 2010). Early introductions or high rates of release could lead to extinction and delayed introductions could result in the pest damaging the crop before the control agent has the chance to populate (Stavrinides, et al. 2010), resulting in crop damage exceeding the economic threshold.

The aim of this survey was to establish the population cycles of both phytophagous and predatory (i.e. pest and beneficial) mites occurring in South African vineyards. This was done by collecting vine samples every two weeks over a period of two years. The survey included the inspection of motherblocks, nurseries and commercial vineyards. By inspecting all three main components of vineyard development, a clearer understanding of the occurrences can be reached. This survey therefore aims to confirm the seasonal presence of mite pests, for which further management can be determined and hopes to provide specific guidelines at different planting stages (i.e. motherblocks, nurseries and commercial plantings), if necessary.

3.2 MATERIALS AND METHODS

The fieldwork was conducted in vineyards in the Winelands region of the south Western Cape Province, South Africa. Sampling sites included four conventional farms in the Wellington region (Chapter 2). Each farm included a nursery, motherblock and commercial vineyard from which samples were collected. Nursery material is planted at the start of each season; October/November and kept for six to eight months. Motherblocks are replaced every ten years. The nurseries were used for cultivating a range of different cultivars. The commercial vineyards were between 10 and 30 years old. Each farm had their own management approach with a standard spray programme (Appendix A).

3.2.1. Experimental design

The survey was conducted over two-years. Samples were collected every second week from November 2016 until April 2018. No samples were collected during the dormant season, during winter and spring (June 2017 – October 2017). Ten random vine branches and sub-branches were collected in each vineyard planting. Whilst sampling, damaged vine leaves or leaves displaying symptoms were preferred above healthy leaves. Vine branches were cut with sterilised garden shears, wrapped in towelling paper and placed in zip-lock plastic bags. This was done to prevent the leaves from perspiring and wilting. This allowed the leaves to stay fresh at 5°C for up to six weeks. During each visit to the four farms, each containing a commercial block, nursery and motherblock, a total of 120 vine samples was collected at each planting.

3.2.2. Sampling and laboratory work

All the vine samples collected at each site were processed by running them through a Leedom engineered mite brushing machine (Fig 2.1). The mite brushing machine has previously proven effective with mite population studies (Henderson & McBurnie, 1949). The machine contains two bristles that comb through the veins and hairs of the leaf, and allowing the mites to fall onto a Perspex plate. The guidelines of Krantz & Walter (2009) were followed by slide mounting the mites with a polyvinyl alcohol medium. Identification was conducted with a Leica DM 2500 microscope with phase contrast using x1000 magnification with oil induction. Identification to family level was done with the help of descriptive keys (Lindquist, et al. 2009; Walter, et al. 2009; Zhang, 2003) and identified to species with the guidance of acarologists Prof. Eddie Ueckermann (retired) and Davina Saccaggi (Department of Agriculture, Forestry and Fisheries).

3.3 RESULTS AND DISCUSSION

The population fluctuations for predatory and phytophagous mites in commercial vineyards, motherblocks and nurseries from 2016 – 2018 are given in Figure 3.1. *Euseius addoensis* (McMurtry) and *Typhlodromus praeacutus* van der Merwe were the two dominant predators that were present in low numbers throughout the seasons in the commercial vineyards (Fig. 3.1.A). *Brevipalpus lewisi* McGregor was absent at the start of the season (November 2016 – January 2017) and rapidly increased from February 2017 to March 2017 and started to decline from April 2017 onward until February 2018 where the population started to grow again. *Brevipalpus lewisi* reached its highest numbers in March 2017 and again in March 2018. Predators *E. addoensis* and *T. praeacutus* do not seem to overlap as *B. lewisi* peaked just before the end of the season, whereas *E. addoensis* and *T. praeacutus* had the greatest abundance at the start of the season.

In the motherblocks, the appearance of predators *Neoseiulus barkeri* Hughs, *T. praeacutus* and an unknown *Typhlodromus* species, were not synchronized with *B. lewisi* (Fig 3.1. B). *Typhlodromus praeacutus* only occurred once in February 2018, *Typhlodromus* sp. in January 2018 and *N. barkeri* in April 2018. *Brevipalpus lewisi* first appeared in February 2017 and started to decline in April 2017 and is absent from May 2017 until January 2018 where it reached its highest population number and started to decline thereafter. Motherblocks displayed the lowest predatory diversity. *Brevipalpus lewisi* did not co-occur once with any of the predatory mites found in the motherblocks (Fig 3.1. B).

In the nurseries (Fig 3.1. C), the predators were sporadic. Nursery predatory mites only occurred during the middle of the vine season (January – March). *Neoseiulus barkeri* was only seen once in February 2018 and *E. addoensis* was present from January to March 2017 and absent until April 2018. *Pronematus ubiquitous* McGregor was present during January 2017 and February 2017 and *T. praeacutus* was only seen in April 2018. *Brevipalpus lewisi* was absent until January 2017, where it peaked during February 2017 and it was present until April 2017 and started to decline. *Brevipalpus lewisi* reappeared January 2018, it peaked again in February 2018 and persisted until April 2018. *Brevipalpus lewisi* did not share a seasonal population cycle with any of the other predatory mites.

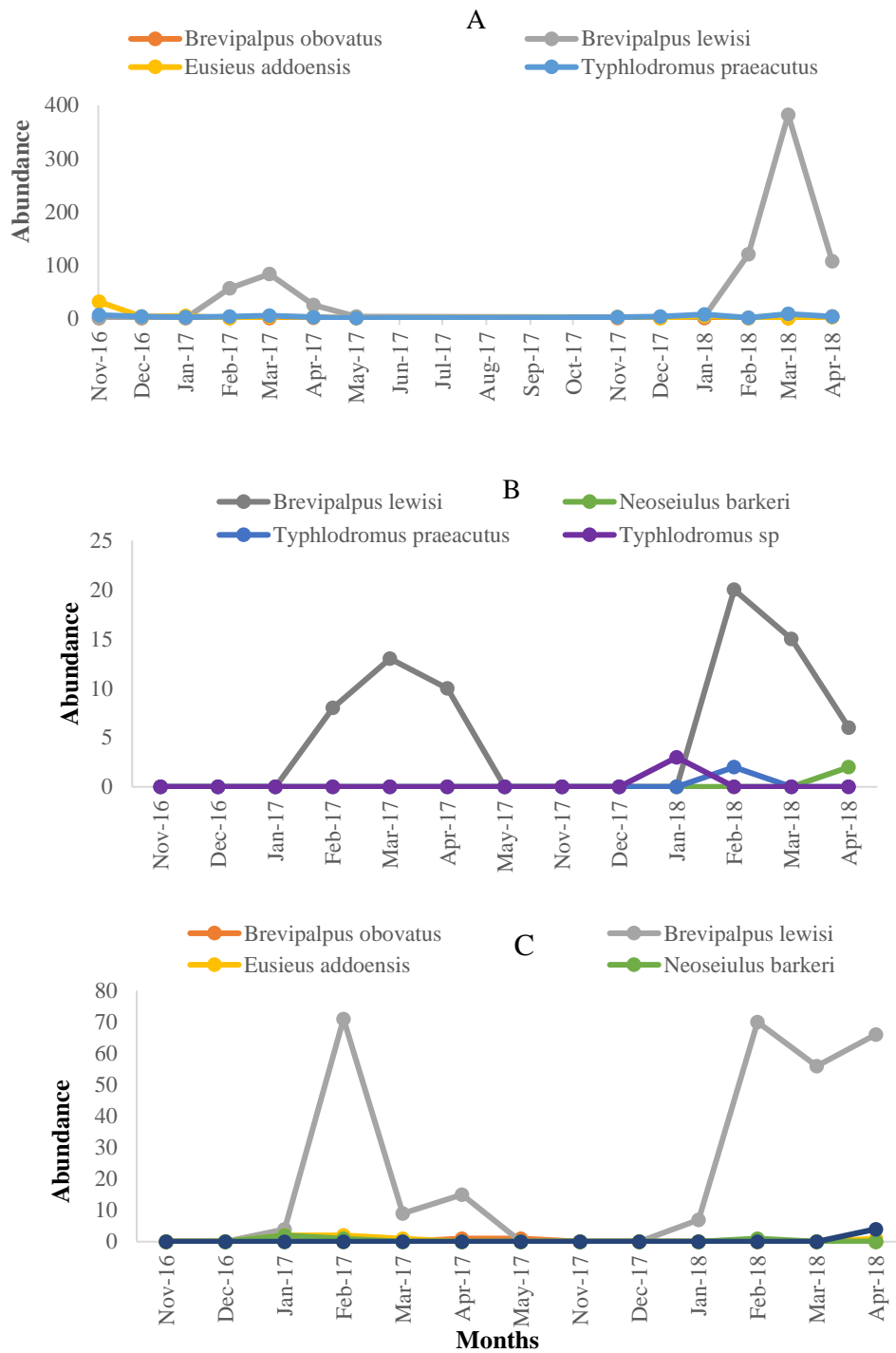


Figure 3.1: The seasonal cycles of the main predatory and phytophagous mites found on commercial vineyards (a), motherblocks (b) and nurseries (c) from November 2016 – May 2017; November 2017 – April 2018.

Phytoseiid mites are categorised according to their food preference into four main categories (McMurtry & Croft, 1997). *Euseius addoensis* is a type IV phytoseiid mite, which means this species in this genus are specialised pollen feeders, successful predators, but also have the ability to successfully live and reproduce on pollen (McMurtry & Croft, 1997, McMurtry, et al. 2013). The genera *Typhlodromus* are Type Ib and IIIa while *Neoseiulus* are type II, IIIb and IIIe phytoseiidae (McMurtry & Croft, 1997, McMurtry, et al. 2013). Type II mainly prey on Tetranychidae (McMurtry & Croft, 1997, McMurtry, et al. 2013). Thus, there could be interspecific competition between *E. addoensis* and *T. praeacutus* as specialized pollen feeders tend to be more aggressive predators (Maoz, et al. 2016). Tydeidae like *P. ubiquitous* and *T. grabouwi* tend to live off of a range of plant sources and small organisms (Gerson, et al. 2003). *Tydeus grabouwi* is not a dominant predatory mite and was most likely preyed upon by the other predators (Gerson, et al. 2003).

Persistence in predatory species confirms they have the capacity to survive on a variety of prey, successfully propagate and compete with other predatory mites (Duso & Vettorazzo, 1999). The interspecific competition between predators could have a healthy outcome on the predator-prey dynamics within the community; as the coexistence of two or more predators in a community enhances the chance of controlling a pest (McMurtry, et al. 1970; Croft & McRae, 1992; Roseheim, et al. 1995). This seems to apply to *E. addoensis* and *T. praeacutus* found in commercial vineyards.

Brevipalpus lewisi experienced a population spike towards the end of the grape-growing season (March/April). This was seen in nurseries, motherblocks and commercial vineyards. This is an adaptive character in *Brevipalpus* species (Kennedy, 1995). It is called a grouping development; where the developmental time, life span and oviposition are altered based on the population size and food availability (Kennedy, 1995). When the population density is high, mites will develop faster before all the food is exhausted, to prevent the consequences of overcrowding where life span is significantly reduced (Kennedy, 1995). *Brevipalpus lewisi* dominated throughout the monitoring period; neither the predators present nor the pesticides appear to have suppressed

populations. This is common behaviour for an invasive species like *B. lewisi* and since its discovery in 2015 in South Africa, it has quickly adapted and spread (Saccaggi, et al. 2017). Despite not showing forms of physical damage, it could affect the natural mite complex in our vineyards and thus, a suitable management strategy should be developed for sustainable control as it could pose a threat to grapevine and other crops in the future.

A pest management strategy should always start with confirming “why is the pest a pest?” and should address the underlying weakness in the agronomic practice that allowed this organism to reach pest status (Lewis, et al. 1997). Minimal pruning of grapevine has been shown to be effective in attracting predatory mites and keeping phytophagous pests at bay (Pennington, et al. 2017). *Brevipalpus lewisi* have been effectively controlled in Victoria, Australia via pruning (Buchanan, et al. 1980). This is a low-cost, low risk, biological management strategy that improves natural pest control by attracting a high density of predatory mites.

3.4. CONCLUSION

Euseius addoensis was the main predatory mite at each vineyard planting with *B. lewisi* being the dominant phytophagous pest. *Euseius addoensis* is a generalist feeder endemic to South Africa while *B. lewisi* is an invasive crop pest. Due to the relatively low abundance of predatory mites in all three vine development phases, it was not possible to establish synchronization in life cycle between predators and phytophagous mites from this survey. This indicates that *B. lewisi* does not have any effective natural enemies in our vineyards.

Brevipalpus lewisi was detected in South Africa with its predator, *Agistemus collyerae* Gonzales-Rodrigues. *Agistemus collyerae* is a predator of *Brevipalpus* and is native to Australia (Saccaggi & Ueckermann. 2018). A few *A. collyerae* was collected during the survey, but not enough to establish any seasonal occurrences. Despite having a natural predator of *B. lewisi* present, currently it would not be effective enough as a means of control.

During this survey, no visible signs of damage were recognised. Yet, *B. lewisi* is spreading and increasing, thus it is essential to continue monitoring its distribution and track potential damage. Frequent, routine-based inspections should be done on vineyards in the Western Cape and other provinces in South Africa involved in the grapevine industry like the Northern Cape and Limpopo. Currently *B. lewisi* is only found on grapevine (Saccaggi & Ueckermann, 2018), but it is a threat to other crops especially citrus as it is also a vector for Citrus leprosis virus (Rodrigues & Childers, 2013; Lovisolo, 2001). Monitoring our fruit crops and citrus orchards would thus also be beneficial. The most immediate risk of *B. lewisi* is for transportation of nursery material to other parts of the country not containing *B. lewisi*. This should encourage monitoring of the pest. The optimal time to monitor *B. lewisi* would be to start in the beginning of the season, around October, and then focus particular attention towards the end of the season (March/April) as they are most abundant then. This will give an indication of their dominance in the vineyard and would be able to ensure managers to plan for management actions for the following season.

Long-term monitoring can be used to establish economic thresholds (Pringle, 2006). Economic thresholds make up one of the main components of Integrated Pest Management (IPM). IPM is based on controlling arthropod pests by only applying treatments when and where necessary. For IPM to be applied, effective monitoring, substantial knowledge on the species, sampling and defined procedures should be in place (Pringle, 2006). This study also concluded that the predatory mite populations did not increase with the phytophagous mites (Pennington, et al. 2017). Miticides should not be applied whilst predatory mites and other natural enemies are abundant. In this case, it is best not to apply treatment during January to March.

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CHAPTER 4

INVESTIGATING WEEDS AND COVER CROPS IN VINEYARDS AS POTENTIAL ALTERNATE HOSTS FOR MITES ASSOCIATED WITH VINES

4.1. INTRODUCTION

Weedy plants form an important aspect of agro-ecosystems. Reductions in their diversity and abundance by means of chemical or mechanical treatments may negatively affect the occurrence of pest and beneficial organisms (de Sousa Saraiva, et al. 2015). Weeds also tend to harbour mites that eventually migrate to the main crop (de Sousa Saraiva, et al. 2015). This includes pests and beneficial mites. Weeds and cover crops both act as an additional host plant and food source for phytoseiids and phytophagous mites. Mites tend to migrate to weeds and cover crops during winter months. In South Africa, the mite complex in ground cover in vineyards is less well known. Grapevine farmers do not follow the same practices in managing the plants that grow on the floor between the vine rows, but the presence or absence of these plants affects the acari pest complex in vineyards (de Villiers, et al. 2010).

Herbicides are the most common method in effectively treating unwanted weeds (Rao, 2000). However, the impact that these treatments have on non-target species like pests, natural enemies and other arthropods with undefined feeding habits are rarely considered (Scheider, et al. 2009; Zhao, et al. 2013). The long-term effect of these practices could lead to changes in invertebrate food webs (Pereira, et al. 2007) which in turn could influence the occurrence and distribution of predatory mites due to the lack of alternate food sources (van Rijn & Tanigoshi, 1999). Thus, the conventional practices of weed management may be linked to changes in habitat, loss of vegetation cover, elimination of food sources (Belden & Lydy, 2000) and natural enemies.

There are benefits in allowing weeds to grow between vines. The presence of alternative prey and hosts recreate and enhance corridors for beneficial insect and mite movement (Altieri, et al. 2010) in and around vineyards. Habitat management strategies using non-crop plant species and natural enemies is recognised as an important approach to improve the biological control of pest mites by increasing populations of natural enemies in agroecosystems (Blumberg & Crossley, 1982; Landis, et al. 2000).

Coli, et al (1994) wanted to determine the vegetation composition of commercial apple orchard ground covers and woody borders in Massachusetts, USA to test if certain ground cover and border plants are preferred hosts of plant-feeding and predatory mites. They concluded that mites do have preferences for broad leaf plants and thus would rather migrate to herbaceous or woody plants than grasses. Cover crops are also important for over-wintering phytoseiid mites as it enhances recolonization during spring (James, 1989; 1990). Previous studies in South Africa have found the movement of mites between table grape vineyards and weed plants species to be important for effective biological control as *Tetranychus urticae* Koch (the dominant mite pest) and *Euseius rubicolus* (van der Merwe & Ryke) and *Neoseiulus californicus* (McGregor) (the dominant predatory mites) were found in both ground covers and vineyards (de Villiers & Pringle, 2011). In an earlier study, *Phytoseiulus persimilis* (Athia-Henriot) was found to be present in apple orchards in Elgin, South Africa (Botha & Pringle, 1995), yet, it failed to establish as an effective biological control agent of *T. urticae* (Pringle, 2001). It was further found that certain cover crop species were important for the survival of *P. persimilis* because they hosted *T. urticae* during winter months and therefore provided *P. persimilis* with a constant food source in orchards (Pringle, 2001).

The aim of this study was to assess potential host utilization by mites of various ground covers grown in commercial vineyards, motherblocks and nurseries during winter months; and to determine if ground covers played an equally important role in all three plantings. A survey was therefore conducted whereby weed and cover crop samples were collected during the winter

months (four months) on four conventional farms, each with a motherblock, nursery and commercial vineyard, which aimed to reduce variability as far as possible.

4.2 MATERIALS AND METHODS

4.2.1 Sampling sites and experimental design

During the winter (dormant) season, weed and cover crop samples were collected from four conventional farms in Wellington (Chapter 2). Each farm contained a commercial vineyard, motherblock and nursery, which were approximately 4ha in size. A standard control programme for weed management was used by each farm (Fourie, et al. 2007; Fourie, et al. 2015). Sampling took place once a month from July to October 2017. A sample consisted of an entire weed plant that was removed by hand or with a spade. Thereafter the sample was wrapped in towelling paper and placed in a sealable plastic bag. At each site, at least ten random samples were collected. A different diversity of weeds was growing at each site if there was a higher diversity, more samples were taken to ensure every weed species was sampled. At least four weed samples were collected of each species at each site. Thus, a minimum of 120 samples were collected on each field trip.

4.2.2. Sampling and laboratory work

Each weed sample was brought back to the laboratory and studied under the microscope. All the mites were removed from the weed plants by hand with paint brushes and micro pins and placed into tubes containing 70% ethanol. All the mites were slide mounted following the procedures of Krantz & Walter (2009). Identifications were conducted with a Leica DM 2500 microscope with phase contrast using x1000 magnification with oil induction. The identification of mites to family level was done with descriptive keys (Lindquist, et al. 2009; Walter, et al. 2009; Zhang, 2003) and further identified to species level with the guidance of acarologists Prof Eddie Ueckermann (retired) and Davina Saccaggi (Department of Agriculture, Forestry and Fisheries). All the weed

and cover crop samples were sent to the Compton Herbarium in Kirstenbosch (Western Cape) to be identified.

To confirm if the vineyard mites do migrate to cover crops and weed species; the mites present in the ground cover were compared to mite species occurring on vines in the vineyards. The vineyard mite data was obtained from a previous survey (Chapter 2).

4.2.3 Data analysis

To compare whether any particular mite species were associated with certain weed species, a correspondence analysis was conducted with plant species as column variables and mite species as row variables. The analysis was conducted using Statistica 13.0 (TIBCO Software Inc., Palo Alto, USA).

4.3 RESULTS AND DISCUSSION

The highest mite diversity and abundance was found on weeds in commercial vineyards and the lowest in weeds growing between the nursery material (Fig 4.1). The most frequently occurring mites on the weeds were an *Aplonobia* sp. (phytophagous mite) and *Tydeus grabouwi* Meyer & Ryke (predator) in commercial vineyards, *Tydeus reticoxus* Ueckermann (predator) was the only mite found in the nursery weeds; while motherblock weeds hosted the predators *Graminaseius bufortus* (Ueckermann & Loots) and an *Eupodidae* sp.

The lack of mites in the nursery blocks, which had Triticale (Triticale v. Usugen 18) as cover crop, could be ascribed to the fact that if grass species are planted as cover crop, they were found to harbour fewer mite problems; possibly because these vineyards are slightly cooler, more humid and less dusty (Flaherty, et al. 1982). This practice also usually improves water filtration into soil so that vines are better supplied with moisture. Improving water intake is important because spider

mites tend to favour stressed and dry conditions (Flaherty, et al. 1982). Cover crops in general have been an effective management practice because it improves structure, protects the soil, supports natural enemy populations and reduces the vigour of the vine (Vogelweith & Thiery, 2017). In addition, Coli, et al (1994) showed phytoseiid mites and *T. urticae* showed the least preference for grasses.

The migration of mites to vines depends on weed senescence, weed distribution and diversity (Bostanian, et al. 2012). During the sampling period a smaller total of mites were collected than expected. This could be due to the lack of weed diversity growing in the vineyards and the use of herbicides also preventing the growth of weeds between the vine rows. The main vine predators were not found on the weeds and cover crops; thus they do not appear to migrate to host plants during the dormant vine-growing season.

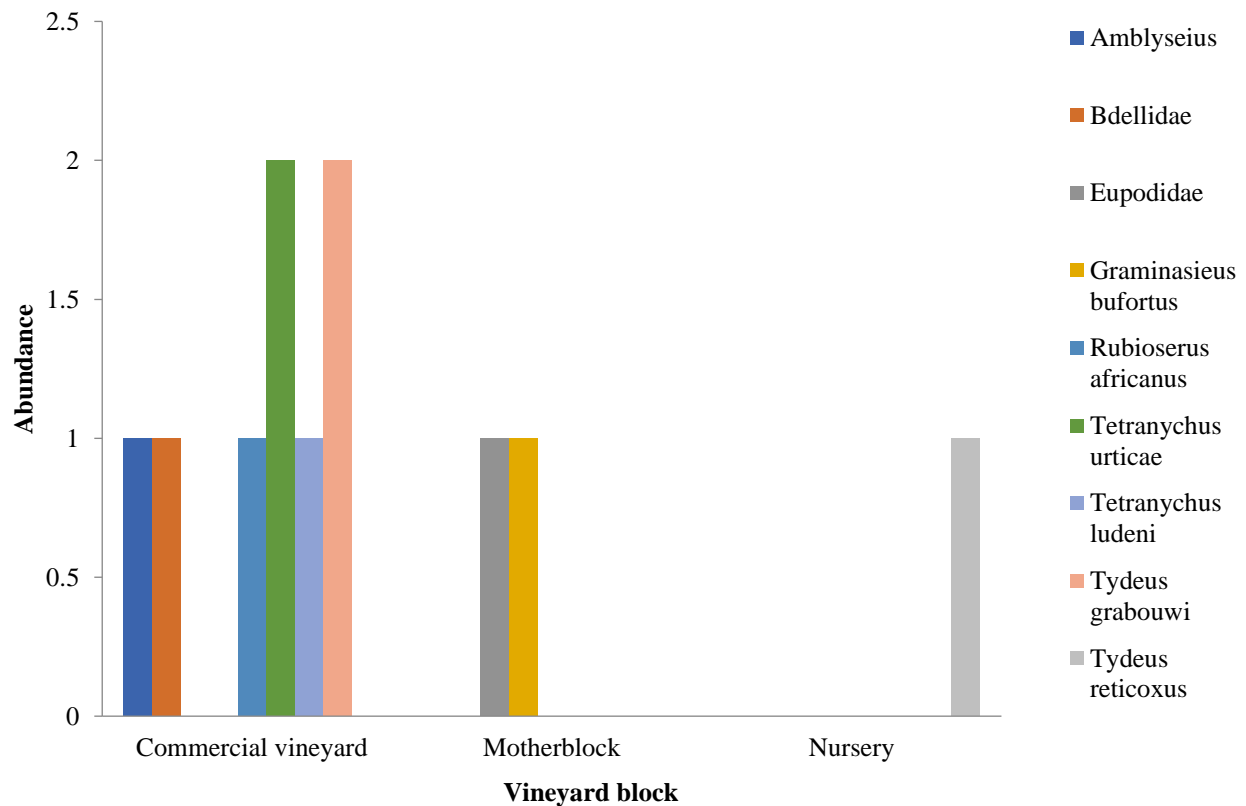


Figure 4.1 Mites that were found on the weed plant species at the three different vineyard plantings from July to October 2017.

Similar studies have shown that mites do tend to migrate from crop to weed and vice versa (de Villiers & Pringle 2010, Botha & Pringle, 1995). *Tetranychus urticae*, *Euseius rubiocolus* (van der Merwe & Ryke) and *Neoseiulus californicus* (McGregor) were abundant in both apple orchards and ground cover (de Villiers & Pringle, 2010). In the findings of the current study, alternative results were potentially due to a combination of influences; chemical treatment of weeds was still being practiced at all the vineyard plantings, and the different weed species could have had a strong influence as preferred weed species mites tend to migrate to might have been absent. The Western Cape falls under a winter rainfall region and underwent a very dry winter in 2016 - this also prevented weeds and cover crops from thriving due to a lack of rain. A minimum of 18mm of rainfall per week during March and April is needed for broad leaf weed species and grass species to reach their maximum growth before winter (Fourie, et al. 2001).

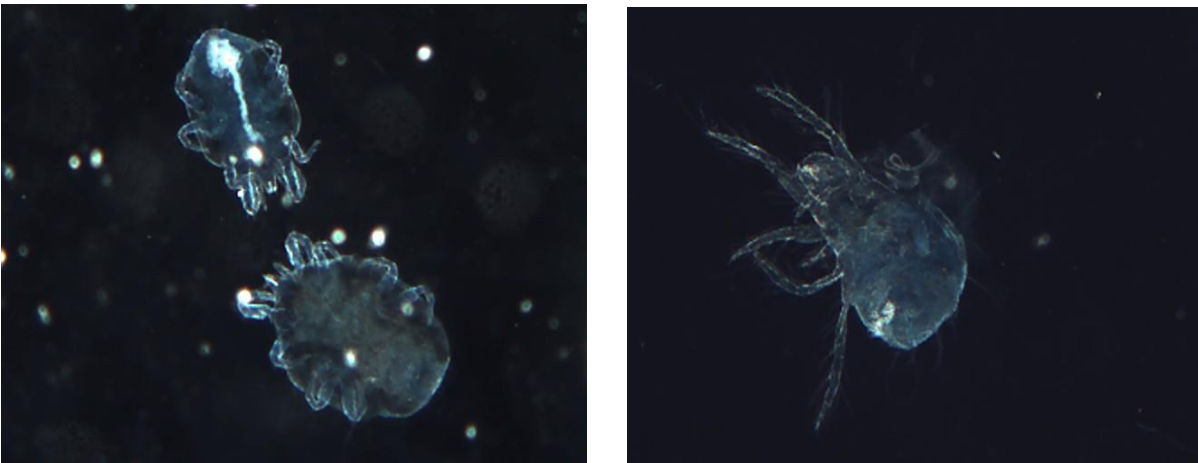


Figure 4.2: Predatory mites *Tydeus grabouwi* (a), phytophagous mite *Tetranychus ludeni* (b). General aspect.

The mites found on the cover crops and weeds differed from the mite species found on the vines (Table 4.1). There were phytophagous and predatory mites both occurring between and on the vines, but the dominant predators and phytophagous mites were absent. Thus, vineyard mites do not appear to migrate to cover crops and weeds during the winter months when sampling was conducted.

Tetranychus ludeni Zacher (Fig 4.2b) was present on ground cover plants and vineyards. The only predatory mites that were found on both vines and weeds, were Bdellidae and *T. grabouwi* (Table 4.1). *Tydeus grabouwi* (Fig 4.2a) feed only on slow-moving and inactive mites that are undergoing moulting, thus it is not considered an effective predator at controlling phytophagous mites (Pringle & Campbell, 2016). *Tydeus* species feed on a range of plant sources like pollen, honeydew, fungi and small organisms (Gerson, et al. 2003; Duso, et al. 2005). Bdellidae, *T. grabouwi* and *T. ludeni* could be the only mites that migrate from the vine to weeds during winter months (Table 4.1).

Tetranychus ludeni, *T. grabouwi* and Bdellidae were present in both categories (Table 4.1). Despite finding a co-occurrence, the mites present are not the dominant predatory mites or main grapevine pests. The dominant predatory mites present on grapevine were *Euseius addoensis* and *Typhlodromus praeacutus* with the main plant-feeding pest being *Brevipalpus lewisi* (Chapter 2).

Table 4.1: The predatory and phytophagous mites that occurred in the vineyards as well as the ground cover from 2016 to 2018.

PREDATORY MITES	VINEYARDS	WEED PLANTS
<i>Agistemus collyerae</i>	√	
<i>Amblyseius</i> sp		√
<i>Anystis baccharum</i>	√	
<i>Balaustium</i> sp	√	
Bdellidae	√	√
Eupalopsellidae	√	
Eupodidae		√
<i>Eusieus addoensis</i>	√	
<i>Graminaseius bufortus</i>		√
<i>Hemicheyletia</i> sp	√	
Iolinidae	√	
<i>Neoseiulus barkeri</i>	√	
<i>Pronematus ubiquitous</i>	√	
<i>Rubioserus africanus</i>		√
<i>Tydeus grabouwi</i>	√	√
<i>Tydeus reticoxus</i>		√
<i>Tydeus</i> sp	√	
<i>Typhlodromus praeacutus</i>	√	
<i>Typhlodromus saevus</i>	√	
<i>Typhlodromus</i> sp	√	
PHYTOPHAGOUS MITES		
<i>Aplonobia</i> sp		√
<i>Brevipalpus californicus</i>	√	
<i>Brevipalpus lewisi</i>	√	
<i>Brevipalpus obovatus</i>	√	
<i>Brevipalpus phoenicis</i> complex	√	
<i>Tetranychus ludeni</i>	√	√
<i>Tetranychus</i> sp	√	
<i>Oligonychus vitis</i>	√	
<i>Tetranychus urticae</i>	√	

The correspondence analysis has shown to be less informative (Fig 4.3). Both dimension 1 and 2 together only accounted for 40% of the variation on the graph, indicating relatively weak associations, and therefore not providing additional information about the mite occurrence on the weed plant species. This weak association could indicate that the mites show no real preference for any weed species, and utilize them at random. It should be noted that no mites were found on the grass, *Triticale* (*Triticale* v. Usgen 18) and all weeds with mites were broadleaf species.

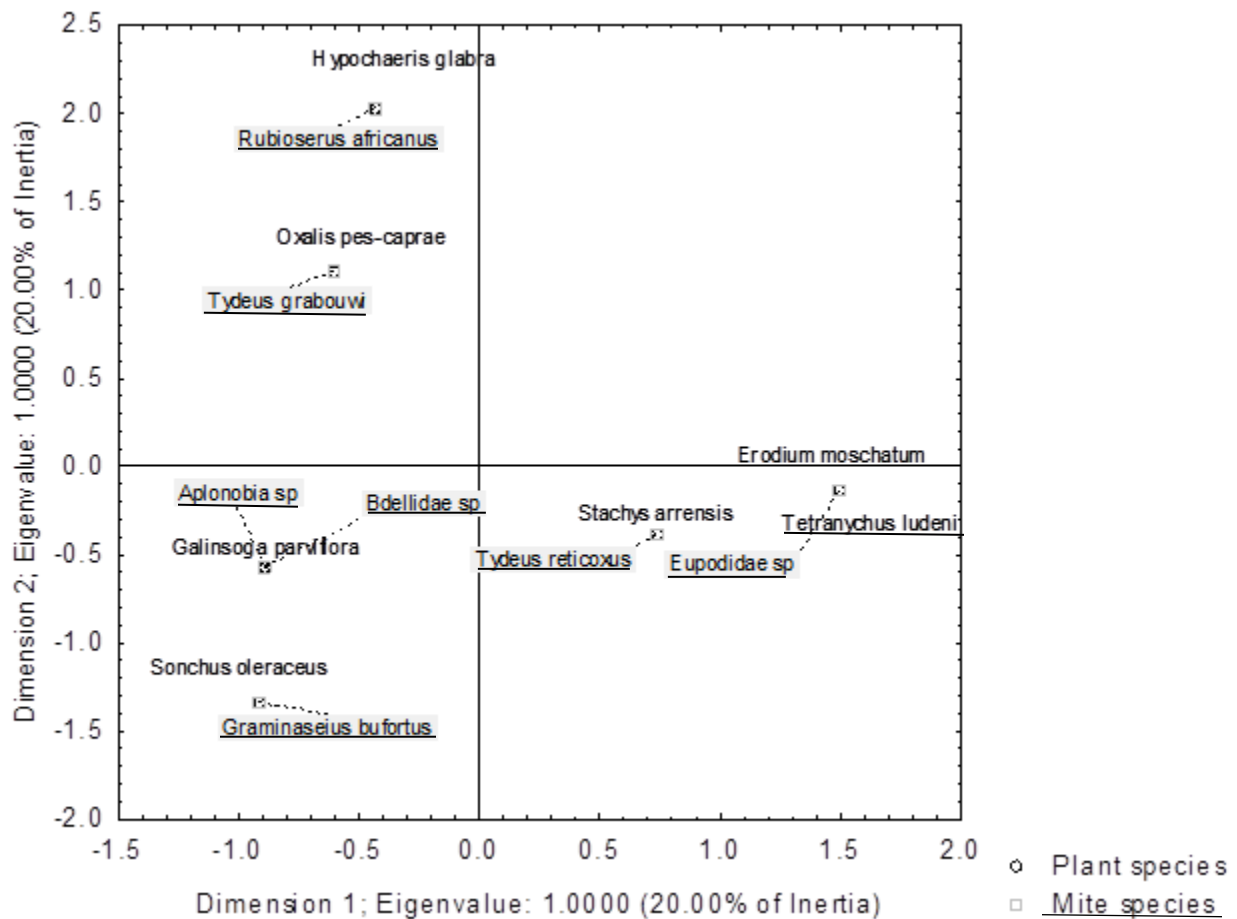


Figure 4.3: The correspondence analysis displaying the mite species association to the weed plant species occurring in commercial vineyards, motherblocks and nursery material during July to October 2017.

The total abundance of mites found on each weed species is given in Fig 4.4, with relatively few mites recovered from weeds. All the mites were predatory except for *Aplonobia* sp and *Tetranychus ludeni*, which are both spider mites.

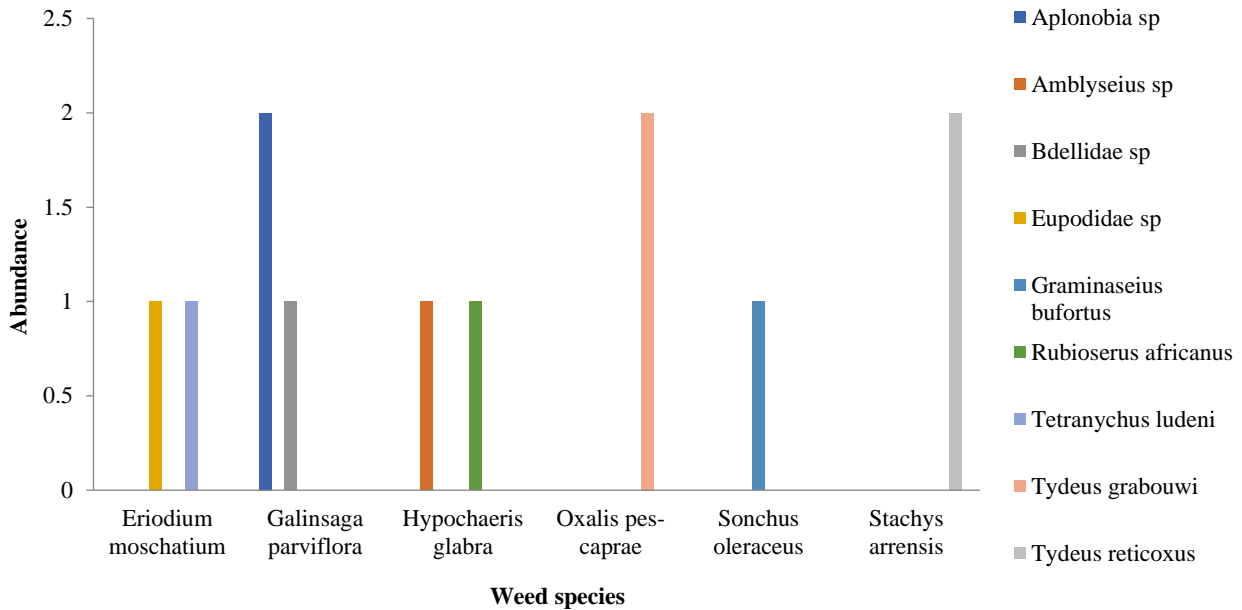


Figure 4.4: The number of mites present on broadleaf weed plant species collected from July to October 2017 at commercial vineyards, motherblocks and nurseries in the Wellington region, South Africa.

No two weed species harboured the same mite species (Table 4.2). Very low abundances of mites were found on these weed species, which may be improved with longer sampling over more seasons. The data do indicate that a higher diversity of weeds, harbor a higher diversity of mites at low abundance, which is important for a more stable system (Diaz & Cabido, 2001).

Table 4.2: List of weed plant species found at each of the three vineyard plantings from July 2017 to October 2017.

WEED PLANT SPECIES

Coronopus didymus L.
Cotula turbinata L.
Erodium moschatum (L.)
Euphorbia peplus
Galinsoga parviflora Cav.
Helmintholoca echioides
Hypochaeris glabra L.
Hypochaeris radicata
Lepidium africanum (Burn.f.) DC. Subsp. *africanum*
Lupinus angustifolius L.
Malva parviflora
Medicago polymorpha L.
Melia azedarach
Oxalis pes-caprae L.
Picris echioides
Plantago lanceolata
Raphanus raphanistrum L.
Senecio pterophorus
Sonchus oleraceus L.
Stachys arvensis L.
Tagetes minuta L.
Triticale (*Triticale* v. Usgen 18)
Vicia berghalensis L.
Vicia sativa subsp. *Sativa*

4.4 CONCLUSION

The aim of this survey was to confirm if vineyard mites utilise ground cover plants like weeds and cover crops during the winter months when the vines are dormant. Commercial vineyards had the highest diversity of weed species present and therefore it had the highest diversity of mite species present. The lowest diversity in both weed species and mite species was seen in nurseries. The mites that were found on both weeds and grapevine were the predators *T. grabouwi* and *Bdellidae* and phytophagous mite *T. ludeni*. Each weed species harboured different mite species. Thus, a higher weed diversity is encouraged to provide a more stable system (ie. prevention of pest peaks and greater diversity of predators).

Ground cover plants have been shown to be beneficial to the agricultural industry as they can act as corridors between crops, encouraging movement of beneficial mites and insects (Altieri, et al. 2010). Incorporating natural vegetation also leads to more predatory mites present in your crop (Altieri, 1994; Luna & House, 1990; Olkowski, et al. 1991). Phytoseiid mites have shown to be effective at controlling mite pests on a range of crops, including grapevine (McMurtry & Croft, 1997) with high densities of these predators present in weedy and native vegetation (Boller, et al. 1988; Tsolakis, et al. 1997; Kreiter, et al. 2000; Tixier, et al. 2000a, b; Duso, et al. 2004; Barbar, et al. 2005). Thus, these plants can act as reservoirs for natural enemies, alternate hosts and food resources. Due to the close connection between mite development and plant traits, the plant composition can affect the diversity and abundance of phytoseiid mites (Tixier, et al. 1998, 2000b; Kreiter, et al. 2001).

Therefore, the plant species likely determined the mite species composition. Due to the drought situation, there may not have been sufficient weeds available for mites to migrate to. This could be why there were not many mites present in the ground cover and the reason why mites from the grapevine did not migrate to the weedy plants. The low inertia of the correspondence analysis suggests that mite species did not show any preference to any specific ground cover species during the sampling period. This study shows that the dominant phytophagous mites did not migrate from the vineyard to alternate plant hosts such as weedy plants and cover crops in the current study, but it may be different if sufficient rain in winter provided a more diverse and dense ground cover. It is therefore recommended to repeat this survey during more abundant rainfall years to confirm if these trends are indeed rainfall related.

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CHAPTER 5

DISCRIPTION OF A NEW PHYTOSEIIDAE SPECIES

Subgenus *Typhlodromus* Scheuten

Typhlodromus Scheuten 1857: 111

5.1 INTRODUCTION

Typhlodromus falls within the family Phytoseiidae and the subfamily Typhlodrominae. Phytoseiidae consist of 2436 species in 91 genera (Demite, et al. 2014). Typhlodrominae have 732 described species in 23 genera. The genus consists of two subgenera; *Typhlodromus* (*Typhlodromus*) and *Typhlodromus* (*Anthoseius*).

Several species of the genus *Typhlodromus* are important controllers of phytophagous mite populations in orchards and vineyards (Kemmitt, et al. 2015).

Typhlodromus vary between selective and generalist predators (McMurtry, et al. 2013). *Typhlodromus* is a common predator on fruit crops with some species being effective at controlling pests. *Typhlodromus pyri* Scheuten have been shown to regulate the European red mite (*Panonychus ulmi* Koch) and the Apple rust mite (*Aculus schlechtendali* (Nalepa)) on apple in New Zealand and the United Kingdom (Wearing, et al. 1978; Solomon, et al. 1993). *Typhlodromus pyri* has also shown potential in controlling eriophyids and tetranychids in Italy (Duso & Vettorazzo, 1999). *Typhlodromus pyri* is capable of surviving on alternate food sources like plant sap, fungi spores and pollen (Johnsen & Hansen, 1986; Croft, et al. 1995; Ripka, 1998, Pozzebon & Duso, 2008; Pozzebon, et al. 2009).

Species of this genus form an important part in integrated pest management programmes (Croft, 1990; McMurtry & Croft, 1997; Desneux, et al. 2007). *Typhlodromus exhilaratus* Ragusa and *Typhlodromus phialatus* Athias-Henriot have been recognised as effective at protecting vineyards in Southern Europe (Kreiter, et al. 2000; Moraes, et al. 2004).

5.2 MATERIALS AND METHODS

Samples of grapevine, *Vitis vinifera* L. were collected bi-monthly at four farms in Wellington (-33.936179; 18.862899) (Western Cape, South Africa). Each farm contained a commercial vineyard, motherblock and nursery. Samples were collected from November 2016 to May 2017 and November 2017 to April 2018. Ten random vine branches and sub-branches were collected at each block, with a total of 12 blocks sampled. Samples were separately packaged and sealed in plastic bags, returned to the laboratory and kept in the refrigerator (10°C) until further inspection. Laboratory work entailed running the vine leaves through a mite brushing machine (Fig 5.1); the machine has two brushes that comb the mites off the leaf from which the mites fell onto a Perspex plate. The plate was then examined under a stereo microscope and mites were collected into 70% ethanol. Mites were cleared in lactic acid and slide mounted in PVA solution following the general protocols in Krantz & Walter (2009). The mites were identified with a compound microscope with phase contrast and measured with a Zeiss Image Analysing System, Zen 2.3 lite. All measurements were in micrometers (μm). The Phytoseiidae database Ferragut & Ueckermann (2012), Tixier, et al (2016) and Stathakis, et al (2012) were used in confirming the new species.

Type specimens were deposited in the National Collection of Arachnida (NCA) in Pretoria, South Africa.



Figure 5.1. The Leedom engineered leaf brushing machine.

5.3 RESULTS AND DISCUSSION

5.3.1. Materials examined

The subgenus *Typhlodromus* was described as follows: Dorsal setae j1, j3, j4, j5, j6, J2, z2, z3, z4, z5, Z4, Z5, s4, s6, S2, S4, r3 and R1 present. Ventral setae JV1, JV2, JV3, JV4, JV5, ZV1, ZV2, ZV3 present. JV3 present or absent. Setae S5 are absent. Z4 and S4 are transversely aligned.

FEMALE (N = 5) (Fig 5.2):

Dorsum (Table 5.1). Dorsal shield 322 (316 – 330) long and 162 (160 – 181) wide. With 4 pairs of solenostomes. Setae j1 22 (19 – 23), j3 31 (30 – 32), j4 16 (16 – 20), j5 16 (16 – 20), j6 22 (19 – 23), J2 20 (23 – 25), z2 22 (18 – 22), z3 26 (23 – 29), z4 24 (24 – 31), z5 19 (18 – 20), Z4 47 (47 – 55), Z5 69 (69 – 71), s4 30 (31 – 33), s6 35 (34 – 37), S2 39 (39 – 43), S4 41 (40 – 46), r3 21 (21 – 27), R1 30 (24 – 30). Setae serrate, except j1, j4, z5 smooth, and J5 and R1 sometimes smooth. Peritreme extending to level between j3 and z2.

Venter (Table 5.1). Sternal shield mostly smooth, with few lateral striae; posterior margin with medium lobe between ST1 – ST2 31 (31 – 37), ST2 – ST2 57 (56 – 60). Genital shield smooth; distance between ST5 – ST5 54 (54 – 60). Ventrianal shield striate, pentagonal, with anterior margin straight, 96 (96 – 108), 84 (84 – 99) wide at level of ZV2 77 (74 – 88) wide at level of anus, pre-anal pores absent. Caudoventral setae smooth JV5 60 (54 – 63).

Spermatheca. Calyx saccular with distal half thick walled. Atrium incorporated in calyx.

Legs. Macrosetae sharp-tipped: Sge IV 25, Sti IV 25, St IV 46 (42 – 50). Chaetotaxy: genu II 2-2/0, 2/0-1; genu III: 1-2/1, 2/0-1.

Table 5.1: Comparisons between measurements (in μm) of the specimens (total five; all female)

	HOLOTYPE	PARA SPECIES (FEMALE) (SLIGHTLY DAMAGED)	PARA (F)	PARA (F)	PARA (F)	PARA (F)
LENGTH OF DORSAL SHIELD	322	317	316	330	318	306
WIDTH OF DORSAL SHIELD	162	174	167	168	181	160
st1 - st2	31	37	35	31	34	32
st2 - st2	57		56	60	61	60
st5 - st5	54	58	60	56	55	55
VENTRAL ANAL PLATE	96	108	105	103	107	140
B. Zv2 - Zv2	84	87	85	99	89	92
B. ANAL OPENING	77	74	81	84	5	88
JV5	60	63	61	58	54	57
st IV	46	47	43	50	42	52
sti IV	25	24	24	24	24	28
sge IV	25	23	23	27	25	24
j1	22	23	20	19	20	19
j3	31	31	31	32	30	32
j4	16	18	17	19	17	20
j5	16	18	20	19	18	18
j6	22	22	23	23	19	22
J2	20	23	24	24	25	24
z2	22	18	18	19	19	18
z3	26	28	23	29	26	27
z4	24	26	27	26	25	31
s4	30	32	33	33	33	31
s6	35	34	34	38	38	37
S2	39	41	41	40	39	43
S4	41	40	46	41	43	40
Z4	47	50	55	50	48	47
Z5	69	71	69	71	70	69
J5	5	6	6	7	6	7
z5	19	20	18	19	19	20
r3	21	22	25	27	28	27
R1	30	26	24	26	25	26

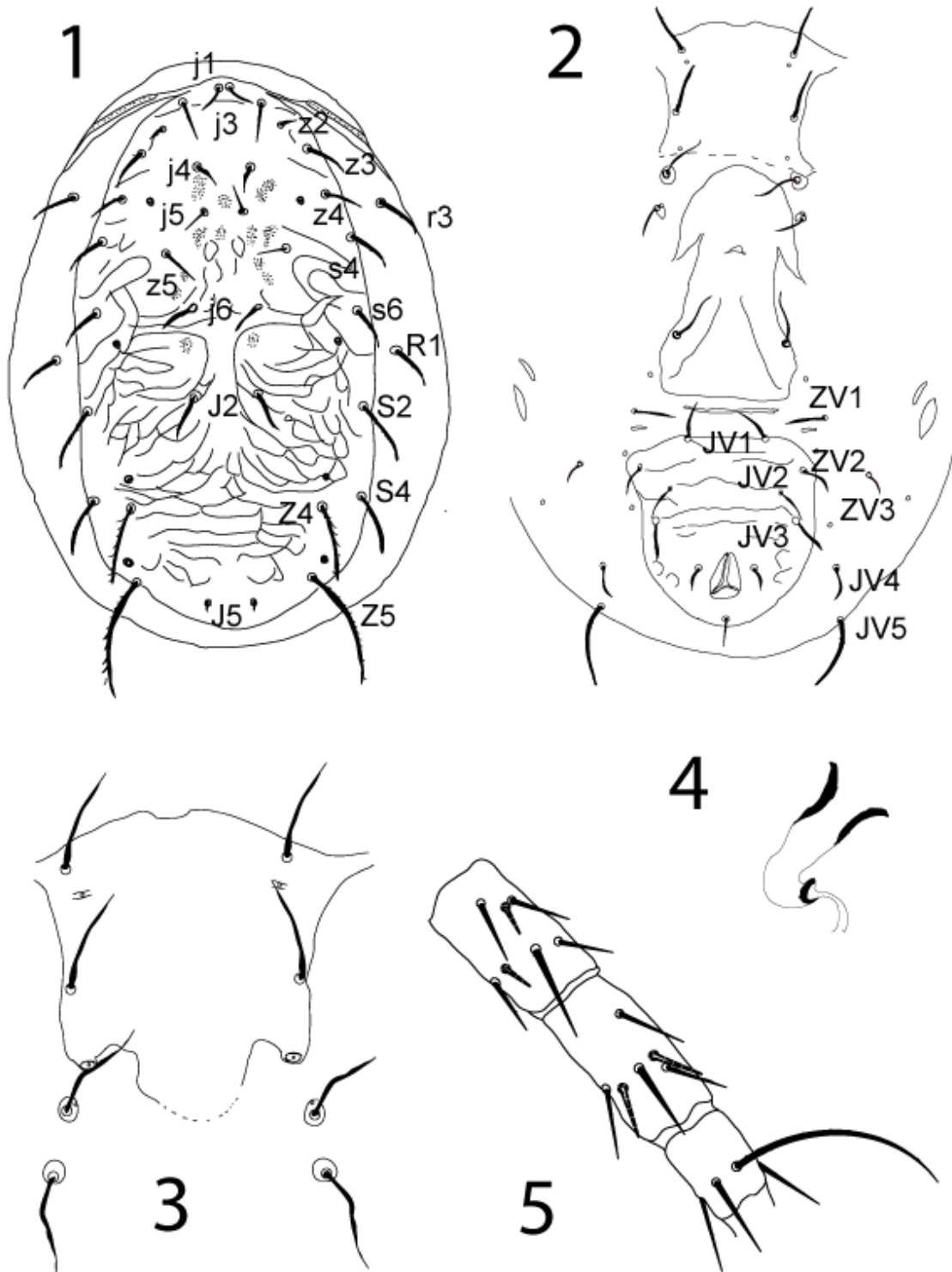


Figure 5.2: Dorsal (1) and ventral (2) view of idiosoma. Spermatheca (4) and genu, tibia and tarsus of leg IV (5).

The holotype female and four paratype females were collected from *vitis vinifera* L. Two females were collected in Wellington at Cordiersrus farm (33° 36'13.21'' S; 19° 0'46.68'' E) on the cion motherblock; January 2018. The other three females were also collected in Wellington, but on the commercial vineyard at Uitkyk farm (34° 24'1.50'' S; 19° 13'43.13'' E) between February and March 2017. All specimens were collected by Mia Vermaak.

5.3.2. Discussion

This species belongs to a group of species with 4 pairs of solenostomes (*gd2*, *gd6*, *gd8*, *gd9*) on the dorsal shield, ventrianal shield pentagonal and without preanal pores. We reduced the 29 species with similar characters to 23 based on synonyms and possible synonyms suggested in Ferragut & Ueckermann, 2012; Stathakis, et al. 2012 and Tixier, et al. 2016.

This *Typhlodromus* species differs from the 23 species as follows: *T. (T.) octogenipilus* Kreiter, et al.; *T. (T.) sirikariensis* Stathakis, et al.; *T. (T.) antakyensis* Stathakis & Döker, et al. and *T. (T.) mazarii* Allam, et al. have eight setae on genu II. *T. (T.) setubali* Dosse and *T. (T.) moroccoensis* Denmark have six setae on genu II. In *T. (T.) laurae* Arutunjan and *T. (T.) knisleyi* Denmark setae Z4 is .5 the length of Z5 but with this new species Z4 is about .7 the length of Z5. It also differs from these two species in the shape of the spermathecal, which is short (16-17), saccular and thick walled but longer tube-like/saccular with only distal half thick walled in the new species.

The calyx of the spermatheca of *T. (T.) personatus* Karg, *T. (T.) bichaetae* Karg and *T. (T.) cotoneastri* Wainstein is proximally slender, tube-like and flared distally but in the new species it is saccular/tube-like with only distal half thick walled. Calyx of spermatheca of *T. (T.) exhilaratus* Ragusa and *T. (T.) atlanticus* Ferragut with a short neck and small bulbous atrium, setae *j1* and *j3* subequally long and peritreme reach to level between setae *j1* and *j3* but, in the new species the atrium is incorporated in the saccular calyx, setae *j3* are longer than *j1* and the peritreme reach to

between j_3 and z_2 . *T. (T.) atlanticus* further differs in that setae Z_4 are clearly shorter than distance to Z_5 and the latter two setae are also shorter than those in the new species, 27-33 and 36-41 versus 47-55 and 69-71, respectively. Calyx of spermatheca of *T. (T.) beglarovi* Kuznetsov and *T. (T.) olympicus* Papadoulis & Emmanouel is also with a short neck and small bulbous atrium which differs from that of the new species as already mentioned in the previously.

Both these two species differ from the new species in that setae Z_4 are clearly shorter than the distance to setae Z_5 instead of extending to Z_5 . The following nine species resemble the new species in that the calyx of the spermatheca is without a neck or constriction between it and the atrium. However, *T. (T.) klimenkoi*, Kolodochka differs from them all in the short peritreme reaching to a level between setae z_3 and z_4 and in that the peritreme is not uniformly stippled but only with a central core. *Typhlodromus (T.) tiliae* Oudemans differs from the new species in that setae Z_4 is shorter than the distance to Z_5 and peritreme reach to a level between z_2 and z_3 (j_3 and z_2 in new species). *Typhlodromus (T.) floresiensis* Ferragut, *T. (T.) morellensis* Ferragut and *T. (T.) mutatus* Kolodochka can be distinguished from the new species in that setae Z_4 are also shorter than distance to Z_5 and setae Z_4 and Z_5 are clearly shorter than 40 (19-30) and 70 (36-47), respectively. The bell-shaped calyx of the spermatheca, setae j_1 and j_3 equally long and shorter setae Z_4 (33-36 vs 47-55) and Z_5 (50-54 vs 69-71) distinguished *T. (T.) erensti* Ragusa & Swirski from the new species.

The peritreme extending to between setae j_1 and j_3 versus j_3 and z_2 in new species, shorter setae Z_4 (38 vs 47-55) and Z_5 (54 vs 69-71) and short, broad, thick walled and tubular calyx of spermatheca of *T. (T.) phialatus* Athias-Henriot separate it from the new species. The new species, however, is similar to *T. (T.) athiasae* Porath & Swirski, but the long, thick walled tubular calyx of the spermatheca distinguishes it from the new species, only the distal half of the calyx of the latter is thick walled.

Specimens of this new species were collected with other phytoseiidae; *Typhlodromus preaecutus* van der Merwe, *Typhlodromus saevus* van der Merwe and *Euseius addoensis* (McMurtry) as well as Tenuipalpidae *Brevipalpus lewisi* McGregor.

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CHAPTER 6

GENERAL CONCLUSION

This study provides knowledge in the identification of phytophagous and predatory mites occurring in vineyards, including their seasonal cycles, as well as the confirmation of the presence of the invasive *Brevipalpus lewisi* McGregor as a pest on grapevine in the Western Cape. These findings relate to the commercial vineyards, motherblocks, nurseries and an organic vineyard that were surveyed from 2016 to 2018.

In the general introduction attention was given to the importance of mites in agriculture. The predatory and plant-feeding mites known to occur in grapevine were explained and the known beneficial and the detrimental families and species occurring on grapevine identified. The importance of biological control and Integrated Pest Management (IPM) of mites in agriculture was discussed. From this review, it was clear that information on mites in vineyards is lacking, leading to the formulation of the main objectives of the current study, namely: strengthening the knowledge of the occurring mite diversity in commercial vineyards, motherblocks and nurseries; to identify any pest species present in the vineyard plantings; compare a conventional commercial vineyard mite composition to that of an organic commercial vineyard; to determine the seasonal population cycles of predatory and phytophagous mites in vineyard plantings; as well as to determine if vineyard mites migrate to alternate host plants during winter time.

In Chapter 2, the predatory and phytophagous diversity of mites in commercial vineyards, nurseries and motherblocks were established. A surprisingly higher diversity of both predatory and phytophagous mites was present in commercial vineyards with the lowest in motherblock material. *Brevipalpus lewisi* was the dominant phytophagous pest in commercial vineyards, motherblocks and nurseries. Tetranychidae were in comparison not as abundant and quite sporadic in their temporal occurrence. *Brevipalpus lewisi*, which was first found in South Africa in 2015, was

confirmed to be a new invasive pest in vineyards in the Western Cape. Its population size by far surpassed that of the other plant-feeding mites and other *Brevipalpus* species. Jones (1967) and Buchanan, et al (1980) have reported that in Victoria, Australia, early in the season *B. lewisi* would only be present at the base of the grape canes and later would move on to the green tissues where it caused scarring of the grapes and stems. Continued feeding resulted in vines wilting and drying out. None of these symptoms were observed during the course of this study. Similarly, no physical symptoms of damage were observed during the two years of data collection. Over the past three years, *B. lewisi* quickly established itself without obstacles. As yet, it does not hold any threat to the industry as it does not cause serious damage to the vineyards. This poses a phytosanitary threat. National borders are not as effective in controlling the spread of pests, especially mites (Africander, 2018). The major pests of concern are fruit flies, bud mites (Eriophyidae), codling moth and the false codling moth. The export and control of table grapes are also a greater priority than wine grapes (Africander, 2018; www.agri-intel.com).

The dominant predatory mites found in this study were *Euseius addoensis* McMurtry and *Typhlodromus praeacutus* van der Merwe in the nursery material and commercial vineyards, and *T. praeacutus* and *Neoseiulus barkeri* Hughes in motherblocks. During the survey, a new phytoseiid mite of the *Typhlodromus* family was discovered, and described for the first time (Chapter 5). It is distinguishable from other *Typhlodromus* species as it has shorter Z4 setae than Z5 and both are longer compared to other species. Half of its calyx is thick-walled and has a short saccular spermatheca. A high predatory diversity, based on relative abundance, was also found in the organic commercial vineyard. *Euseius addoensis* was by far the most abundant predatory mite species. Other abundant predators were *T. saevus* and *Pronematus ubiquitous* McGregor. No plant-feeding mites were observed during the organic vineyard survey, which displayed a more ‘balanced’ system where the phytophagous mites were suppressed to undetectable levels.

The diversity of predatory mites in the conventional commercial vineyards shows potential as biocontrol agents. Commercial vineyards tend to be dominated by specialist phytoseiid mites (Hanna, et al. 1997) with generalist phytoseiids being more abundant in unsprayed vegetation

(Boller, et al. 1988). A summary of the phytophagous and predatory mites occurring in South African vineyards based on previous studies and the current survey results, are listed in Table 6.1, with the mites that were present in this study highlighted in bold. In the present study, the phytoseiid mites in the sprayed vineyards were all classified as generalists (McMurty & Croft, 1997), with similar diversity in both the conventional vineyards as well as the organic vineyards monitored in this study. Generalist phytoseiid mites not only feed on mites but utilize other food sources, which include tydeid mites, eriophyid mites, pollen and honeydew (see Chapter 1), which act as bridging hosts when one food source is absent (McMurty & Croft, 1997). We can conclude that the conventional farms were more diverse than expected and the organic farms less diverse than expected. The surveyed organic vineyard did make use of treatments such as applying sulphur which tends to affect mite abundance and diversity. Based on the results of this study, it would be best to apply treatments for *B. lewisi* before the end of the season, as that is when they were most abundant. With that, it would be important to keep the predatory mites into account which were most abundant during January to March. There are no registered miticides for Tenuipalpidae and Tetranychidae (www.agri-intel.com).

Table 6.1: A summary of the established phytophagous and predatory mites that occur in South African vineyards based on the current study as well as previous research.

PHYTOPHAGOUS VINEYARD MITES		
SUPER FAMILY/ FAMILY	SPECIES	REFERENCES
<i>Tetranychidae</i>	<i>Oligonychus coffeae</i> Nietner	Smith Meyer & Rodrigues, 1966; Smith Meyer, 1974; 1981, 1987, 1996; Jeppson, et al. 1975; Annecke & Moran, 1982; Smith Meyer, et al. 1987, 1989, 1990
	<i>Oligonychus mangiferus</i> (Rahman & Sapra)	Moutia 1958; Smith Meyer 1974, 1987; Jeppson, et al. 1975; Annecke & Moran 1982
	<i>Oligonychus vitis</i> Zaher & Shehata	
	<i>Panonychus citri</i> McGregor	<i>Panonychus citri</i> , [s.a.]
	<i>Panonychus ulmi</i> (Koch)	Smith Meyer 1974, 1981, 1987; Jeppson, et al. 1975; Annecke & Moran 1982; Pringle, et al. 1986; Smith Meyer, et al. 1990; Botha, 1993
	<i>Schizonobia viticola</i> Meyer	Smith Meyer, 1987; Petrushov & Belyaeva, 1987
	<i>Tetranychus kanzawai</i> Kishida	<i>Tetranychus kanzawai</i> , [s.a.]
	<i>Tetranychus ludeni</i> Zacher	Smith Meyer & Ryke, 1959
	<i>Tetranychus urticae</i> Koch	Coates, 1974; Smith Meyer, 1974, 1981, 1987, 1996; Jeppson, et al. 1975; Duncombe, 1977; Jordaan, 1977; Dippenaar-Schoeman & Meyer, 1979; Annecke & Moran 1982; Botha, 1984; Botha, et al. 1986; Pringle, et al. 1986; Meyer, et al. 1987, 1989, 1990; Botha, 1993; Du Toit, 1993; Smith Meyer & Honiball, 1998
<i>Eriophyoidea</i>	<i>Colomerus vitis</i> (Pagenstecher)	<i>Colomerus vitis</i> , [s.a.]
	<i>Eriophyes vitis</i> (Pagenstecher)	Petrushov & Belyaeva, 1987, Smith Meyer, 1981
<i>Tenuipalpidae</i>	<i>Brevipalpus californicus</i> (Banks)	<i>Brevipalpus californicus</i> , [s.a.]
	<i>Brevipalpus lewisi</i> McGregor	Saccaggi, et al. 2017
	<i>Brevipalpus obovatus</i> Donnadieu	
	<i>Brevipalpus phoenicis</i> (Geijskes) complex	
<i>Tarsonemidae</i>	<i>Polyphagotarsonemus latus</i> (Banks)	<i>Polyphagotarsonemus latus</i> , [s.a.]
PREDATORY VINEYARD MITES		
<i>Anystidae</i>	<i>Anystis baccharum</i> Linnaeus	
<i>Iolinidae</i>	<i>Pronematus ubiquitous</i> McGregor	
<i>Phytoseiidae</i>	<i>Euseius addoensis</i> McMurtry	de Villiers & Pringle, 2011
	<i>Neoseiulus californicus</i> (McGregor)	de Villiers & Pringle, 2011
	<i>Neoseiulus barkeri</i> Hughes	
	<i>Typhlodromus praeacutus</i> van der Merwe	
	<i>Typhlodromus saevus</i> van der Merwe	
	<i>Typhlodromus pyri</i> Scheuten	
<i>Stigmaeidae</i>	<i>Agistemus collyerae</i> Gonzalez-Rodriguez	Saccaggi & Ueckermann, 2018; unpublished data, 2016
	<i>Agistemus tranatalensis</i> Meyer	
<i>Tydeidae</i>	<i>Tydeus grabouwi</i> Meyer & Ryke	de Villiers, 2006

*mites indicated in bold were found in the present study

Phytoseiid mites can be an essential element in pest management programs (McMurtry, 1992). *Neoseiulus* species have been successfully incorporated into various management plans. (McMurtry & Croft, 1997). *Neoseiulus californicus* is able to adapt to fluctuating prey populations and temperature changes and thus provides consistent pest suppression (Croft, et al. 1998; Greco, et al. 2005; Escudero & Farragut, 2005). *Neoseiulus californicus* has also proven to be successful at keeping *Tetranychus urticae* below threatening levels in strawberry fields in south eastern United States (Fraulo & Liburd, 2007). *Neoseiulus cucumeris* (Oudemans) is commonly used to control the western flower thrip, *Frankiniella occidentalis* (Pergande) in greenhouse cucumber in the Netherlands (Messlink, et al. 2005). *Typhlodromus* and *Amblyseius* species have also been successfully used as control agents against spider mites (McMurtry & Croft, 1997). *Typhlodromus doreenae* have shown potential to regulate Tenuipalpidae on grapevines in Australia (James & Whitney, 1991). It is also confirmed that *Amblyseius aberrans* can control tetranychid populations to a low population density (Duso, et al. 1991). The seasonal cycles established for the main predatory and plant-feeding mites at all three components of vineyard development would help develop IPM strategies as treatments can be target-based and applied more precisely. Although the main predator, *E. addoensis* and the main pest, *B. lewisi*'s seasonal cycles are not well synchronised – further research can determine how best to utilise *E. addoensis* in controlling other potential threats, like thrips (Thysanoptera), and the determination of seasonal population cycles of potential predators like *A. collyerae* for *B. lewisi* can be measured. It is likely that *B. lewisi* and *A. collyerae* were introduced into South Africa together but Saccaggi & Ueckermann (2018) also found first detections of *A. collyerae* dating back to 1991. *A. collyerae* could have been preying on *B. lewisi* as they were detected together. Yet *A. collyerae* has not established itself as a dominant mite predator – this could be due to competition with the large diversity of predatory mites which are present in our vineyards. Although only a few *A. collyerae* were present in the present study, it was been shown to prey on *B. lewisi*, unlike *E. addoensis* and *T. praeacutus* that do not show preference for *B. lewisi*. *Agistemus collyerae* did occur during the seasonal time when *B. lewisi* was most abundant, but it was detected only twice in very low numbers in commercial vineyards in March 2017 and February 2018.

Regular monitoring of *B. lewisi* is important to assess the current distribution in the Western Cape and if necessary, prevent any further spread, rather than trying to implement management after the mite has already become established on a broad scale. Large scale surveys in the Western Cape were not an objective of this study, but the present results indicate that *B. lewisi* may already be well established in this Province. Scheduled surveys should be done on fruit crops in South Africa, targeting other Provinces. Currently *B. lewisi* is only present on grapevine in the Western Cape and Northern Cape (Saccaggi, et al. 2017). It has not been found on citrus, yet there is a risk of establishment on citrus as citrus is a host and the mite vectors Citrus leprosis virus (Lovisolo, 2001). Citrus leprosis-like symptoms have been reported in South Africa, but vectors and agents have not been established (Rodrigues & Childers, 2013).

Factors that were limitations in the study were: time constraints, working without extra assistance and having, to follow farmers' directions, and a lack of ecological information on mites. Although two years of data collection was adequate, more factors could have been included in an extended study such as climate. To be able to expand the study, a whole team collecting data would be needed to make investigations in vineyards between provinces logistically feasible. There is a general lack in available information regarding mite ecology as they have not been studied in enough detail to be able to predict or substantiate their actions, functions and impacts during the research study. Improvements could be made by extending the time period of the survey and to include more vineyards and monitoring for more seasons. The study only surveyed one organic vineyard without any organic motherblocks or nurseries. By extending or adding more organic farms more comparisons can be made and better conclusions could be drawn between the mite diversity and the farmers chosen management strategy. By adding more seasons, the average time period for phytophagous and predatory mites present in the vineyards can be calculated. Additionally, including microclimatic data would also be beneficial as it can indicate to what extent weather conditions are driving mite behavior and abundance. It will test mites' degree of sensitivity towards temperature/humidity changes. Incorporating leaf age and the nitrogen concentration in leaves will create a more in depth look at the nutrient preference of the mites. Combining this with seasonal cycle patterns would lead to more robust conclusions as all these

components are linked. For example, an increase in nutrient levels in leaves leading to higher leaf surface temperature and lowered plant defences, makes for a more attractive environment for mites (Mattson & Haack, 1987). Spider mites *Tetranychus pacificus* McGregor and *Eotetranychus willamettei* (McGregor) distributions are highly dependent on leaf age (Hanna, et al, 1996). By sampling leaves at the base (oldest) middle and top (youngest) one can compare the mite herbivore distribution within a plant. Photosynthesis and nitrogen levels tend to decline with age (Williams, 1987). *Tetranychus urticae* have been known to thrive on high nitrogen levels (van de Vrie, et al. 1972). The low abundance and diversity of spider mites from the current study was unexpected and should be investigated further. This observed pattern may be due to the dominant presence of *Brevipalpus* species, which outnumbered Tetranychidae. It was also found that *B. lewisi*, *B. californicus*, *B. phoenicis* complex and *B. obovatus* co-occurred which is unlike their previously-observed behavior where they rarely have a shared distribution (Childers, et al. 2003). In addition, *Brevipalpus* species might be outcompeting the spider mites in our vineyards.

The alternate host plant survey has proven to be less successful. Based on previous studies mites do tend to migrate from ground cover to the vine and orchards (de Villiers & Pringle, 2011; Botha & Pringle, 1995; Pringle, 2001). Alternate plant species usually carry a high diversity of predatory mites (Boller, et al. 1988; Tsolakis, et al. 1997; Kreiter, et al. 2000; Tixier, et al. 2000a, b; Duso, et al. 2004; Barbar, et al. 2005). Although there were some shared mite species found on both vine and weed species, they were not the main predatory or phytophagous mites occurring in the vineyards. Movement between weeds and vine were most frequent on *Oxalis pes-caprae*, *Eriodinium moschatium* and *Galingsaga parviflora*, indicating the importance of having ground cover in vineyards. However, alternate host species for dominant grapevine mites (both predators and phytophagous mites) could not be identified. Year-round sampling covering a larger sample size in different climatic zones would yield more potential alternate hosts. While the present study was limited by the location of motherblocks and nurseries, which are mostly found in the Wellington area. About 80% of the motherblocks and nurseries in South Africa are located in Wellington due to the ideal climate and soil content. A more general survey of commercial vineyards would be more valuable to locate alternate hosts. Monitoring the weeds and cover crops

during the season can release more pollen and thus support more generalist (pollen-feeding) phytoseiids (Moaz, et al. 2011). In addition, the continuation of weed management during winter months and the drought had a definite impact on the weed diversity and thus the mite composition on the weeds. This highlights the importance of monitoring for mites on an ongoing basis.

The response of predatory mites to pesticides depends on the alternative food resources available (Fountain & Medd, 2015). Weeds not only act as refuges but are a host to more phytophagous mites and pollen, which in turn attract predators. The provision of pollen has shown to alleviate the effects of certain pesticides on predatory mites (Pozzebon, et al. 2014; Wackers, et al. 2007; Lundgren, 2009; Lu, et al. 2014). Consequently, untreated refuges associated with the cropping system can reduce the development of pesticide resistance (Lewis, et al. 1997). This also suggests that one can incorporate biological control with chemical control in vineyards. Pesticides must be applied in such a way that phytoseiid mites would be able to re-enter the sprayed areas (Fountain & Medd, 2015). Repeatedly applying pesticides would be detrimental to predatory mite populations. Over the long term, the presence of weedy vegetation could improve crop yield (Dennis & Fry, 1992; Jones, et al. 1995; Cooley & Autio, 1997; Kiss, et al. 1997). This approach will also improve local fauna and flora diversity and the preservation and quality of the landscape as well as the conservation of energy and renewable resources (Vereijken, et al. 1986; Wijnands & Kroonen-Backbier, 1993).

The majority of the objectives set out for this study have been met; the status of *B. lewisi* has been determined as well as the predatory and phytophagous mites occurring at all three vineyard plantings in commercial vineyards and the organic vineyard's mite diversity was also established. *Brevipalpus lewisi* does not pose a threat to our vineyards as yet, as it does not cause physical damage and there are no signs of Citrus leprosis virus. Second, the alternate host survey showed there is definite movement between ground cover and grapevines, even though it was not the dominant predatory and phytophagous mites. These mites are just as important as they tend to services like preying on other small organisms as well as a food source to larger predators.

The use of pesticides in the study area was not as intensive on mites as there were still a healthy abundance of mites present during the season on grapevine. The farmers did not make use of any miticides either. The best time to implement treatments would be as a pest arises, based on sound monitoring protocols (de Villiers & Pringle, 2007). This approach is more sustainable as it will prevent mites from building pesticide resistance, it will keep the diversity of vineyard mites healthy and only applying treatments when the predators are incapable of suppressing the pest will prevent unnecessary chemical applications. Based on the predatory mites' seasonal cycles it would be best not to apply treatments at the start of the growing season (November, December) as *E. addoensis*, *T. praeacutus*, *Pronematus ubiquitous* and *Neoseiulus barkeri* were most abundant during the beginning of the year (i.e. between January and March). There is a fine balance between applying chemicals for treating a pest without disrupting their natural enemies.

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APPENDIX A**SUMMARY OF CONVENTIONAL VINEYARD SPRAY TREATMENT PROGRAMME**

GROWTH STAGE	TARGET	PRODUCT	AMOUNT/100L	VOLUME
5-10cm	Downy mildew	Dithane, Hyperphos	200 - 400g	250L/ha
	Downy mildew	Agphos	400g	250L/ha
	Powdery mildew	Penconazol, Phosper	22.5 - 60ml	250L/ha
10-20cm	Downy mildew	Dithane, Hyperphos	200 - 400g	250L/ha
	Downy mildew	Agphos	400g	250L/ha
	Powdery mildew	Penconazol, Luna expierence	18 - 22.5ml	250L/ha
20-40cm	Downy mildew	Dithane	200g	500L/ha
	Downy mildew	Agphos	400ml	500L/ha
	Mealy bug	Movento	100ml	500L/ha
	Powdery mildew	Cosavet	600g	500L/ha
Bloom start	Downy mildew	Dithane, Dimothozeb	200g	500L/ha
	Downy mildew	Agphos, Hyperphos	400ml	500L/ha
	Powdery mildew	Cosavet, Luna expierence, Cropbio	600g	500L/ha
	fruit set	Boor	200g	500L/ha
Full bloom	Downy mildew	Dithane	200g	500L/ha
	Downy mildew	Agphos	400ml	500L/ha
	Powdery mildew	Penconazol	22.5ml	500L/ha
	Bor resistance	Boron	100g	500L/ha
Set	Downy mildew	Dithane	200g	500L/ha
	Downy mildew	Agphos	400ml	500L/ha
	Powdery mildew	Cosavet	600g	500L/ha
After set	Downy mildew	Dithane	200g	500L/ha
	Downy mildew	Agphos	400ml	500L/ha
	Powdery mildew	Penconazol	25ml	500L/ha
After harvest	Powdery mildew	Cosevet	800g	500L/ha

APPENDIX B**ORGANIC COMMERCIAL VINEYARD SPRAY PROGRAMME**

Plant date	Cultivar	Hectares	Sulfostar (treatment for downy and powdery mildew)	Copstar (treatment for downy and powdery mildew)		Potassium bicarbonate (treats potassium deficiency)	Bio-Impilo (builds resistance and inhibits parasite investatio)	
				0.35	Active			Active/Ha
1999	Cabernet Sauvignon	3.18	42.40	37.00	4.44	1.40	0.00	20.80
1998	Cabernet Sauvignon	2.38	34.28	29.83	3.58	1.51	0.00	17.38
1999	Shiraz	3.03	42.73	33.40	4.01	1.32	0.00	23.03
2000	Merlot	4.30	65.38	50.00	6.00	1.40	0.00	27.90
1999	Merlot	5.36	72.37	63.40	7.61	1.42	0.00	38.26
	Chenin Blanc	3.21	43.63	39.00	4.68	1.46	0.00	21.00
2012	Mix	0.30	4.20	3.54	0.42	1.41	0.00	2.32

APPENDIX C

KEY TO THE VINEYARD MITES IN THE WESTERN CAPE

Ueckermann, E .A. & Vermaak, M.

1. With one pair of dorsolateral or ventrolateral stigmata posterior to coxae II, chelicerae chelate; coxae of all legs free, usually movable; tritosternum present.....Mesostigmata.....2
 - Stigmata opening between bases of chelicerae or on anterior prodorsum; tritosternum absent; chelicerae rarely chelate, fixed digit usually regressed movable digit usually a hook, knife, needle or stylet-like structure, cheliceral bases sometime fused medially; palpi simple or modified into a thumb-claw process, sometimes reduced;; coxae of all legs integrated with venter.....Trombidiformes.....7

2. Seta z3 and s6 absent.....3
 - Either or both z3 and s6 present, at least one of setae Z1, S2, S4 or S5 present.....5

3. Sternal shield with median posterior projection; seta JV1 and ZV2 transversally aligned.....Euseius.....Seta z2 much shorter than distance to z4; setae Z4 at most 20 µm long; spermotheca slightly bulged near atrium, then narrow and flares towards vesicle.....*Euseius addoensis* (Van der Merwe & Ryke) (fig 1(b))
 - Sternal shield without posterior projection; setae JV1 and ZV2 not transversely aligned...4

4. Setae s4, Z4 and usually Z5 not distinctly longer than other dorsal seta. Legs II and III mostly without macrosetae, genu III rarely with a macro seta...*Neoseiulus*.....Atrium bifurcate, calyx a broad tube, sometimes with a small bulge adjacent to atrium; male with four pairs of pre-anal setae.....*Neoseiulus barkeri* (Hughes)
- Seta s4, Z5 and usually Z4 clearly longer than other dorsal setae; macrosetae present on legs II and III; spermatheca with atrium bifurcate or vacuolate, calyx bulged adjacent to atrium, then narrows and flares towards vesicle.....*Graminaseius bufortus* (Ueckermann & Loots)
5. Setae S5 absent, with setae S2, S5, Z4, Z5, J2, J5...*Meyerius*..... Setae Z4 longer than half length of setae Z5, setae s4 much longer than s6 and s2, calyx of spermatheca disc-shaped connected to atrium with a short pedicel.....*Meyerius litus* (Ueckermann & Loots)
- Setae S5 present.....*Typhlodromus (Anthoseius)*.....6
- Setae S5 absent;.....*Typhlodromus (Typhlodromus) spiceae* sp.n.
6. Setae S5 much shorter than S4. Calyx of spermatheca elongate, bell-shape; genu II with 8 setae.....*Typhlodromus (Anthoseius) praeacutus* Van der Merwe
- Setae S4 and S5 equally long; calyx bell-shaped; genu II with 7 setae.....*Typhlodromus (Anthoseius) saevus* Van der Merwe
7. Prodorsum with one or two pairs of trichobothria.....8
- Prodorsum without trichobothria.....16

8. Prodorsum with two pairs of trichobothria.....9
 - Prodorsum with one pair of trichobothria.....12
9. Palptarsus distally with two long setae forming a fork; palptarsus always five segmented.....Bdellidae.....tibia II without a trichobothria.....*Bdella* sp
 - Palptarsus ending in a claw, palp three to five segmented or palptarsus forming a thumb-claw complex, with tibia or tibia with three spines distally10
10. Palptarus ending in a claw, palp three to five segmented...Cunaxidae.....Palp always with five segments, palp telofemur with one or more apophyses, dorsal shields usually reticulate; setal formula of coxae II – IV 1 -3 -1, basifemur III without solenidia; trichobothrium on tibia IV much longer than distance to distal articulation facet.....*Rubioscirus africanus* Den Heyer
 - Palp with thumb-claw complex or three spines distally on tibia.....11
11. Palp with thumb-claw complex; prodorsum with two trichobothria longitudinally arranged on a schlerite; eyes present; body and legs densely covered with setae.....Erythraeidae..... palp tibial claw with a small tooth..... *Balaustium* sp.
 - Palptibia distally with three closely associated spines; first pair of trichobothria on a small naso, second pair on prodorsal shield, body broadly oval, body and legs less densely covered with setae Anystidae..... Peritreme on anterior margin of prodorsum reticulate

and flared distally; prodorsal shield kidney-shaped with two pairs of setae and one pair of trichobothria *Anystis baccharum* (Linnaeus)

12. Tarsus I with claws and an empodium13

- Tarsus I without claws and an empodium replaced by four long setae; trichobothria I and II without setae *Pronematus*..... Tarsus I as long as or longer than tibia I, two terminal setae on tarsus I longer than segment, all terminal setae serrated along entire length; members of ventral setae half as long as distance between them and longitudinally aligned*Pronematus ubiquitous* (McGregor)

13. Setal formula of femora 3 -3 -2 -1 *Brachytydeus* sp

- Setal formula of femora 3 -3 -1 -114

14. All dorsal setae, except trichobothria, similarly shaped, setae serrated with caudal setae relatively longer; leg coxae with subcutaneous reticulations....*Tydeus reticoxus* Ueckermann

- Dorsum with two types of setae.....15

15. All dorsal setae except some on prodorsum and trichobothria short and leaf – like.....*Tydeus munsteri* Meyer & Ryke

- Only four pairs of caudal setae long and spatulate..... *Tydeus grabouwi* Meyer & Ryke

16. Retractable stylophore present with moveable digits very long and stylet-

- like.....17
- Retractable stylophore absent, moveable chelicerae shorter, stylet-like or needle-like.....22
17. Palp with thumb-claw complex, palp five segmented; tarsi I and II with duplex setae.....Tetranychidae.....18
- Palp linear without thumb-claw complex, vary from one to five segments; tarsi I and II without duplex setae; body dorso-ventrally flattened...Tenuipalpidae.....20
18. Tarsus I with duplex setae, well separated; empodium ending in a tuft of hairs.....
Tetranychus19
- Tarsus I with duplex setae approximate; empodium claw-like with proximoventral hairs...
OligonychusTarsus two with three setae posterior to duplex setae; aedeagus of male bent ventrad at a right angle.....*Oligonychus vitis* Zaher & Shehata
19. Female with proximal pairs of duplex setae distal to proximal setae; axis of aedeagal knob more or less parallel with shaft, knob with anterior and posterior small projections dorstal striae of female with lobes varying from triangular to broadly semi-circular.....*Tetranychus urticae* Koch
- Female with proximal pair of duplex setae more or less in line with most proximal setae; axis of aedeagal knob parallel to shaft but without posterior projections; dorstal striae of female with lobes triangular.....*Tetranychus ludeni* Zacher

20. Opisthosoma with six pairs of dorso-lateral setae (c3, ds, e3, f3, h2, h1 (f2 absent)).....21
21. Tarsus II with two solenidia, prodorsum with uneven reticulations...
.....*Brevipalpus phoenicis* complex
- Tarsus II with single solenidion; prodorsum with even reticulations laterally, but devoid of median reticulations; spermatheca terminates into a round vesicle with short finger-like projections around entire perimeter..... *Brevipalpus obovatus* Donnadieu
- Opisthosoma with seven pairs of dorso-lateral setae (c3, d3, e3, f2, f3, h2 and h1)...22
22. Tarsus II with two solenidia; prodorsum evenly reticulated...*Brevipalpus californicus* (Banks)
- Tarsus II with one solenidion, prodorsum weakly to strongly wrinkled or folded medially, can also appear like eareolae, laterally with elongate cells forming a reticulation, opisthosoma with V-shaped folds posterior to e1 – e1; spermatheca terminating into a small round vesicle with a series of short projections and clear internal “bubble”.....*Brevipalpus lewisi* McGregor (Fig i(a))
23. Chelicerae fused with subcapitulum to form a gnathosomatic capsule. Peritremes on dorsum gnathosoma; palptarsus usually with comb-like setae; dorsum covered by none to three shields Cheyletidae.....
- Dorsum with two shields, fan-shaped setae and two eyes; peritremes forming an inverted U.
.....*Cheletomimus (Hemicheyletae)* sp.

- Cheliceral bases usually contiguous, but not completely fused; peritremes absent; dorsum completely or partially covered by shields; comb-like setae absent.....Stigmaeidae..... prodorsum with three pairs of setae, median shield on opisthosoma with five pairs of setae, all shields reticulated, dorsal setae short and barbed, becoming gradually longer posteriorly; formulae of genu 3 -0 -0 -0, femora 4 -4 -2 -2 and tibia 6 -6 -6 -4.....*Agistemus collyerae* Gonzales-Rodrigues (Fig. i(c))

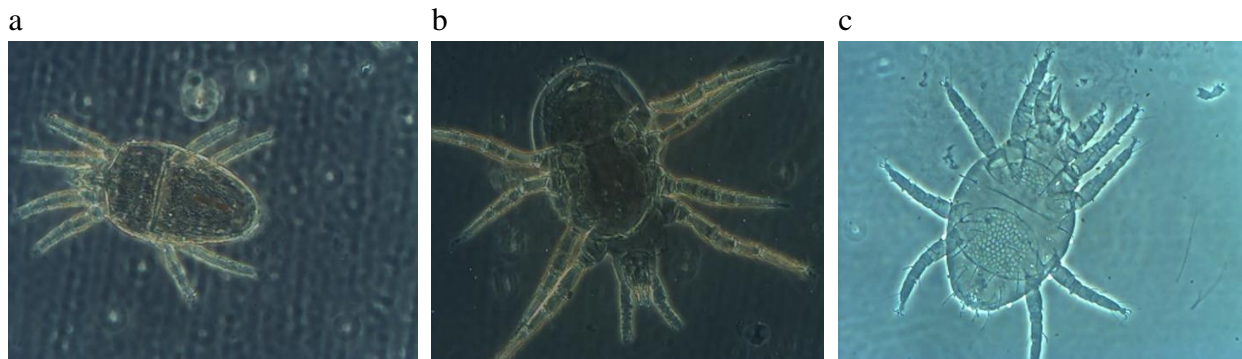


Fig i: Phytophagous mite (a) Brevipalpus lewisi and predatory mites (b) Euseius addoensis and (c) Agistemus collyerae taken with Leica DM 2500 microscope. General aspect.

APPENDIX D

Table i: A list of all the mites found at each site throughout the entire study; from 2016 to 2018. The list includes the four conventional farms (1 – 4) and the organic vineyard (5). Site information is not provided on request by growers.

MITE SPECIES/GENUS/ FAMILY	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5
<i>Brevipalpus lewisi</i>	112	18	829	342	0
<i>Brevipalpus phoenicis</i>	0	0	3	0	0
<i>Brevipalpus obovatus</i>	2	0	7	1	0
<i>Brevipalpus californicus</i>	1	0	2	2	0
<i>Tetranychus</i> sp	1	0	0	1	0
<i>Tetranychus urticae</i>	0	0	2	0	0
<i>Tetranychus ludeni</i>	0	1	0	0	0
<i>Oligonychus vitis</i>	0	0	0	1	0
<i>Typhlodromus</i> sp	0	0	0	0	1
<i>Typhlodromus saevus</i>	0	0	0	1	12
<i>Typhlodromus praeacutus</i>	45	0	0	10	1
<i>Euseius addoensis</i>	50	2	3	1	91
<i>Meyerius litus</i>	0	0	0	0	15
<i>Neoseiulus barkeri</i>	0	0	0	3	0
<i>Graminasieus bufortus</i>	0	0	0	0	0
Ionlinidae	1	0	1	1	0
<i>Tydeus munsteri</i>	0	0	0	0	1
<i>Tydeus</i> sp	1	0	0	0	1
<i>Tydeus grabouwi</i>	3	0	0	0	3
<i>Tydeus reticoxus</i>	0	0	0	0	2
<i>Brachytydeus</i> sp	0	0	0	0	0
<i>Agistemus collyerae</i>	1	0	6	0	0
Bdellidae	1	0	0	0	0
<i>Balaustium</i> sp	2	0	1	2	0
<i>Amblysieus</i> sp	0	0	0	0	0
<i>Hemicheyletia</i> sp	0	0	0	1	0
<i>Rubioserus africanus</i>	0	0	0	0	1
<i>Anystidae</i> sp	0	0	0	0	0
<i>Anystis baccarum</i>	0	0	0	1	0
Eupalopsellidae	0	0	1	0	0
Eupodidae	0	0	0	0	0
<i>Pronematus ubiquitus</i>	3	0	2	0	7
Oribatida	1	0	1	1	0
<i>Tyrophagus</i> sp	3	1	9	0	0
Glycophagidae	0	0	1	0	0

