

Quantifying the pollination ecosystem service provided by *non-Apis* bees in tomato production

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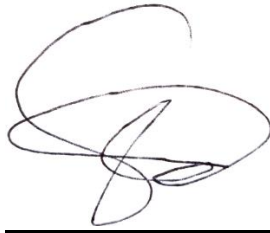
DECLARATION

In submission of this dissertation I, Retang Nkakole Kgaphola, declare that the work contained in this submission is entirely my own original work except where otherwise indicated or acknowledged and I am the author of this dissertation and have never submitted this work (or part of) for obtaining any qualification.

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Date: November 2020

Signature



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DEDICATION

This dissertation is dedicated to my family, my beloved mother Makelle Concelia Kgaphola (Pebetse), my sisters Tshegofatso and Matsie, my brother Monare and my nephews Mosa, Lesedi and Thero and my niece Amogelang. It has not been an easy journey, but your support and prayers kept me going.

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ABSTRACT

Pollination provided by bee species is a key contributor to the reproductive success of a wide variety of crops. In South Africa, tomato (*Solanum lycopersicum*) is among the most important cash crops. Tomato flowers are self-fertile and self-pollinating. The anthers of tomato flowers are poricidal and connivent, though tightly held together in a cone by interlocking hairs along their edges, bees that can perform vibratile pollination can access pollen grains from these flowers. During buzz pollination, some species of bees cling on the anthers of tomato flowers by means of their mandibles which then results in vibration of the flowers. This biting action cause “bee kisses” (brown marks) on the flowers and these marks can be used to confirm that the flowers have been visited by bees and that pollination might have taken place. There is little information available on the pollination status of tomatoes in South Africa, therefore, the aim of this study was to quantify the pollination ecosystem service provided by bee species to tomato production in the Limpopo Province, South Africa. The objectives of the study were to: 1) determine the effect of field proximity to natural vegetation on bee visitation rates on tomato flowers in four bio-climatic areas, 2) describe bee species that provide pollination ecosystem service to tomato flowers, and 3) determine the effect of pollination provided by bees on the yield and quality of tomatoes. This study was conducted on four farms (Waterpoort, Vreedzaam, Jachtpad and Dikgale) from April to December in 2018 and 2019. Data were collected inside tomato fields along four transects which were separated using four distance intervals. Results showed that bee visitation rates varied between farms. This could be attributed to different planting months/seasons between the various study areas which was directly linked to different temperature regimes as well as different vegetation types. In addition, the distance from the natural vegetation also influenced bee visitation rates. The latter was higher, closer to natural vegetation (< 50 m) and differed significantly from visitation rates recorded at the furthest distance in two of the farms (> 200 m). The potential pollinators collected during the study were largely from the Apidae family, and three genera i.e. *Xylocopa*, *Apis* and *Amegilla*. The Megachilidae with one genus, i.e. *Megachile*, was the second most abundant family. Furthermore, an exclusion experiment was conducted to compare the effect of bee pollination and self-pollination on yield and quality of tomatoes. Bee pollination had a positive impact on the quality of tomato fruit particularly in terms of fruit weight, height and diameter. The fruits harvested from bee-pollinated flowers had a significantly larger size when compared to fruits obtained from the enclosed flowers while there were also fewer malformed fruits. This study highlighted the importance of bee species in tomato production. It is therefore important to develop management practices that will aid in improving bee visitation rates, abundance and diversity in order to improve tomato production.

Key words: bee kisses, buzz pollination, connivent anthers, ecosystem services, fruit yield, fruit quality, poricidal anthers, sonication

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Chapter 1

Background information

1.1 Background information

Tomato (*Solanum lycopersicum* L.) is among the most consumed crops in the world and it is a staple food for millions of people across the globe (Poysa, 1991)). Tomato is consumed in diverse ways including raw, as an ingredient in many dishes and as a drink (Raiola *et al.*, 2014). Furthermore, tomato is a source of essential vitamins, such as vitamins A and C and folic acid (Canene-Adams *et al.*, 2005; Raiola *et al.*, 2014). In South Africa, tomato is a popular and widely grown income generating crop and it is a valuable cash crop for both small scale and commercial farmers (DAFF, 2018). The Limpopo Province is one of the major production areas in the country, with a production area of approximately 3 590 hectares (Moodley *et al.*, 2019). There are several factors that determine tomato yield quantity and quality, such as climatic conditions (Malherbe and Marais, 2015), irrigation methods, fertilizer application, soil properties, and the control of pests and diseases (Huat *et al.*, 2013; Barbosa *et al.*, 2019).

In the early 1890s, pollination ecosystem services were recognized as one of the major factors that affect yield and quality of tomato fruits (Toni *et al.*, 2020). Pollination is an essential ecosystem service. It is defined as the transfer of pollen grains from the male reproductive organs, the anther, to the female reproductive organs, the stigma, and it is a precursor for fertilization (Morandin and Kremen, 2012). Most pollination ecosystem services are provided by living organisms, with bees (superfamily Apoidea, series Apiformes) being the most common pollinators of a variety of crops and wildflowers worldwide (Melin *et al.*, 2014; Saturni *et al.*, 2016). Bees are a highly diverse group with about 20 000 species described globally (Danforth *et al.*, 2006). Bees vary in terms of body size, floral preference, nesting requirements, sociality, and habitat requirements (Torne-Noguera *et al.*, 2014) and are depended on plant species for resources such as nectar and pollen for survival (Kearns and Inouye, 1997). Nectar which is a sweet liquid and a source of carbohydrates and pollen which is a source of proteins for insects such as bees (Kearns and Inouye, 1997).

Pollen bearing the plant's genetic material is transported on the bees' hairy legs and body. When bees fly from one plant to another, some of the pollen lands on the stigma of another plant and pollination might take place (Bascompete *et al.*, 2003; Danforth *et al.*, 2006). Because of their structured ability to collect and move pollen around, bees have been used by crop farmers to assist in the pollination of a variety of crops to improve yield and quality (Bascompete *et al.*, 2003). Honeybees (*Apis mellifera* L.) are the most common and generalist bee species that have been managed for crop pollination (Melin *et al.*, 2014). However, the vast majority of bees are non-*Apis*, and are also important pollinators of crops and wildflowers globally (Brittain *et al.*, 2013). One of the key differences between *Apis* and non-*Apis* bees is that *Apis* bees produce honey in large quantities while non-*Apis* bees, excluding stingless bees, do not (Daly *et al.*, 2012; Melin *et al.*, 2014). Floral morphology plays a key role in determining the kind of pollinators required to pollinate flowers (Ollerton *et al.*, 2011).

Tomato flowers are hermaphroditic, both the male and female reproductive organs are found in the same flower promoting self-pollination; however, agitation of the anthers is required for pollen grains to be released onto the receptive stigma (Bell *et al.*, 2006; Amala and Shivalingaswamy, 2017; Silva-Neto *et al.*, 2017). This can be done by wind, but insufficient wind may result in poor fruit set and quality. Previous research showed that vibratile pollinating bee species are essential pollinators of tomatoes and produce higher yield and quality when compared to the effect of wind only (Greenleaf and Morandin, 2006; Toni *et al.*, 2020).

Within the Limpopo Province, ZZ2 is the main producer of fresh tomatoes and they supply at least 40-60% of fresh tomatoes to the national fresh produce market (NAMC, 2012). ZZ2 tomato production and availability is not seasonal, it is operational for 365 days a year. Approximately 5 to 10% of the tomatoes goes directly to supermarkets such as Pick and Pay, Checkers, Shoprite and Spar (NAMC, 2012). ZZ2 produces tomatoes in different regions in the Limpopo Province, namely Mooketsi, Polokwane, Waterpoort and Malaladrift along the Limpopo River (www.zz2.co.za). Tomato production at ZZ2 is divided into two cultivation systems which are open fields and net-houses (Personal observation).

The production of tomatoes within ZZ2 has been moving from open fields to net-houses with the aim to improve the yield and quality of tomatoes. Currently, the production area for net-houses is approximately 150 hectares while the open fields cover about 1 800 hectares (P. Novela, Mooketsi, 2018, personal communication). A net-house structure is used as a barrier for insects or pests that may cause damage to tomatoes, such as red spider mites (*Tetranychus urticae*) and white flies (*Trialeurodes vaporariorum*), however, while protecting the crop from pests, it also limits the access of beneficial insects such as bees that play a role in pollinating tomato flowers. Therefore, to improve production, mechanical techniques such as leaf blowers are used to assist with the pollination of tomato flowers in the net-houses, this because of the total absence of bees and wind (Figure 1.1). However, the open field production, where bees are allowed to visit the tomato flowers, their contribution to yield and quality of ZZ2 tomatoes is poorly known (J. de Bruyn, 2018, personal communication). It is therefore important to measure and understand the pollination ecosystem services provided by these species of bees in tomato production.



Figure 1.1: Tomato pollination in a net-house using a leaf blower to pollinate. Photo credit: Wiam Haddad, 2019.

1.2 Problem Statement

To ensure sustainable production of tomato crops, effective pollination services are needed. Since buzz pollinating bee species are important in this context, information should be generated on the aspects that will allow protection of these species and to quantify their importance in the production system. For example, little or no information is available regarding the effect of buzz pollinating bee visitation rates on tomato production, both in quality and quantity, the frequency of bee visits on tomato flowers, whether pollination is consistent across geographic regions in the Limpopo Province, and whether the natural vegetation plays a significant role in bee visitation rates in tomato fields.

1.3 Aim and objectives of the study

1.3.1 Aim of the study

The contribution of bee species to yield quantity and quality of tomatoes within ZZ2 is not known. Therefore, the aim of this study was to quantify the pollination ecosystem service provided by buzz pollinating bees to tomato crops in selected areas of the Limpopo Province.

1.3.2 Objectives of the study

The objectives of the study were to:

- I. determine the impact of distance from natural vegetation on bee visitation rates to tomato flowers in four bio-climatic regions of Limpopo Province.
- II. identify the species of bees that provide the pollination ecosystem service to tomatoes
- III. determine the effect of pollination services provided by non-*Apis* bees on yield and quality of tomato crops by conducting an exclusion experiment

1.4 Thesis outline

This thesis starts with a broad general introduction and eventually zooms in to more specific analyses. The first chapter introduces the device key concept, rationale and literature review on topics presented in the thesis. In the second chapter a rapid technique was developed to measure bee visitation rates in tomato fields taking proximity of field to natural vegetation into consideration. This study was conducted in four bio-climatic regions to investigate whether bee visitation rates are constant

throughout the regions. The third chapter identifies the potential pollinators of tomato flowers. The fourth chapter aims at linking the bee visitation rates to tomato production by conducting an exclusion experiment. Finally, in chapter five we conclude the thesis by summarising our findings and discussing further research questions that may add to the current work. Figure 1.2 illustrates the flow of concepts and information from coarse to fine scale throughout the thesis.

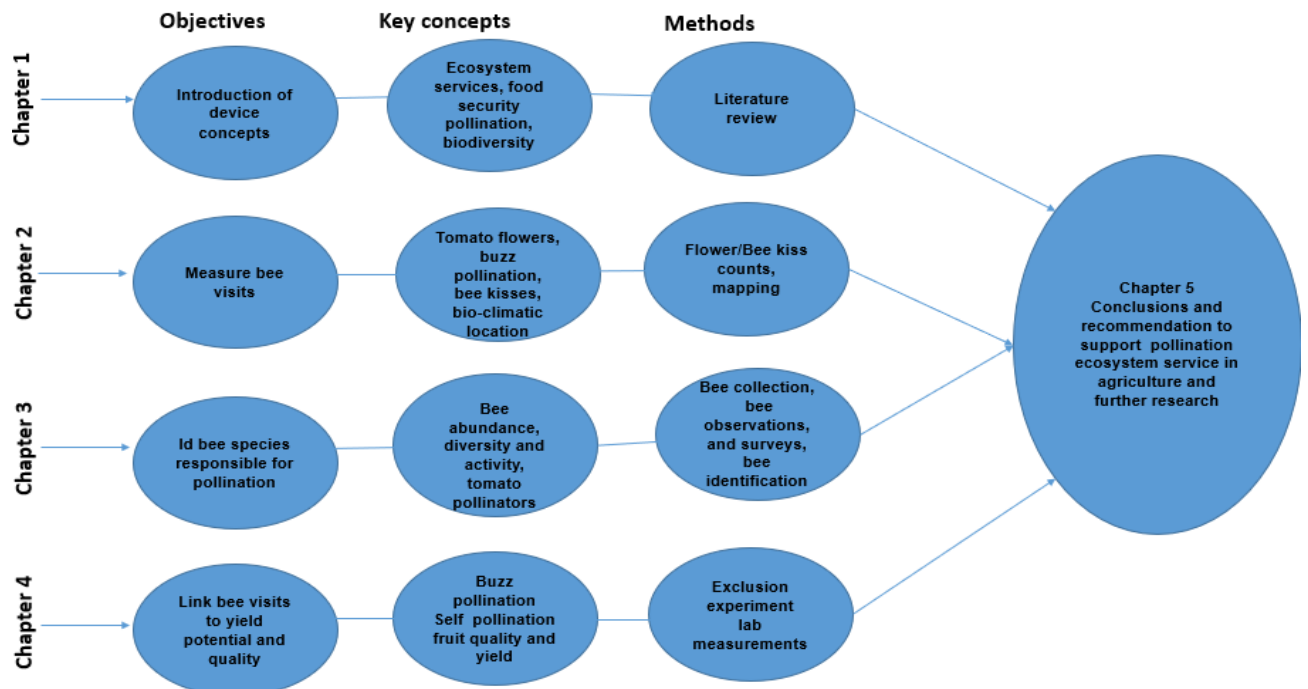


Figure 1.2: Schematic diagram outlining the flow of the thesis.

1.4 Literature review

1.4.1 Tomato

1.4.1.1 Tomato origin and distribution

Tomato (*Solanum lycopersicum* L.), one of the most popular produced and extensively consumed vegetable crops in the world, is native to South and Central America (Nesbitt and Tanksley, 2002). The world's production of tomatoes is approximately 162 million tonnes with China being in the lead with 50 million tonnes followed by India with 17,5 million tonnes. The other top producing areas include USA, Brazil, Mexico, Italy, Portugal, Spain, Turkey, Egypt, Iran, Uzbekistan, Russian Federation, Ukraine and Nigeria (Raiola *et al.*, 2014). The production area for tomatoes has increased by at least 47% during the period 1992-2002 (Saavedra *et al.*, 2017). In South Africa, tomato crop is regarded as the second largest vegetable crop after potatoes (*Solanum tuberosum* L.) (Moodley *et al.*, 2019). Tomatoes contribute to at least 24% of the total vegetable production in the country (DAFF, 2010). The most recent census reported that at least R630 million of the revenue was generated from about 4 523 hectares of tomatoes (DAFF, 2018). Within South Africa, the Limpopo Province is one of the major production areas, with a production area of at least 3 590 hectares, followed by the Mpumalanga Province, with a production area of 770 hectares and the southern parts of the Eastern Cape with a production area of approximately 450 hectares (DAFF, 2018).

1.4.1.2 Conditions required for tomato production in South Africa

Although tomatoes are produced in many parts of South Africa, there are distinctive guidelines and requirements documented with regard to the conditions needed for optimum tomato production (DAFF, 2010). Production requirements are summarized in Table 1.1 and they include climatic conditions, soil preparation and soil type, fertilizer applications, irrigation and pest and disease control.

Table 1.1: A summary of production requirements to optimize tomato production in South Africa.

Climatic conditions	Tomato is an annual plant that is classified as a warm season crop and the optimum temperatures for growth, yield and fruit quality ranges between 20 °C and 24 °C. Temperatures below 10 °C cause serious damage to both the plant and the fruit (DAFF, 2010). Hot and dry winds may result in excessive flower drop while continuous moist, rainy weather conditions promote the occurrence and spread of diseases (DAFF, 2010).
Soil preparation and types	Tomatoes grow moderately in a wide range of soil types however a very high level of soil fertility is required for a profitable production (DAFF, 2010). Some of the soil factors that influences tomato production are nutrient composition, pH, water holding capacity, compaction and herbicide residues (DAFF, 2010).
Fertilizer applications	The amount of fertilizer application on tomatoes is influenced by the fertility status of the soil (OECD, 2017). Tomato plants are heavy feeders of a variety of plant nutrients which include nitrogen, phosphorus and potassium (DAFF, 2010). Nitrogen is one of the essential macro-nutrients and it improves flower and fruit set. The second most required nutrient is potassium, which influences colour, taste, firmness, sugar content, acids and solids of the fruit (DAFF, 2010). Phosphorus is the third most important macro-nutrient, and promotes root development, fruit set and early flowering.
Irrigation	Excess irrigation after a long dry period may result in fruit cracking. In addition, irrigating plants late in the season may result in watery fruits of poor quality (DAFF, 2010). Irrigation schedule is important and it is usually determined by the size of the root system. The majority of tomato roots occurs in the top soil (500-600 mm). The amount of water supplied varies depending on the climatic conditions of the area (DAFF, 2010).
Pest and disease management	Tomatoes are attacked by a wide variety of pests and diseases. Although the severity of attack and infection varies between regions and seasons, control measures are required in virtually every planting (DAFF, 2010). The majority of the pests and diseases are controlled by means of registered chemicals (DAFF, 2010; 2018).

1.4.1.3 Dietary importance of tomato

Tomato is rich in essential nutrients and minerals (Canene-Adams *et al.*, 2005), are high in water and contain low calories and are an important source of both Vitamin A and C (Canene-Adams *et al.*, 2005; Bhowmik *et al.*, 2012). The fruits are also good sources of lycopene, which is an antioxidant that contributes to reduction of several cancer risks (Hanson and Yang, 2016). In addition, tomatoes contain phytochemicals such as carotenoids and polyphenols which are thought to reduce cardiovascular diseases (Canene-Adams *et al.*, 2005).

1.4.1.4 Morphology of tomato plants

The tomato plant can either be a perennial or a semi-perennial plant, however, commercially it is considered an annual crop (Brewer *et al.*, 2007). Tomato plants are generally branched and can grow up to 60-180 cm tall. The leaves are usually hairy, pinnately compound and are approximately 45 cm long (Shamshir *et al.*, 2018). Tomato crops are either cultivated in open fields or in protected areas such as greenhouses and net-houses, optimally around 45 days are necessary for tomato growth, from germination to anthesis. It takes about 90-100 days to reach the beginning of fruit ripeness (Brewer *et al.*, 2007). The number of days within each stage depend on the cultivar and environmental factors such as air temperature, light conditions, soil conditions and nutrients. Tomato plants go through five growth stages (Figure 1.3) (Shamshir *et al.*, 2018).

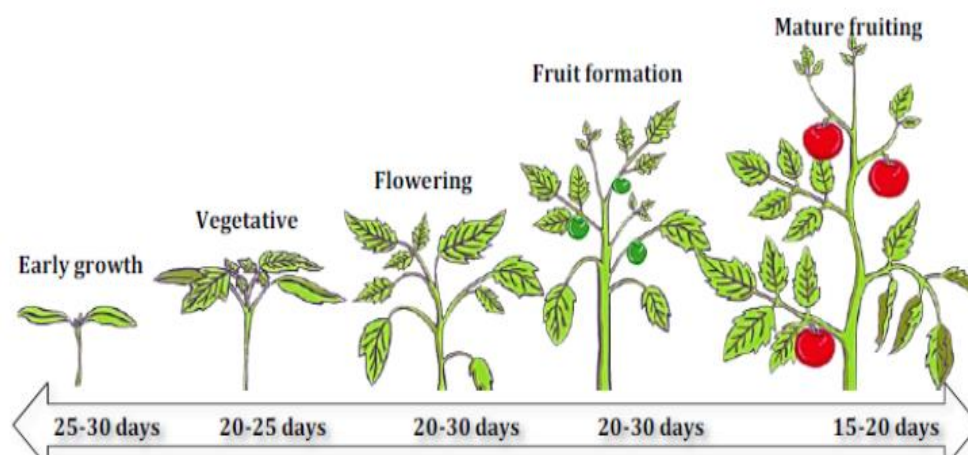


Figure 1.3: The five growth stages of a tomato plant. (Shamshir *et al.*, 2018).

Similar to the majority of plant species, fruit formation in tomatoes begins with flowers whereby the majority turn into fruits that contain seeds (Shamshir *et al.*, 2018). Tomato flowers are hermaphroditic, generally about 1.5 to 2 cm in diameter and the corolla and anthers are yellow (Toni *et al.*, 2020). The biological function of a flower is to provide mechanisms for reproduction to take place (Smit and Combrink, 2005; Matsuki *et al.*, 2008; Willmer *et al.*, 2017). Sexual reproduction in plants begins with the production of male and female gametes, and for fertilization to take place the pollen grains need to be transferred from the anther (male) to the stigma (female) (Watanabe, 2014). There are different mechanisms by which pollen grains can be transferred from the anther to the stigma, depending on the floral morphology of the plant (Wolff *et al.*,

2008; Deprá *et al.*, 2014; Cardinal *et al.*, 2018; Kovács-Hostyánszki *et al.*, 2019). In tomatoes, pollen grains are produced inside the connivent anthers and agitation of the flowers are required for the pollen grains to be released from the anthers (Figure 1.4) (Toni *et al.*, 2020).

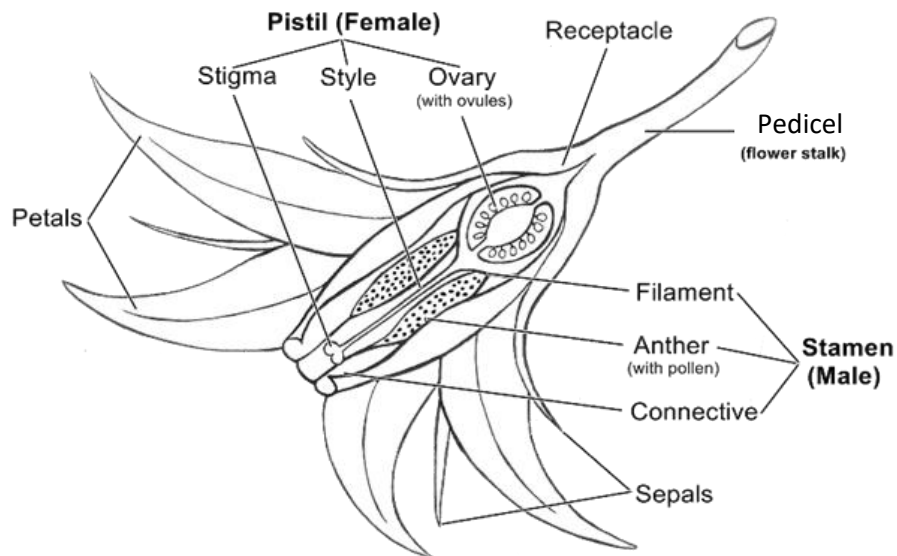


Figure 1.4: The morphology of a tomato flower. (<https://www.biobeas.co.nz/>).

It is well known that pollination ecosystem services provided by insects such as bees play a key role in ensuring that pollen grains are released from the connivent tomato anthers to the stigma and therefore may result in formation of fruits (Greenleaf and Kremen, 2006; Toni *et al.*, 2020). The rest of this chapter will focus on pollination ecosystem services with special reference to buzz pollination, the influence of pollination on yield and quality of crops (tomato and other crops), diversity of pollinators and factors influencing pollination services.

1.4.2. Pollination

1.4.2.1 Pollination as ecosystem service

Ecosystem services are defined as the benefits that living organisms derive directly and indirectly from the natural environment for free (Costanza *et al.*, 1997). Ecosystem services are grouped into four categories (Figure 1.5), i.e. provisioning, cultural, regulating and supporting services (Power, 2010; Palm *et al.*, 2014; MEA, 2015). The natural environment has been provides both human beings and other living organisms with ecosystem services (Farber *et al.*, 2002; Wakindiki and Kirambia, 2011; Blowers *et al.*, 2018). However, human interventions have altered the capacity of the natural

environment to provide these services (Melin *et al.*, 2014; Bennett and Chaplin-Kramer, 2016). Previously, studies have focused on estimating the economic value of ecosystem services in order to use them sustainably (Kremen *et al.*, 2002; Steffan-Dewenter *et al.*, 2005; Zhang *et al.*, 2007; Swinton *et al.*, 2015).

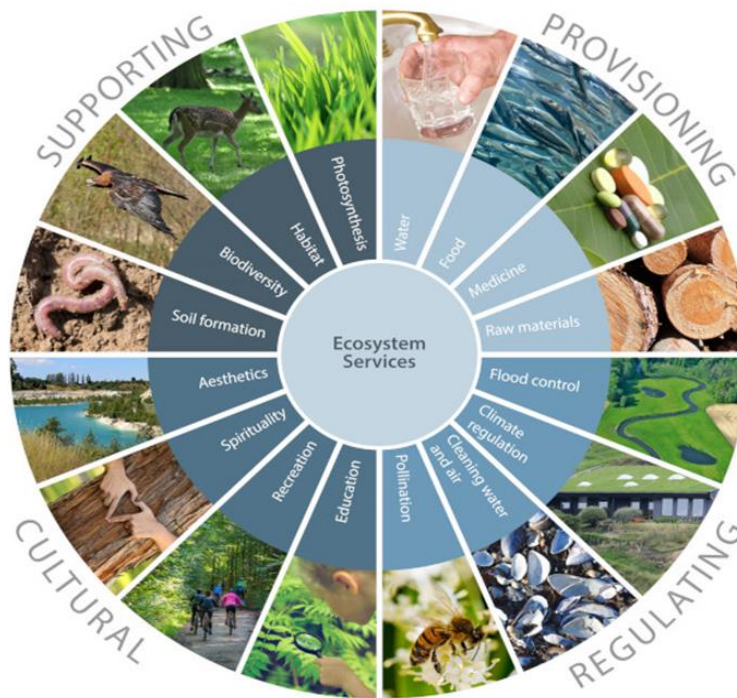


Figure 1.5: Categorisation of ecosystem services. (<https://envs.au.dk/en/research-areas/society-environment-and-resources>).

Pollination is one of the essential ecosystem services (Melin *et al.*, 2014). It is regarded as a regulating ecosystem service and it is defined as the third-party mediated transfer of pollen grains from the anther (male reproductive organs) to the stigma (female reproductive organs) between or within individuals of a particular plant species (Ollerton *et al.*, 2011; Melin *et al.*, 2014). Pollination success results in fertilized flowers that produce seeds which in some instances enable the associated plant species to produce and form fruits (Smit and Combrink, 2005; Lihoreau *et al.*, 2016). Pollination plays a key role in plant species persistence, global food security and ecosystem resilience (Gordon *et al.*, 2010; Power, 2010; Redhead *et al.*, 2020). Ollerton *et al.* (2011) reported that at least 90% of the world's plant species are dependent on pollination services for reproductive success, including at least one third of crop species.

Lack of efficient pollination services will therefore not only result in adverse ecological consequences and shifts in plant diversity and community composition, but it will also affect food security (Winfree *et al.*, 2008; Olynyk, 2017).

There are two methods by which pollination can occur, namely abiotic and biotic methods (Palm *et al.*, 2014; Kovács-Hostyánszki *et al.*, 2019). An example of an abiotic pollination method is wind pollination (anemophily), a form of pollination whereby pollen grains are transferred by wind between or within plant species (Hanna, 1999; Lone *et al.*, 2017). Lone *et al.* (2017) reported that less than 15% of the world's plant species are wind pollinated and that this is more common in savannas and deciduous forests. Wind pollinated plants usually have small flowers, no petals and no special odours, colours or nectar (Cook, 1988). Some examples of wind pollinated plants are grasses and include cereal crops such as maize, wheat, rice and barley (Sluijs and Vaage, 2018). Although less than 15% of the world's plant species are wind pollinated, these crops provide the majority of calories in the human diet (Sluijs and Vaage, 2018). Water pollination (hydrophily) is important for the pollination of some aquatic plant species, these plant's pollen grains are released into the water and water currents act as pollen vectors (Cook, 1988). Although abiotic mechanisms are important for reproductive success of some plants, the majority of plant species depend on biotic mechanisms for pollination (Bascompte *et al.*, 2003; Ollerton *et al.*, 2011; Melin *et al.*, 2014; Veldtman, 2018).

Biotic pollination mechanisms are provided by living organisms such as sun birds, bats, moths, butterflies, bees and flies and they are collectively referred to as pollinators (Kremen *et al.*, 2002; Bascompte *et al.*, 2003; Klein *et al.*, 2012; Vaughan *et al.*, 2014). Pollinators vary in size, floral preferences, shape and behaviour (Bascompte *et al.*, 2003; Power, 2010; Ollerton *et al.*, 2011; Vaughan *et al.*, 2014). In addition, pollinators play a vital role in maintaining biodiversity and vibrant ecosystems for plants, humans and the pollinators themselves (Winfree and Kremen, 2009; Melin *et al.*, 2014; Lihoreau *et al.*, 2016). There has been recent evidence of declines in pollinator abundance and diversity globally which has generated concern (Ollerton *et al.*, 2003; Greenleaf and Kremen, 2006; Winfree *et al.*, 2008; Klein *et al.*, 2012; Melin *et al.*, 2014; De Palma *et al.*, 2016).

Among the biotic pollinators, insects, particularly bees, are the most efficient and common pollinators of wide variety of crop species (Wolff *et al.*, 2008; Melin *et al.*, 2014; Vaughan *et al.*, 2014).

1.4.2.2 Importance of pollination services for crop production

It is estimated that approximately 35% of the crops that provide at least 75% of food globally are dependent on pollination ecosystem services, the majority of pollination services are being provided by bees (Klein *et al.*, 2007; Melin *et al.*, 2014; Stein *et al.*, 2017).. This includes crops that supplies the majority of micro-nutrient rich fruits, vegetables, nuts, seeds and oils (Gordon *et al.*, 2010; Melin *et al.*, 2014). In addition, pollinator mediated crops are of key importance in supplying essential nutrients to the human food supply, for example at least 90% vitamin C, 100% lycopene and almost 100% of the antioxidants β -cryptoxanthin and β -tocopherol (Sluijs and Vaage, 2018).

Pollination services also play an important role in improving the yield quantity and quality of crops. For example, Stein *et al.* (2017) reported that pollination services provided by honeybees and wild-bees significantly increased the yield quantity and quality of cotton and sesame in Burkina Faso, West Africa, while Roubik (2002) noticed that pollination by non-native honeybees increased yield of coffee by 50% in Panama. Furthermore, lack of sufficient pollination may result in deformed and non-developing fruit which will therefore reduce yield quantity and the market value of the fruit (Figures 1.6 and 1.7). A study conducted by Abrol *et al.* (2017) on strawberries found that pollination services are not only important for yield of fruit but also essential for physiological processes that results in improved fruit appearance, prolonged shelf life and commercial value of strawberries.



Figure 1.6: The effect of pollination on fruit weight in strawberries; pollinated strawberry becomes bigger (left) compared to a non-pollinated one (right). From (<https://www.thestar.com.my/lifestyle/living/2017/09/23/stingless-bees-brazil/>).

Similar results were observed in other crops such as blueberries (Isaacs and Kirk, 2010), mangoes (Huda *et al.*, 2015) and tomatoes (Toni *et al.*, 2020) (Figure 1.7).

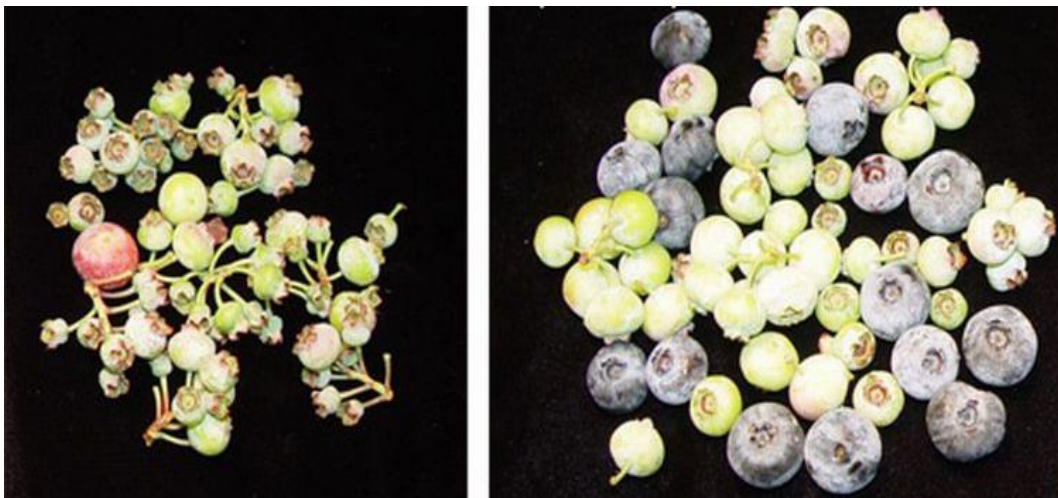


Figure 1.7: Comparison of lack of efficient pollination (left) and sufficient pollination (right) in blueberries (Isaacs and Kirk, 2010).

In South Africa, the demand for pollination services has increased for the majority of crops. Melin *et al.* (2014) reported that there are only nine studies and four published in peer-reviewed journals, assessing and discussing the importance of pollination in crop production. Despite the evidence base linking pollination services efficiency to crop yield, this aspect is still poorly known for most South African agricultural systems.

1.4.2.3 Pollination mechanisms

The extent to which crops depend on pollination services for reproduction is dependent on the morphology, phenology and behaviour of the flowers and varies between plant species (Abrol *et al.*, 2017). Plants are either dioecious (male and female organs occur on different plants), monoecious (male and female organs occur in the same plant but on different flowers) or have hermaphrodite flowers (with male and female organs in the same flower, these organs may mature at the same time or at different times) (Abrol, 2011). Depending on the floral morphology, plants can either depend on self-pollination or cross-pollination mechanisms for reproduction to take place (Klein *et al.*, 2007).

During self-pollination, the pollen grains from one flower can fertilize the ovule of the same flower (Abrol *et al.*, 2019). Self-pollination usually occurs in flowers in which the stamens and carpels mature at the same time and are positioned in a way that the pollen can potentially land on the stigma. This mechanism is known to be efficient as it does not usually require external forces to assist in the transfer of pollen grains to the stigma. In addition, the plant involved does not have to provide pollinators with nectar and/or pollen. In contrast, in cross-pollination the male and female reproductive organs usually mature at different times or are found on different flowers and these pollen grains have to be transferred from the anther of one plant to the stigma of another plant of the same species. In addition to self and cross-pollination, there is another mechanism by which pollen grains can be transferred from the anthers to the stigma referred to as buzz pollination. Buzz pollination is a type of pollination used by some bee species to collect pollen grains from flowers by means of vibrations (Arroyo-Correa *et al.*, 2019). Buchman and Hurley (1978) reported that although buzz pollination has been known for at least 100 years, more research has to be conducted with regard to this mechanism. In this context, tomato is a crop that requires buzz pollination in order for pollen grains to be moved from the anthers to the stigma.

1.4.2.4 Buzz Pollination

Buzz pollination occurs in plants with flowers showing the morphological syndrome for this type of pollination (Buchman and Hurley, 1978). Buzz pollination is a relatively rare pollination mechanism and it is estimated that approximately 6% of flowering

plants are buzz-pollinated. This strategy has evolved in some of the flowering plants whereby the plants have developed floral morphologies that conceal floral rewards such as nectar and pollen (Buchman and Hurley, 1978; Cardinal *et al.*, 2018; De Luca *et al.*, 2019). For most buzz-pollinated plants, pollen grains occur inside connivent anthers or inside closed corolla tubes with small apical openings which usually limit direct access to the pollen (Buchman and Hurley, 1978). In order to access pollen from buzz-pollinated flowers, vibrations are required and this is usually performed by certain types of pollinators (Cardinal *et al.*, 2018). According to De Luca and Vallejo-Marín (2013), it is thought that certain bees are the only animals that are capable of collecting pollen from flowers using vibrations. However, more research has to be done in order to find out if any other arthropods can also perform buzz pollination. There has been a surge of interest in the study of buzz pollination worldwide because of the importance in production of some essential crops. For example, it has been widely recognized that although tomato flowers are self-pollinating, buzz pollination can improve production in terms of yield and fruit quality (Toni *et al.*, 2020). Cardinal *et al.* (2018) reported that globally at least 74 out of the 508 recognized bee genera are able to extract pollen using buzz pollination (Figure 1.8). However, the majority of bees, including honeybees, cannot perform buzz pollination (Greenleaf and Kremen, 2006; Al-Abbadi, 2010; Santos *et al.*, 2014).

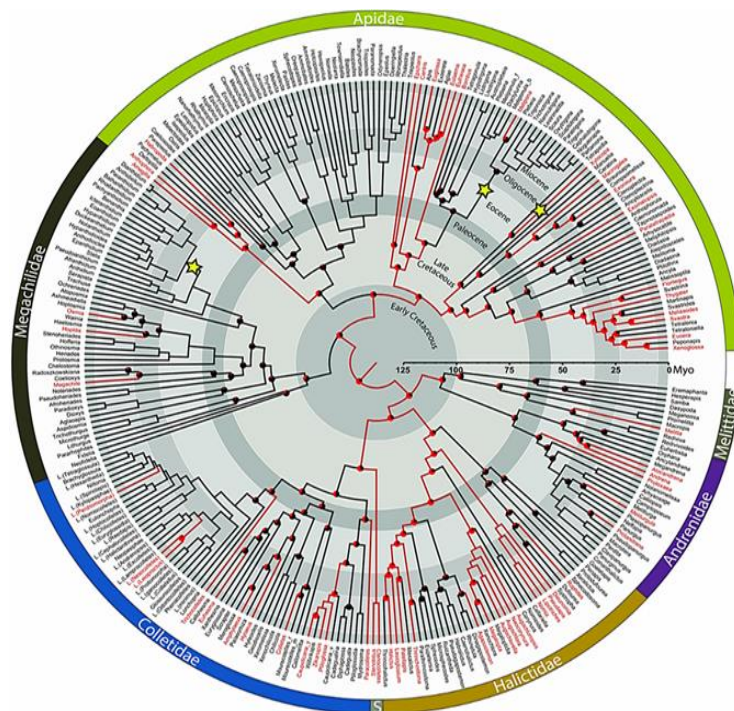


Figure 1.8: The taxa marked in red indicate lineages with at least one bee species capable of buzz pollination (Cardinal *et al.*, 2018).

1.4.2.4.1 Buzz pollination in tomatoes

The morphology of a tomato flower is such that pollen grains are kept in poricidal anthers and the latter must receive some external agitation to be released for pollination availability (Greenleaf and Kremen, 2006; Winfree and Kremen, 2009; Garcia *et al.*, 2015; Amala and Shivalingaswamy, 2017; Cardinal *et al.*, 2018). This morphology allows only bees that can buzz pollinate to access tomato pollen grains (Cardinal *et al.*, 2018). Bees access pollen grains from poricidal flower anthers by vibrating them, they grip onto the anthers using their mandibles and move their flight muscles rapidly, causing the flower and anthers to vibrate, dislodging and forcing pollen grains out of the apical pores of the anthers (Figure 1.9) (Greenleaf and Kremen, 2006; Al-Abbadi, 2010). The tight grip may lead to distinct bruises (Figure 1.10) on the anthers clearly indicating bee visitation (Greenleaf and Kremen, 2006; Franceschinelli *et al.*, 2013; Deprá *et al.*, 2014; Cardinal *et al.*, 2018). It is these distinct marks left by pollinators on tomato flowers that enabled the quantification of bee visits presented in this study. These marks are colloquially referred to as “bee kisses” (Figure 1.10).



Figure 1.9: Buzz pollinating bee gripping on the anther cone of a tomato flower. From (<http://www.wildbeeproject.com/blog/2015/7/18/vegetables-and-bumblebees>).



Figure 1.10: A tomato flower with a red circle indicating a bee kiss. Photo credit: Wiam Haddad, 2019.

1.4.2.4.2 Impact of buzz pollination on tomato production

Tomatoes are cultivated in different cropping systems, which are open fields and protected areas (greenhouses and net-houses) and this usually influences their production (Hanna, 1999; NAMC, 2012; Malherbe and Marais, 2015). Tomatoes grown in protected areas usually require supplementary pollination because of the total absence of bee pollinators and only mild air movement (Hanna, 1999; NAMC, 2012). Vergara and Foncesa-Buendia (2012) reported that in Mexico, bumble bees, particularly *Bombus ephippiatus*, are the efficient pollinators of greenhouse cultivated tomatoes and they significantly increase the yield and quality of the fruit when compared to manual pollination. Furthermore, Hogendoorn *et al.* (2000) indicated that pollination by blue banded Amegilla bees (*Amegilla cingulata*) can result in a 20% increase in tomato weight when compared to manual pollination. For tomatoes grown in open fields, some producers believe that the action of sufficient wind in the open areas is adequate to cause anthers to release pollen (Al-Abbadi, 2010; Klatt *et al.*, 2013). However, previous research has shown that bees contribute significantly to yield and quality of open field tomatoes (Greenleaf and Kremen, 2006; Al-Abbadi, 2010; Depra *et al.*, 2014; Silva-Neto *et al.*, 2017). In a study conducted by Green and Morandin (2006), they found that *Bombus vosnesenkii* and *Anathophora urban* were the common visitors of tomato flowers and they contributed to the production of open field grown tomatoes in Northern California.

Although, buzz pollinating bees have shown to be effective pollinators of tomatoes, there is limited research with regard to tomato pollination in South Africa (Melin *et al.*, 2014; Gous *et al.*, 2017). In numerous countries, several species of bumble bees are used for pollination of tomatoes in both protected areas and open fields (Melin *et al.*, 2014). Bumble bees (Melin *et al.*, 2014) do not occur in South Africa and tomato production in protected areas is mainly depended on manual labour which is associated with high labour costs (R. Kgaphola, 2020, Personal observations). Suggestions have been made that South Africa would benefit from introducing *Bombus terrestris* (buff-tailed bumblebee) and managing their populations for tomato production, however, bringing bumble bees in the country can potentially harm the South African biodiversity and agriculture (Melin *et al.*, 2014). Gous *et al.* (2017) reported that an indigenous carpenter bee, *Xylocopa scioensis* could potentially be used for tomato pollination, however, there has not been research conducted to support this suggestion.

1.4.3 Biodiversity of bees

The family Sphecidae, series Apiformes, consists of a highly diverse group of insects (Figure 1.11) with approximately 20 000 species of bees described globally (Ollerton *et al.*, 2011; Liss *et al.*, 2013; Vaughan *et al.*, 2014). Bees vary in terms of behaviour, ranging from solitary to highly eusocial. In addition, bees differ in terms of their floral preference, body size and shape, nesting biology and foraging behaviour (Michener, 2001). Solitary bees make approximately 85% of the bee population (Batra, 1984). As the name suggests solitary bees do not belong to a colony. The females of solitary bees make their individual nests and provide food for their offspring with no help from other bees. Some of the female solitary bees make nests near other females of the same species, and there are therefore called aggregates. Although these aggregates share the same nests each female provides for her own nests. Some examples of solitary bees are of the genus, *Megachile*, *Nomia*, *Xylocopa* and *Amegilla* (Batra, 1984). In contrast to solitary bees, highly social bees, for example stingless bees and honeybees are usually found in colonies. In highly social bees there is usually division of labour within the colony, for example egg laying individuals, and pollen and nectar collectors.



Figure 1.11: Eight bee species of the series Apiformes. From (<https://www.northernpublicradio.org>).

Bees spend most of their life cycle visiting plants for resources such as nectar, pollen, leaves, resins and oils. Because bees are depended on plant products for almost their complete life cycle, they are frequent visitors of plants (Matsuki *et al.*, 2008; Johnson and Anderson, 2010; Gous *et al.*, 2017; Olynyk, 2017). Bees have several ways of obtaining resources, some bees are generalists while others are specialists (Bascompte *et al.*, 2003; Melin *et al.*, 2014; Vaughan *et al.*, 2014) . Generalist bee species gather resources from a wide variety of plants whereas specialists usually gather resources from a narrow range (Allsopp *et al.*, 2008; Melin *et al.*, 2014; De Palma *et al.*, 2016; Veldtman, 2018). These resources are used as a source of food, to build and protect their nests (Matsuki *et al.*, 2008; Johnson and Anderson, 2010; Gous *et al.*, 2017; Olynyk, 2017).

During flower visits, bees carry relatively large amounts of pollen and as they move from one plant to the next, the pollen is spread and some of it lands on the stigma of the next flower (Larson and Barrett, 1999; Wolff *et al.*, 2008; Johnson and Anderson, 2010). In addition, bees also assist in the pollination of some plant species that are known as self-fertile, for example in crops such as peppers which are self-pollinated, bees assist in moving pollen grains that are hidden in the anthers to the associated

stigma through buzz pollination (Winfree *et al.*, 2008; Vinícius-Silva *et al.*, 2017; Amjad *et al.*, 2018; Rosi-Denadai *et al.*, 2020).

Previous research has indicated that bees are more effective pollinators in comparison to other insects because of their frequent visit to plants and their structural ability to carry relatively large amounts of pollen on their bodies (Matsuki *et al.*, 2008; Munyuli, 2011). As a result of their ability to move pollen between plants or within plants, bees have been used for years for the pollination of variety of crops (Kremen *et al.*, 2002; Melin *et al.*, 2014; Gous *et al.*, 2017).

Apis mellifera (honeybees) are the most common generalist pollinators and are used in the pollination of wide variety of crops and wild flowers (Power, 2010; Ollerton *et al.*, 2011). According to Melin *et al.* (2014), Klein *et al.* (2017) and Veldtman (2018), the majority of fruit tree farmers especially avocados, apples and pears are depended on honeybees for crop production. Studies by Klatt *et al.* (2013), Stein *et al.* (2017) and Fikadu (2019) have also reported that honeybee pollination improved yield and quality of various crops, such as red onion, ginger, apple, strawberries, blueberries, coffee, and pears. Unlike most other species of bees, honeybees can be managed, fed, bred, kept in boxes and can be moved from one area to another to assist in pollination services (Melin *et al.*, 2014). Colonies of honeybees are kept throughout the year to meet pollination services.

In the Western Cape, South Africa, pollination services by honeybees is estimated R980 million per year (Melin *et al.*, 2014; Gous *et al.*, 2017). The original emphasis on honeybees for crop pollination was based on their convenience and effectiveness in intensive agricultural systems and because honeybees can be moved between areas for pollination services (Melin *et al.*, 2014; Martin *et al.*, 2019). However, agricultural demand for honeybees may outstrip supply and greater demand for high value crops such as nut crops may further increase (Steffan-Dewenter *et al.*, 2005; Carvalheiro *et al.*, 2011; Vaughan *et al.*, 2014; Tamburini *et al.*, 2019). Additionally, there have been reports over the past 15 years with regard to honeybees declining (Julier and Roulston, 2009; Melin *et al.*, 2014).

The vast majority of bee species are non-*Apis* species and because they are usually not recognized in crop production, they are often overlooked as pollinators (Matsuki *et al.*, 2008; Ollerton *et al.*, 2011; Melin *et al.*, 2014; Vaughan *et al.*, 2014). Non-*Apis* refers to any other bee species that are not honeybees (*A. mellifera*) and includes a wide variety of bees that are solitary, semi-social and eusocial (Vaughan *et al.*, 2014). One of the non-*Apis* bees that are used for the pollination of crops in some countries are *Bombus* spp. (family Specidae, series Apiformes) (bumble bees) (Steffan-Dewenter *et al.*, 2002; Vaughan *et al.*, 2014). Steffan-Dewenter *et al.* (2002) and Martin *et al.* (2019) reported that over 1 million bumble bee colonies were sold worldwide in 2006 for crop production and because of the increasing need for pollinators, the use of non-*Apis* as pollinators has been increasing and becoming more common globally. Farmers in the United States are using solitary bees such as the wood and ground nesting-alkali bees (*Nomia melanderi*), leaf cutting bees (Megachilidae) and blue orchard bees (*Osmia lignaria*) for pollination of crops, such as almond, alfalfa seed and sweet cherry (Bosch and Kemp, 2002; Vaughan *et al.*, 2014).

In most African countries, the use of non-*Apis* bees as crop pollinators is not yet recognized. However, in Kenya, development of management strategies to use solitary bees such as *Xylocopa calens*, *Xylocopa incostans* and *Xylocopa flavorufa* for crop pollination is being investigated (Vaughan *et al.*, 2014). Furthermore, recent research has highlighted the importance of bee diversity in crop pollination and the importance of the interaction between *Apis* and non-*Apis* bee species (Melin *et al.*, 2014; Vaughan *et al.*, 2014). However, a balanced perspective on the importance of both *Apis* and non-*Apis* bees for pollination service is lacking globally (Melin *et al.*, 2014). Munyuli (2011), Melin *et al.* (2014) and Willmer *et al.* (2017) reported that there is growing evidence indicating that a diverse complex of bees provides improved pollination services to crops. Both *Apis* and non-*Apis* pollinators are exposed to different environmental pressures that have resulted in shifts in their diversity and abundance. These environmental stresses include climate change, land use changes, pests and diseases and pesticides use (Klein *et al.*, 2017; Belsky and Joshi, 2019). Below are some of the factors that affects bee diversity, communities, and abundance.

1.4.4 Factors influencing bee diversity, communities and abundance

1.4.4.1 Climate change

Changes in climatic conditions, particularly increased global temperatures has an adverse impact on pollination services as it influences plant-pollinator interactions (Bosch and Kemp, 2002) .

Abrha (2018) indicated that the decline in pollinators as a result of climate change have been reported for honeybees and bumble bees. The biological impact of increasing temperatures is dependent on the sensitivity of individual species to temperature changes (Bosch and Kemp, 2003; Grote, 2014; Giannini *et al.*, 2017; Abrha, 2018). Giannini *et al.* (2017) reported that some insects are sensitive to temperature changes because increase in temperatures may exceed the species' thermal tolerance levels which may have an effect on species distribution and abundance in affected areas. They also indicated that overall pollinator abundance and diversity will decrease in most of Brazil by the year 2050 as a result of climate change. Climate change can potentially affect pollinator species by changing its diversity and abundance which will further influence crop productivity and biodiversity of plant species (Bennett and Chaplin-Kramer, 2016).

1.4.4.2 Land use changes

Land use changes, involving the removal of the natural and semi-natural vegetation has a potential impact on bee richness, abundance, community and diversity (Winfree *et al.*, 2008; Klein *et al.*, 2007; Olynyk, 2017). The majority of land use changes are associated with agricultural intensification, whereby the land is transformed into crop fields (Kremen *et al.* 2002; Zhang *et al.*, 2007; Carvalheiro *et al.* 2011; Melin *et al.* 2014). Gous *et al.* (2017) found that the diversity, richness and abundance of bees are greater in sites with arable land compared to those under intensive animal husbandry. In addition, Guzman *et al.* (2019) reported that the diversity of bees in vineyards was less when compared to the nearest natural vegetation because of lack of floral diversity due to monoculture.

Bee species respond differently to land use changes. Bees that bore holes into wood and use other resources such as leaves to seal their nests may be affected negatively

by land use changes (Kremen *et al.*, 2002; Greenleaf and Kremen, 2006; Winfree *et al.*, 2008). In addition, nests of solitary bees may also be constructed in abandoned rodent nests which may be more plentiful in natural vegetation than in croplands (Greenleaf and Kremen, 2006; Morandin and Kremen, 2013)

The majority of the studies on the negative impacts of land use on bees have been focused on wild bees, particularly non-*Apis* bees (Greenleaf and Kremen, 2006; Winfree *et al.*, 2008; Santos *et al.*, 2014; Amala and Shivalingaswamy, 2017). Furthermore, land use changes can be used to predict changes in the community and diversity of bees globally because bees are known as indicator species for environmental changes (Carvalho *et al.*, 2011; Munyuli, 2011; Klein *et al.*, 2017; Olynyk, 2017; Blowers *et al.*, 2018; Kovács-Hostyánszki *et al.*, 2019).

1.4.4.3 Pesticides use

Almost a quarter of the world's terrestrial surface has been converted to agricultural land onto which applications of pesticides and other agrochemicals are often done (Kremen *et al.*, 2002; Melin *et al.*, 2014). Applications of pesticides in agricultural production has enabled farmers to increase crop production because pesticides assist in the control of pests and diseases (Kremen *et al.*, 2002; Melin *et al.*, 2014; Vaughan *et al.*, 2014; Klein *et al.*, 2017). However, studies have indicated that the overuse and application of pesticides harm bees. Bees react differently to pesticides than most other insecticides because bees have largely different life and feeding strategies and are highly mobile (Klein *et al.*, 2017; Bareke & Addi, 2019).

Vaughan *et al.* (2014) reported that the majority of studies investigating the impact pesticides impacts on bees have been focused on honeybees, this is because some researchers are of the opinion that honeybees are extremely sensitive when compared with other bee species. However, recent studies have reported susceptibility in other bee species especially to chemicals that were considered safe for honeybees (Vaughan *et al.*, 2014; Klein *et al.*, 2017). Some pesticides, for example pyrethroids and neonicotinoids have been reported to alter the activities of cytochrome P450 mediated enzymes in bees (Vaughan *et al.*, 2014). Furthermore, this may hinder pollination services thus influencing crop production. It is therefore important to understanding and documenting the reaction of bees towards different pressures

especially in agricultural landscapes (Winfree *et al.*, 2008; Deprá *et al.*, 2014; Gous *et al.*, 2017).

1.5 Synopsis

This chapter examined the overall importance of pollination ecosystem services in crop production, briefly discussed different pollination methods, biodiversity of bees and pollination ecology of tomatoes. The following chapters will report in detail on the pollination status of tomatoes in Limpopo Province, South Africa by answering the following questions,

- does distance from the natural vegetation influence bee visitation rates and are bee visitation rates constant throughout the four bio-climatic regions studied?
- which bees are the predominant visitors/pollinators of the tomato crops?
- does pollination provided by bee species improve tomato production both quality and yield quantity?

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Chapter 2

The effect of field proximity to natural vegetation on bee visitation rates to tomato flowers in various bioclimatic-regions in the Limpopo Province

Abstract

Bee visitation in crop fields are influenced by several factors such as landscape changes, availability of resources and the continuous use of pesticides. In this study, the effect of crop field distance from natural vegetation on non-*Apis* bee visitation rates on tomato flowers was investigated in four different regions. Observations were made on four farms in the Limpopo Province, South Africa, over the 2018 and 2019 seasons. Data were collected in tomato fields along four transects which were separated using four distance intervals. These distance intervals were as <50 m, 51-100 m, 101-200 m and >200 m from the natural vegetation in each study area. Results showed that bee visitation rates varied among farms. This could be partly attributed to different planting months/seasons between the various study areas. In addition, the distance from the natural vegetation also influenced bee visitation rates. The latter was higher closer to natural vegetation and differed significantly from visitation rates recorded at the furthest distance in two of the farms. The findings from this study can be used to evaluate/plan the planting seasons/months at the ZZ2 agribusiness while further studies are necessary to examine the effect of other factors such as weather conditions, the use of pesticides, plant height, floral diversity in the natural vegetation and bee diversity and abundance on bee visitation rates.

Key-words: bee kisses, bee visitations, connivent anthers, natural vegetation, pollination

2.1 Introduction

Plants and pollinators have a mutualistic relationship in which both parties are reliant on each other for survival, plants for reproduction and pollinators for pollen, nectar, oil and other rewards (Aizen *et al.*, 2009; Melin *et al.*, 2014; Gous *et al.*, 2017). Plants are usually visited by different pollinators depending on their floral morphology (Ollerton *et al.*, 2011; Melin *et al.*, 2014). Insects, particularly bees, are the most common pollinators of a wide variety of flowers and crops across the globe (Aizen *et al.*, 2009). Bees are active foragers, and they collect various substances such as oil and nectar from plants (Melin *et al.*, 2014; Gous *et al.*, 2017). During their visits, pollen become attached to their bodies and as they move from one flower to the next, they spread the pollen and pollination which may result in fertilization, takes place (Gous *et al.*, 2017). Pollination is an important service in agricultural production (Aizen *et al.*, 2009; Melin *et al.*, 2014). It is estimated that at least one third of food produced relies on bee pollination for its production (Melin *et al.*, 2014).

A decline in crop-pollinator interactions may negatively affect crop production and thus have an impact on food supply (Veldtman, 2018). The majority of agricultural landscapes lack sufficient resources such as nesting materials and food for these insects because of changes in climatic conditions, lack of biodiversity, continuous use of pesticides and loss of natural and semi-natural vegetation (Greenleaf and Kremen, 2006; Klein *et al.*, 2012; Melin *et al.*, 2014; Gous *et al.*, 2017). Carvalheiro *et al.* (2006) found that agricultural fields characterized by monoculture practices and lack of floral diversity resulted in limited food resources for bee pollinators, thus affecting pollination services. In addition, multiple studies have also reported that floral resource availability had a positive effect on bee activity thus improving yield and quality of the majority of crops (Kremen *et al.*, 2002; Klein *et al.*, 2012; Lowenstein *et al.*, 2014).

Establishing a resource-rich environment through planting a wide variety of flowering plants to create artificial natural habitats can help support beneficial insects such as bees in agricultural landscapes (Lowenstein *et al.*, 2014). Furthermore, Blaauw and Isaacs (2014) reported that higher proportions of natural vegetation within agricultural landscapes resulted in improved crop pollination in these areas.

Efficiency of pollination is dependent on the frequency of bee visits to flowers and their effectiveness to transport and spread pollen (Zurbuchen *et al.*, 2010; Young *et al.*, 2018). Therefore, if the environment is not conducive for the establishment and persistence of bee species, problems arise with regard to pollination of agricultural crops (Klein *et al.*, 2012; Melin *et al.*, 2014). For example, Classen *et al.* (2015) reported that higher levels of bee-flower interactions occur when temperatures are higher, and this supports the hypothesis that temperature influences diversity by constraining resource exploitation in ectotherms. Numerous studies have documented the relationships between pollinator diversity and improved yield. Increased pollination because of higher visitation rates is obtained by refining practices that are ecologically friendly and promote on farm pollinator conservation (Kremen *et al.*, 2003; Klein *et al.*, 2007; Munyuli, 2011; Melin *et al.*, 2014; Gous *et al.*, 2017; Veldtman, 2018).

Tomatoes are regarded as one of the most valuable cash crops for both small and commercial scale farmers in South Africa and almost 54% of tomatoes in the country are produced in the Limpopo Province by ZZ2 tomato farms (Malherbe and Marais 2015). Tomatoes have complete flowers, which means the anthers (male) and the stigma (female) are found on the same flower which promotes self-pollination (Toni *et al.*, 2020). However, anthers of tomato flowers are connivent and open poricidally. They require agitation/shaking for the pollen to be released to the receptive stigma of the same flower (Greenleaf and Kremen, 2006; Al-Abbadi, 2010; Franceschinelli *et al.*, 2013; Deprá *et al.*, 2014; Santos *et al.*, 2014).

Previous research has shown that some species of the non-*Apis* bees particularly solitary bees, are essential pollinators of tomatoes. Additionally, bee pollination leads to improved yield and quality when compared to the effect of wind only (Greenleaf and Kremen, 2006; Al-Abbadi, 2010; Franceschinelli *et al.*, 2013; Deprá *et al.*, 2014; Santos *et al.*, 2014). Some non-*Apis* bees have the ability to produce efficient vibrations through a syndrome known as buzz pollination while honeybees (*Apis mellifera*) cannot effectively do buzz pollination (Greenleaf and Kremen, 2006, Gous *et al.*, 2017). During buzz pollination, non-*Apis* bees rapidly contract their indirect flight muscles, producing strong vibrations. By firmly gripping the fused anthers with its mandibles, these vibrations are very effectively transferred from the bee to the flower that forcibly releasing pollen from the inside of the flower anthers. Some of this pollen

fall on the bodies of the bees and some fall on the stigma (Greenleaf and Kremen, 2006; Al-Abbadi, 2010; Franceschinelli *et al.*, 2013; Deprá *et al.*, 2014; Santos *et al.*, 2014). This method of clasping the flower is known as “buzzing” and it leaves very distinct brown marks on tomato flowers (refer to Chapter 1, Figure 1.10). These marks are known as “bee kisses” (Greenleaf and Kremen, 2006; Deprá *et al.*, 2014; Cardinal *et al.*, 2018). It is widely recognized that the presence of bee kisses indicates that flowers were visited by buzz pollinating bees (Toni *et al.*, 2020).

Although tomato is one of the important cash crops in South Africa, there is little information that currently exists on the pollination status of this crop. Therefore, the aim of this study was to determine the effect of proximity to the natural vegetation on bee visitation rates on tomato flowers and to assess whether the visitation rates differ between farming areas.

2.2 Materials and methods

2.2.1 Study area

The study was conducted on four tomato producing farms located in different climatic zones of the Limpopo Province, South Africa (Figure 2.2). The farms were Waterpoort (22° 53' 53.93" S, 29° 37' 58.73" E), Vreedzaam (23° 41' 17.50" S, 29° 59' 46.16" E), Jachtpad (23° 31' 15.47"S, 30°14' 28.41" E) and Dikgale (23°40' 59.38" S, 29° 45' 21.25"E) (Figure 2.1). The weather conditions at the four farms during the 2018-2019 period are indicated in Table 2.1. The four study areas fall in different vegetation types as described by Mucina and Rutherford (2011).

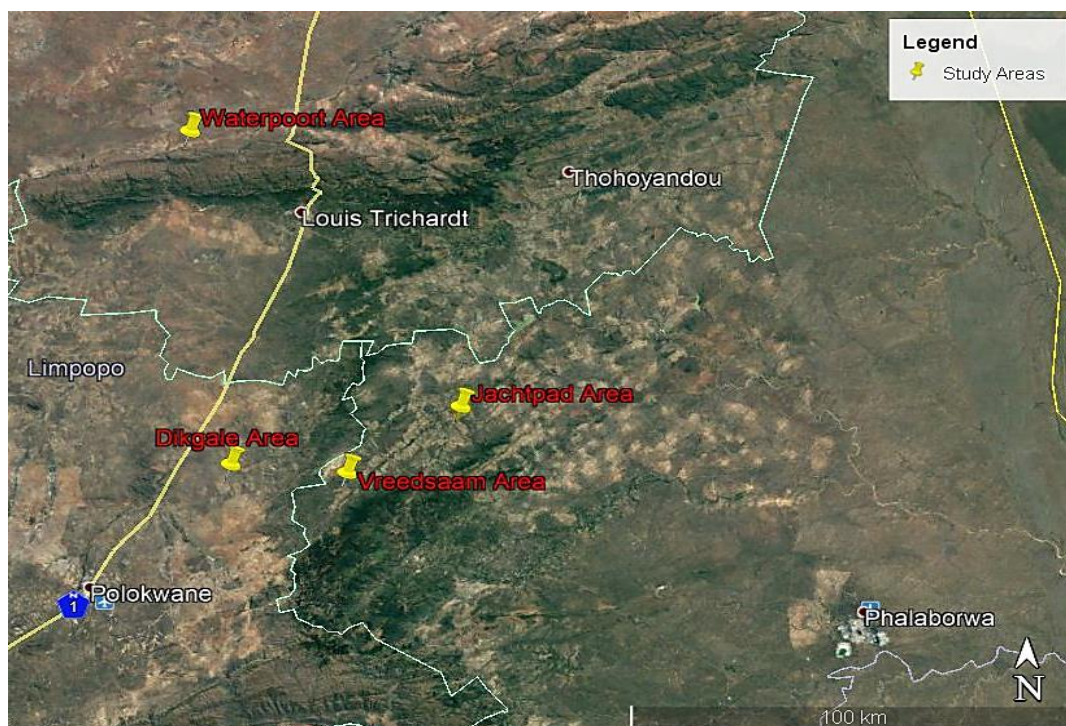


Figure 2.1: Map indicating the four study areas (Waterpoort, Jachtpad, Vreedzaam and Dikgale) in the Limpopo Province where bee visitation on tomato flowers were assessed.

Table 2.1: Weather conditions of the four study areas across Limpopo Province for the months that visitation rates of bees to tomato flowers were collected.

Region	Climatic zone	Min Temp (°C)	Max Temp (°C)	Low wind Speed (km/h)	High wind speed (km/h)	Low humidity (%)	High Humidity (%)	Rainfall (cm)
Waterpoort (2018)	BSh	17.62	31.16	4.71	30.61	29.55	68.45	1.02
Waterpoort (2019)	BSh	15.70	30.34	6.21	30.32	35.69	76.91	0.92
Jachtpad (2018)	Bsk	15.98	28.92	3.22	24.44	48.59	88.48	1.80
Jachtpad (2019)	Bsk	14.84	28.95	2.82	25.09	43.97	85.06	1.22
Vreedzaam (2018)	Bsk	15.08	29.07	1.42	15.67	40.75	84.45	0.70
Vreedzaam (2019)	Bsk	13.93	28.89	2.82	25.09	43.97	88.55	1.42
Dikgale (2018)	Bsk	18.23	35.67	2.83	22.04	42.55	88.55	1.42
Dikgale (2019)	Bsk	20.12	33.55	6.34	32.82	53.44	73.75	1.49

BSh = Hot semi-arid climate; BSk = Tropical and subtropical steppe climate

2.2.1.1 Waterpoort

Waterpoort is located in the transition between the Soutpansberg (SPB) Mountain Bushveld and the Musina Mopane Bushveld. SPB Mountain Bushveld is mountainous, with the highest mountains in the west, and an increasing number of lower mountain

ridges towards the east (Mucina and Rutherford, 2011). The natural vegetation consists of a dense tree layer and a poorly developed grassy layer (Figure 2.2). Information on the planting season and data collection period in the area are provided in Table 2.2. The main vegetation variations within the SPB are subtropical moist thickets, moist bush clumps, relatively open savannah and arid mountain bushveld. The Musina Mopane Bushveld, has undulating plains from around Baines Drift and Alldays in the west, remaining north of the SPB. The main vegetation of the area is open woodland to moderately closed shrubland dominated by *Colophospermum mopane*. Some of the important plant taxa from both the vegetation areas are listed in Table 2.3. (Mucina and Rutherford, 2011).



Figure 2.2: The grassy area is a fallow tomato field in the Soutpansberg Mountain Bushveld. Photo Credit: Retang Kgaphola, 2020.

Table 2.2: Planting period, tomato production period and bee visitation collection period for the four farms.

Farms	Planting months	Production period (months)	Data collection period
Waterpoort	Feb-March	8	Apr-Jul
Vreedzaam	May-Jun	8	Jul-Oct
Jachtpad	May-Jun-	7	Jul-Oct
Dikgale	Aug-Sep	8	Oct-Dec

2.2.1.2 Vreedzaam

Vreedzaam farm is situated between the Tzaneen Sour Bushveld (Mucina and Rutherford, 2011). The vegetation in the Tzaneen Sour Bushveld consists of tall open bushveld, well-developed and tall grass occurring on low to high mountains, with undulating plains mainly at the base of the lower middle slopes of the north east (Figure 2.3A and B). Information on the planting season and data collection period in the area are provided in Table 2.2. Some of the important plant taxa found in this area are listed in Table 2.3.



Figure 2.3: (A) Tzaneen Sour Bushveld on the left and (B) a tomato field in the same area. Photo credits: Wiam Haddad, 2019 (A) and Retang Kgaphola, 2019 (B).

2.2.1.3 Jachtpad

Jachtpad is situated in the Mopane Gabbro shrubland (MGS) and this area has a slightly undulating landscape while it is mainly a low shrub layer with two main structural variations both dominated by *Colophospermum mopane* (mopane tree) in a shrubveld with a few larger shrubs and trees including *Senegalia nigrescens* (knob thorn) (Figure 2.4). Information on the planting season and data collection period in the area are provided in Table 2.2.



Figure 2.4: The Mopane Gabbro bushveld. (Mucina and Rutherford, 2011).

2.2.1.4 Dikgale

In terms of vegetation type, Dikgale is situated in the Polokwane Plateau Bushveld. The vegetation consists of moderately undulating plains with a short open tree layer with well-developed grass plains and occasional trees at higher altitude (Figure 2.5). Information on the planting season and data collection period in the area are provided in Table 2.2. Some of the important taxa found in the area are listed in Table 2.3.



Figure 2.5: The Polokwane plateau bushveld. Photo credit: Nyiko Mutileni, 2020.

Table 2.3: Important bee provisioning plant taxa occurring in the vegetation types of the different regions where bee visitation rate observations were conducted

Vegetation Classification	Study site			
	Waterpoort	Vreedzaam	Jachtpad	Dikgale
Mucina and Rutherford	Soutpansberg Mountain and Musina Mopane Bushveld	Tzaneen Sour Bushveld	Mopane Gabbro Shrubland	Polokwane Plateau Bushveld
Trees and Shrubs	Various <i>Vachellia</i> and <i>Senegalia</i> spp. <ul style="list-style-type: none"> • Profuse flowering, source of pollen 	Various <i>Vachellia</i> and <i>Senegalia</i> spp. <ul style="list-style-type: none"> • Profuse flowering, source of pollen 	<i>Colophospermum mopane</i> <ul style="list-style-type: none"> • Important nesting site for bees 	Various species <i>Vachellia</i> and <i>Senegalia</i> <ul style="list-style-type: none"> • Profuse flowering, source of pollen
	Numerous <i>Commiphora</i> spp. <ul style="list-style-type: none"> • Soft wood preferred nesting site for bees 	Numerous <i>Combretum</i> spp. <ul style="list-style-type: none"> • Soft wood important for nesting site 	Various <i>Vachellia</i> and <i>Senegalia</i> (<i>Acacia</i>) spp. <ul style="list-style-type: none"> • Profuse flowering, source of pollen 	<i>Sclerocorya birrea</i> <ul style="list-style-type: none"> • Flowers and fruits are important for provision of bees
	<i>Adonsonia digitata</i> (Baobab) <ul style="list-style-type: none"> • Flowers are important source of pollen and nectar 	<i>Kirkia acuminata</i> <ul style="list-style-type: none"> • Softwood necessary for bee nests • Flowers (pollen and nectar) 	Numerous <i>Combretum</i> spp. <ul style="list-style-type: none"> • Flowers are a good source of pollen for bees 	<i>Euphorbia ingens</i> <ul style="list-style-type: none"> • Source of nectar for bees
Forbs	<i>Tribulus</i> spp. <ul style="list-style-type: none"> • Profuse flowering (pollen and nectar) 	<i>Abutilon</i> sp. and <i>Hibiscus</i> spp. <ul style="list-style-type: none"> • flowers throughout the year (pollen and nectar) 	<i>Argemone ochroleuca</i> <ul style="list-style-type: none"> • Long flowering season • Flowers are good source of pollen 	1 <i>Argemone ochroleuca</i> <ul style="list-style-type: none"> • Long flowering season • Flowers are good source of pollen
	<i>Solanum delagoense</i> <ul style="list-style-type: none"> • Good source of pollen for bees 	<i>Flaveria bidentis</i> <ul style="list-style-type: none"> • Good source of pollen for bees 	<i>Heliotropium</i> spp. <ul style="list-style-type: none"> • Profuse flowers 	<i>Solanum delagoense</i> <ul style="list-style-type: none"> • Good source of pollen for bees
Anthropogenic vegetation	<ul style="list-style-type: none"> • Tomatoes • Potatoes • Few house gardens 	<ul style="list-style-type: none"> • Tomatoes • Mangoes • Blueberries • Avocados • Numerous house gardens 	<ul style="list-style-type: none"> • Tomatoes • Avocados • Cabbage • Few house gardens • Green peppers 	<ul style="list-style-type: none"> • Tomatoes • Potatoes • Numerous house gardens

2.2.2 Experimental layout and data collection

Data on bee visitation rates were collected during two consecutive seasons (2018 and 2019) in four different areas. Data were collected for a period of 12 and 16 weeks after flowering commenced in the 2018 and 2019 seasons respectively.

Each experiment consisted of four transects which were used as a representative sample of the tomato fields. Each transect was either <50 m, 51-100 m, 101-200 m or >200 m away from the nearest natural vegetation while only tomato plants occurred for at least 500 m on the other side of the field (Figure 2.6). The distance from the natural vegetation was measured using a measuring tape and GPS coordinates were

recorded at each point. In this context, we defined natural vegetation as an area that has suffered little disturbance by human factors such as agriculture or forestry and consisted of a diverse population of plants, including grasses, shrubs and trees.

Each transect had four data collection points which were 50 m apart (Figure 2.6). The collection points were represented by an area of 5 m wide between two tomato trellis support poles, also called a chamber, consisting of 12 to 14 tomato plants. In each selected 5 m area, a total of 20-30 open randomly selected tomato flowers were evaluated.

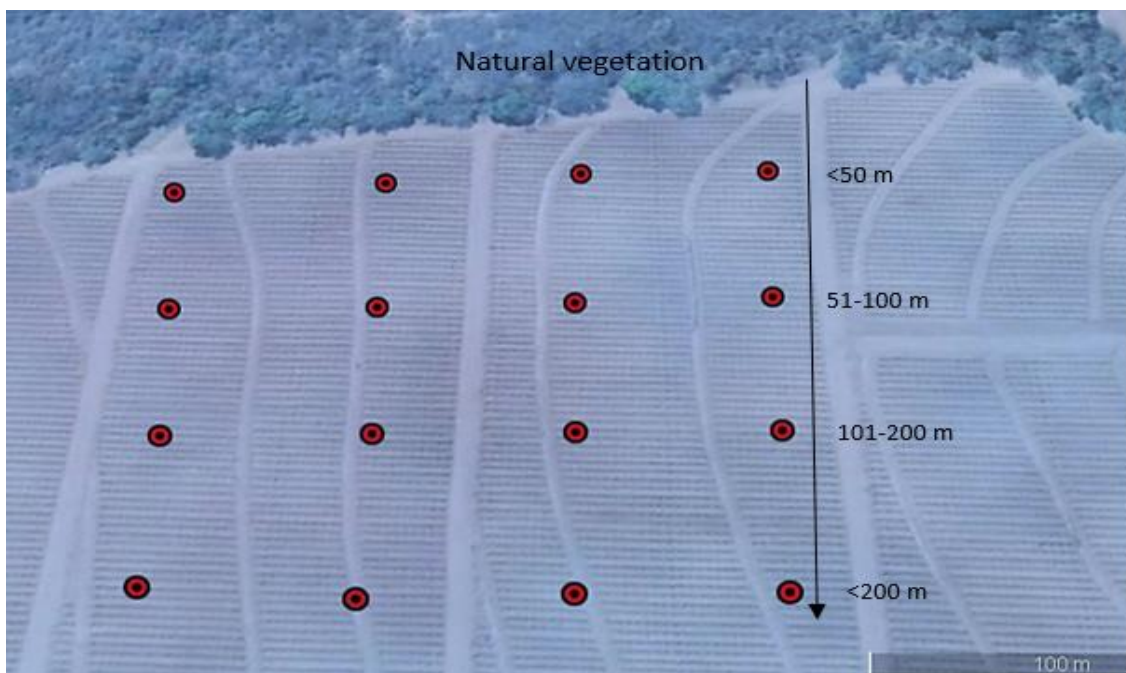


Figure 2.6: Data collection points (red dots), with the arrow indicating how far the points were from the natural vegetation and the red dots indicating data collection points.

The frequency of bee visitation to tomato flowers was determined by counting the number of tomato flowers with and without bee kisses on 20-30 flowers at each point. At each point, the following variables were recorded: number of flowers with and without bee kisses, week of data collection and weather condition (hot, windy, raining, partially cloudy, overcast).

2.2.3 Data Analysis

Data on the percentage of visits by non-*Apis* bees (bee kisses) was analysed by a two-way factorial ANOVA type of hierarchical linear model with blocks per field as the primary unit of measurement. Since all the measurements in a block were regarded

as replicates, all variation within the blocks was taken into account. The covariance matrix was assumed to be unstructured. Sidac multiple comparisons were used as post hoc tests. Further, the effect of location on bee visitation rate was analysed using one-way ANOVA (Statistica 10 (www.satisica.com)).

2.3 Results

2.3.1 Effect of distance from the natural vegetation on bee visitation rates

The trends detected in this study showed that bee visitation rate differed significantly ($P \leq 0.05$) between <50 m and >200 m distance away from the natural vegetation (Table 2.4). The lowest average bee visitation rate (26.2%) was observed at the distance of >200 m away from the natural vegetation, followed by 27.9% in the distance of 100-200 m away from the natural vegetation. The highest bee visitation rate of 33.2% was recorded on flowers <50 m away from the natural vegetation (Table 2.4).

In terms of the effect of the distance from the natural vegetation on bee visitation rate per farm, at Dikgale and Jachtpad no significant differences were observed between the four different distances from the natural vegetation. However, at Vreedzaam significant differences ($P \leq 0.05$) were observed between the incidence of bee kisses at a distance of <50 m and >200 m from the natural vegetation (Table 2.4). At Waterpoort, significant differences ($P \leq 0.05$) were observed in the incidence of bee kisses recorded at a distance of <50 m and those at 101-200 m and >200 m from the natural vegetation (Table 2.4). Furthermore, the average visitation rates differed significantly between the different farms with Dikgale and Jachtpad having significantly ($P \leq 0.05$) lower bee visitation rates than Vreedzaam and Waterpoort (Table 2.4).

Table 2.4: The effect of proximity to the natural vegetation on bee visits to tomato in commercial tomato fields.

Distance (m)	Mean % bee visits ± Standard error (SE)				Average
	Farms				
	Dikgale	Jachtpad	Vreedzaam	Waterpoort	
<50	18.22±4.51a*	19.65±4.00a	32.26 ± 4.04a	62.84 ± 4.09a	33.24 ± 4.16a
51 - 100	14.50 ±6.10a	19.25 ±4.43a	30.32 ± 4.31abc	59.11 ± 4.16ab	30.80 ± 4.75abc
101 - 200	15.89 ± 6.69a	19.28 ± 4.43a	37.09 ± 4.16abc	39.32 ±4.17b	27.90 ± 4.86bc
>200	17.97 ± 4.32a	19.21 ± 4.37a	29.80 ± 4.22bc	38.00 ± 4.10b	26.24 ± 4.25bc
Average	16.64 ± 5.54a	19.34 ± 4.31a	32.36 ± 4.18b	49.82 ± 3.94bc	29.54 ± 4.51b

*Letters within columns that differ indicate a significant difference at $P \leq 0.05$ between the treatments according to Tukey HSD-test.

2.3.2 The effect of location on bee visitation rates

The bee visitation rates recorded at the different farms are presented in (Figure 2.7). The results obtained in this study showed that visitation rates differed significantly ($P \leq 0.05$) across the different farms. The average highest bee visitation rate of 49.8% was observed at Waterpoort farm, followed by Vreedzaam (32.4%) and Jachtpad (19.3%). The lowest bee visitation rate was detected at Dikgale (16.6%).

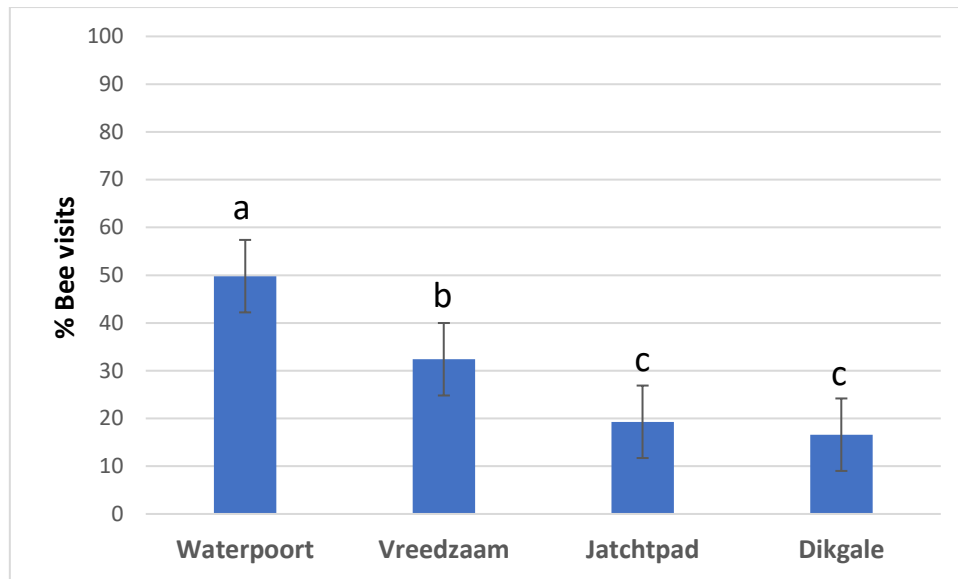


Figure 2.7: Average bee visitation rates per farm over an evaluation period of 12-16 weeks conducted during 2 years. Different letters above bars indicate significant differences ($P \leq 0.05$) between the farms. Bars indicate standard errors.

2.3.3 The effect of plant age on bee visitation rates

At Waterpoort, the percentage bee visitation rates differed significantly ($P \leq 0.05$) between the early (5-9 weeks after planting), middle (9-12 weeks after planting) and late (13-16 weeks after planting) development stages with the highest number during the early stage (48.7%), followed 33.7% at 9-12 weeks and 23.9% at the late stage of development (Figure 2.8A). The highest visitation rates were recorded during April at Waterpoort.

At Vreedzaam, the opposite was observed with significant differences ($P \leq 0.05$) between the three development stages of the tomato plant but a significantly higher percentage of bee visitation rates in the last four weeks (13-16 weeks) (37.8%) was observed compared to the middle stage (21.2%) and the lowest percentages during the early weeks (18.3%) (Figure 2.8B). The highest visitation rates at Vreedzaam were recorded during October.

At Jachtpad, significant differences were only observed for bee visitation rates during the late developmental stage (week 13-16) with 5.5% compared to the other stages ranging from 2.3 to 3.4% (Figure 2.8C). Percentage bee visitations increased over time. The highest percentages at Jachtpad were recorded during October.

At Dikgale, no significant difference was observed in bee visitation rates during the early, middle, or late development stages (Figure 2.8D), with bee visitation rates ranging from 2.3 to 8.8%.

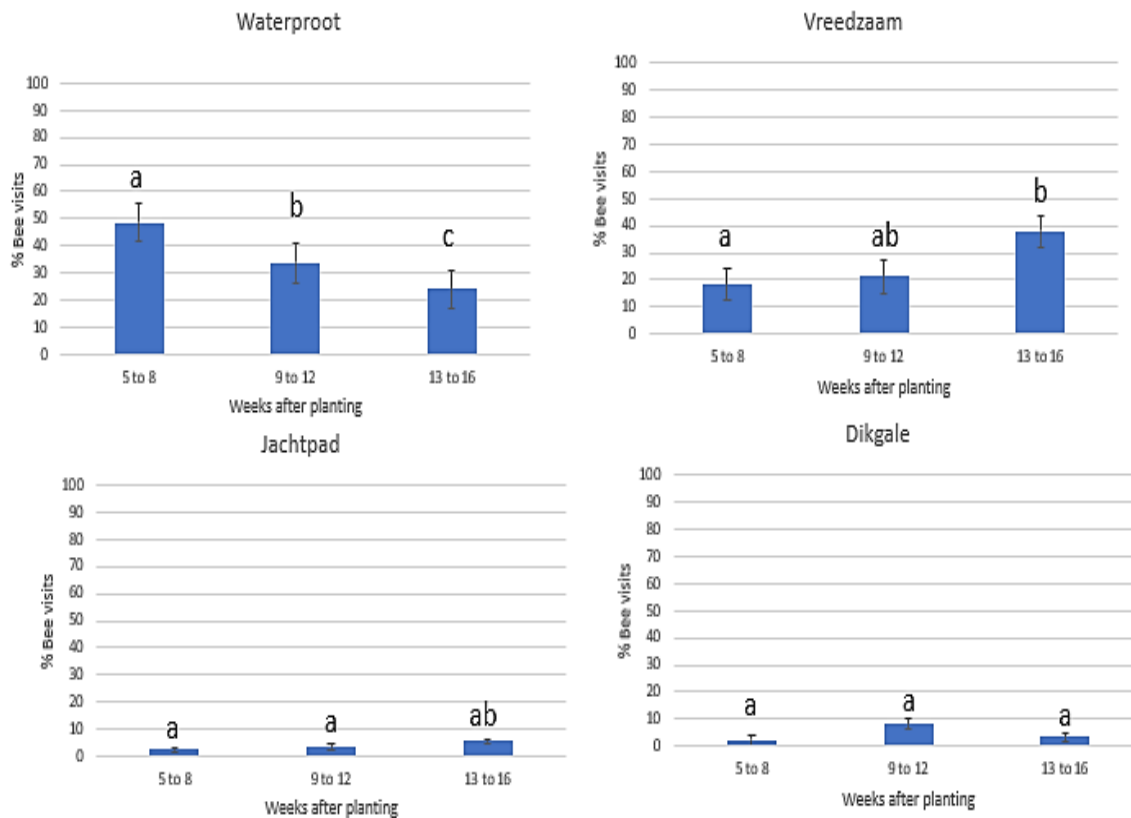


Figure 2.8: Average percentage bee visits recorded during two years from 5 to 16 weeks after planting at (A) Waterpoort, (B) Vreedzaam, (C) Jachtpad and (D) Dikgale. Different letters above bars indicate significant differences ($P \leq 0.05$) between sampling periods on the farms. Bars indicate standard errors.

2.4 Discussion

Pollination ecosystem services are important in the production of agricultural crops because it is the primary mechanism of sexual reproduction in plants (Melin *et al.*, 2014). Kremen *et al.* (2002) reported that there is a positive effect of bee visitation rates on fruit set and the quality of pollinator dependant crops thus suggesting the importance of bees in crop production. In tomato production, high rates of bee visitations to tomato flowers resulted in improved fruit set and increased tomato yield (Greenleaf and Kremen, 2006). Although tomato flowers are self-pollinating, Deprá *et al.* (2014) found that the presence of bee pollinators in tomato fields increased

productivity in terms of yield and quality. In addition, Greenleaf and Kremen (2006), Deprá *et al.* (2014) and Amala (2017) reported that tomato flowers are visited/pollinated by native bees which include non-*Apis* bee species and not the domesticated/managed honeybee (*Apis mellifera*).

Measuring and documenting bee visitation rates in agricultural landscapes is a technique used to determine the pollination ecosystem services in these areas (Munyuli, 2011). The purpose of measuring bee visitation rates and investigating the factors that affect visitation rates in agricultural landscapes helps in building a scope on pollination efficiency and success (Melin *et al.*, 2014). These factors, including availability of natural vegetation, climatic conditions and farming practices, may have an impact on bee visitation rates (Munyuli, 2011). The emphasis of this study was on determining whether bee visitation rates were influenced by distance from the natural vegetation in the four study areas as a primary factor and which other factors played a role in bee visitation rates. Proximity to natural vegetation is one of the major factors that has an impact on bee visitation rates in agricultural landscapes (Kremen *et al.*, 2004; Ruiz-Toledo *et al.*, 2019). Numerous studies have confirmed a positive impact of natural vegetation on bee visitation rates in coffee orchards (Munyuli, 2011), tomato fields (Greenleaf and Kremen, 2006), almond orchards (Klein *et al.*, 2012) and mango orchards (Carvalho *et al.*, 2012).

This study indicated that the proximity of the tomato field to natural vegetation had a significant effect on bee visitation rates and a significant difference was observed between number of bee kisses at <50 m and >200m away from the natural habitat, with higher numbers closer to the natural vegetation. Greenleaf and Kremen (2006) also found that the visitation rate of bumble bees (*Bombus* spp.) pollinating tomatoes depended on the field's proximity to natural habitat. Similarly, Franceschinelli *et al.* (2013) found that landscape context had an influence on native bee's visitation rates in tomato fields and the diversity of pollinators increased with an increase in vegetation cover. The same effect was observed by Fernanda *et al.* (2016) who found that landscape structure influenced the abundance of native bees and honeybees in coffee (*Coffea arabica*) plantations, highlighting the importance of natural vegetation on bee survival. Fernanda *et al.* (2016) further indicated that lack of natural vegetation in these

fields were more likely to affect large bees on a spatial scale and small bees on local scale.

When the different areas were evaluated separately, significant differences were observed in the bee visitation rates between the four distances evaluated. At Waterpoort the results obtained indicated that bee visitation rates were significantly influenced by distance from the natural vegetation. Similar results were obtained at Vreedzaam, although the total number of bee kisses was lower than at Waterpoort. However, at Jachtpad a significant difference was only observed between the closest (<50 m) and furthest distance (>200 m) while at Dikgale no significant differences in visitation rates were observed between any of the four distances. This indicates that distance from the natural vegetation is not the only factor that influences bee visitation rates or abundance of bees.

Other factors seemed to play a role as well and one such factor could be time of flowering, which is directly influenced by the time when the crop is planted. At Waterpoort, tomatoes were planted at the end of February and started flowering in April, with bee visitation rates determined from April until July. This is in contrast to Vreedzaam and Jachtpad where bee kisses were recorded from July to October. At Waterpoort, the number of bee kisses decreased significantly over the three evaluation periods during the growing season with the lowest numbers of bee visitations recorded at 13-16 weeks after planting. This decline could probably be ascribed to higher temperatures and lower humidity during the beginning of observations compared to lower average temperatures later in the season (May and June).

At Vreedzaam and Jachtpad, where bee visitation rates increased over time, an increase in average daily temperature from July to October was recorded. This could indicate that higher bee visitations were observed when higher daily temperatures occurred (Hemp *et al.*, 2015). At Vreedzaam, the differences between the different evaluation periods during the growing season were significant, whereas at Jachtpad a significant difference was only observed between the last evaluation period compared to the two other periods.

Variation in weather/seasonal conditions have an influence on the diversity, activity and abundance of bee species in agricultural fields (Kumar *et al.*, 2002). Klein *et al.* (2003) reported that seasonal changes have an influence on pollinator abundance in various agricultural landscapes. Sihag and Khatkar (1999), Patil and Viraktamath (2001) and Klein *et al.* (2003) also found that bee visitation rates and bee activity varied during different weather conditions depending on the particular site and its conditions. At Dikgale, where observations were conducted from October to December, a warm period of the year with an average temperature 29 °C, no differences were observed in visitation rates between the three observation periods and overall, very low numbers of bee kisses were observed throughout the evaluation period. This could indicate that other factors besides flowering period and temperature played a role at this location. Dikgale is known for more frequent wind compared to the other areas and literature states that wind can have a negative effect on bee visitations (Maxine Nell, Dikgale 2019, Personal communication). For example, Wenner (1963) found that wind speed could impact on bees' ability to successfully pollinate crops. The study was conducted on honeybees whereby wind speed influenced the speed at which honeybees could fly. In addition, Kleinert-Giovannini and Imperatriz-Fonseca (1986) reported that wind speed had an influence on stingless bees (*Melipona marginata*) where it negatively reduced the flight activity of the bees.

In addition, the type of natural vegetation could also have a significant effect in bee visitation rates and bees, since especially native bees need the availability of a nesting site and other materials to cover and protect their nesting sites (Munyuli, 2011). Dikgale is situated in an area consisting of moderately undulating plains with a short open tree layer with well-developed grass plains and a small number of large trees. In contrast, Waterpoort, with the highest number of bee kisses is situated in an area surrounded by well-developed large trees and shrubs. The results obtained indicate that vegetation type most likely has an influence on visitation rates as it can attract more or fewer bees depending on the vegetation type (Klein *et al.*, 2003). Non-*Apis* bees respond negatively to land use changes in terms of increased spatial separation to suitable nesting sites and food source (Kearns and Oliveras, 2009).

Results of this study are partly supported by Greenleaf and Kremen (2006) who reported that bee visitation in tomato crops in Northern California varied per area and

that it was influenced by land management practices. However, in our study the four study areas had the same management practices although there are cultivated in different vegetation areas and are planted at different times of the year. Munyuli (2011), in a study of bee visitation rates in coffee farms, reported that a farm surrounded by a high cover of natural vegetation had a higher chance of bee visitation and thus positively influenced the productivity of coffee. In the same study, it was recommended that coffee farms should be established in areas with a higher biodiversity in the natural vegetation or they should establish vegetation borders around the coffee plantations (Munyuli, 2011).

2.5 Conclusion

It is important to understand the factors that influence bee visitation rates to tomato flowers. Our study represents an important contribution to this direction as it indicated that in one of the largest tomatoes producing areas in the country, there was a variation in visitation rates of tomato pollinators, which was largely affected by climatic conditions when plants are flowering with a higher incidence of bee kisses at higher temperatures and less wind disturbance. Our results indicated the importance of proximity to the natural vegetation on bee visitation rates and this has been confirmed by several studies. This study highlighted the importance of landscape structure in terms of distance of the crop field from the natural vegetation on pollination ecosystem services.

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Chapter 3

Description of bee species that provide pollination ecosystem service to tomato flowers

Abstract

The purpose of this study was to observe and identify species of bees visiting/pollinating tomato (*Solanum lycopersicum*) flowers since very little data is available on the potential pollinators of tomato flowers in South Africa. In this study, surveys were conducted to identify potential pollinators of tomato flowers. Surveys were conducted in four tomato producing areas, i.e. Waterpoort, Vreedzaam, Jachtpad and Dikgale in the Limpopo Province, South Africa, from April to December of 2018 and 2019. The observations at each site took place between 09:00 and 13:00 during the flowering period. At each study area, a tomato field was selected that had adequate flowers and was in close proximity to natural vegetation. Bee observations and samples were done by randomly walking between the tomato rows. The results indicated that the Hymenoptera were the main visitors to tomato flowers. The most represented family was the Apidae while the least represented was the Megachilidae. Within the Apidae, the genus *Xylocopa* was the most abundant visitor to tomato flowers. This can probably be attributed to their ability to access pollen from poricidal tomato anthers using buzz pollination. The second most abundant genus was *Apis* (honeybee) and their frequent visits may be related to the absence of other floral resources in the study areas. The least represented genus in the Apidae was *Amegilla*, and although species within this genus are capable of buzz pollination, only a few species were observed during this study. Other genera observed, although not frequently was *Megachile* which are also known as buzz pollinators. Although tomatoes do not depend exclusively on bee pollination, having knowledge and data on potential pollinators of this crop may assist in improving management practices and conservation strategies for these species.

Key words: bee activities, bee diversity, buzz pollination,

3.1 Introduction

Bees (Hymenoptera: Apiformes) are the predominant pollinators of agricultural crops (Rodger *et al.*, 2004; Willcox *et al.*, 2019). Traits in bees that make them effective

pollinators are their foraging behaviour, physical structure, and the fact that they are dependent on plant products such as nectar and pollen for survival (Kevan and Phillips, 2001). Bees are a highly diverse group of insects with approximately 20 000 species described globally and they vary in terms of behaviour, ranging from solitary to highly social (Ollerton *et al.*, 2011; Liss *et al.*, 2013; Vaughan *et al.*, 2014). In addition, bees vary in terms of their floral preference, body size and shape, nesting biology and foraging behaviour (Michener, 2000). Although, there is a wide diversity of bees globally, honeybees (*Apis mellifera*) are one of the most used bees in crop production (Melin *et al.*, 2014). The original emphasis on honeybees for crop pollination was based on their convenience and effectiveness in intensive agricultural systems. However, because of the increasing demand for agricultural produce, there is a growing demand for pollination services globally (Bosch and Kemp, 2002; Vaughan *et al.*, 2014). Although Melin *et al.* (2014) reported that in South Africa a high demand for pollination services for many crops exists there is little information. Previous studies assessed the importance of different pollinator species for services to crop production. In this context, the focus of this chapter is on investigating potential pollinators of tomato (*Solanum lycopersicum* L.) flowers, which is an important crop in the country.

Tomato pollen is contained in poricidal anthers only accessible to bees that are able to perform buzz pollination. The latter requires specialized movement of flight muscles resulting in the vibration of the flowers to release pollen from the poricidal anthers (King and Buchmann, 2003; Potter and LeBuhn, 2015). Tomato flowers are nectarless, therefore, bees that cannot perform buzz pollination (e.g. honeybees) rarely visit the flowers, especially if there are other floral resources available in the surrounding areas (Potter and LeBuhn, 2015).

According to Toni *et al.* (2020) little information is available on tomato pollination in Africa. Kasina (2007) identified only *Xylocopa calens* and *Halictus* spp. as potential pollinators of tomatoes in Kenya.

It has been established that potential pollinators of tomatoes cause bee kisses (brown marks) after visiting the flowers and that these marks can be used as a proxy for bee visitation to these flowers (Morandin *et al.*, 2001; Toni *et al.*, 2020). The previous

chapter reported on the presence of bee kisses in tomato fields, the variation across different regions of the Limpopo Province and, in addition, the distance from the natural vegetation having an impact on the incidence of bee kisses in some areas.

In this chapter, the aim was to observe and identify which species of bees are responsible for bee kisses on the tomato flowers and which bees were frequent visitors of tomatoes flowers in the Limpopo Province, South Africa. Some of the key questions addressed in this chapter are:

- a. which bee species were the common visitors of tomato flowers?
- b. are the pollinators shared across the different tomato producing areas in the Limpopo Province?
- c. what were the main activities observed in the field, i.e. buzzing the flowers, scouting while flying low or flying high?

3.2 Materials and methods

3.2.1 Study area

The observations and sampling of the pollinators visiting tomato flowers were conducted from April to December in both 2018 and 2019 at four tomato producing areas. These were Waterpoort (22° 53' 53.93" S, 29° 37' 58.73" E), Vreedzaam (23° 41' 17.50" S, 29° 59' 46.16" E), Jachtpad (23° 31' 15.47"S, 30°14' 28.41" E) and Dikgale (23°40' 59.38" S, 29° 45' 21.25" E) in the Limpopo Province, South Africa. The climatic characteristics and the main vegetation types for each location were described in the previous chapter (Chapter 2). Further details on the information of the different study areas, for example planting dates, data collection period and production period were reported in Chapter 2.

3.2.2 Experimental layout

The observations and bee sampling were conducted in one block of a tomato field at each study area. As described in the previous chapter a tomato field covers an area of at least 120 hectares, which is subdivided into 10–15 blocks depending on the structure of the field. One block of approximately 10–12 hectares is divided into at least 200 rows. Each row consists of at least 50 chambers which is the distance between two poles that are 5 m apart, a chamber consists of 12–14 tomato plants.

3.2.3 Observations of bee species activities

The data for observing bee activities were collected by randomly walking between the tomato rows. Bee observations took place at weekly intervals between 09:00 and 13:00. The period between 09:00 and 13:00, according to Silva-Neto *et al.* (2013), is the peak time for floral visitation in tomatoes since that is when the largest amount of pollen for pollination in the anthers is available. Data collection took place from 5 weeks after planting, since this was when the first flowers appeared, until 12 weeks after planting in 2018 and 16 weeks after planting in 2019.

In 2019, the time effort parameter was also recorded. The amount of time spent inside tomato fields was recorded for each observation/sampling event in order to calculate the number of bees observed per hour spend in the field. The total numbers of hours spent per field were dependent on the availability of resources for example, transport and labour. Bees observed per study area were classified as either *Apis* or non-*Apis* in the field.

At each study area time in and time out of the field was recorded. Bees observed were noted (number as well as physical description) and their activity was recorded. Activity was described as: 1) scouting - which took place when the bees were not on the flowers but flying closely to the flowers, 2) buzzing - which was observed when the bees were found on the flower and making a buzzing noise which could be heard close-by, and 3) flying low which involved the bees flying at a lower level (approximately 30–60 cm above ground level).

3.2.4 Bee collection and identification

During these observations, some species were collected using an entomology net and put inside a killing bottle. The samples were then taken to the ZZ2 laboratory for identification. Common bees were not collected every time.

3.3 Results

3.3.1 Observed bee activities

Table 3.1 indicates observed bee activities of the production year 2018 and 2019.

The observed bee activities in the tomato fields were buzzing, scouting, and flying low (Table 3.1). In 2018, buzzing (46.5% of the total activity) was the most common activity, however in 2019, buzzing was only noticed in 28.0% of the activities observed. This activity was performed by the genera *Xylocopa*, *Amegilla* and *Megachile* (Table 3.1) and occurred when the bees were seen gripping onto tomato flowers and shaking it to dislodge pollen from inside the poricidal anthers. Only *Xylocopa* species were observed to cause a brown mark on the flowers after extracting pollen from the anthers. Scouting behaviour was observed in 38.1 and 33.0% of the bee activities in 2018 and 2019 respectively. In this instance, the bees were not seen directly on the flowers but were seen flying next to the flower in search of food or mates. This activity was observed for *Xylocopa*, *Apis*, *Amegilla* and *Megachile*. Flying low, whereby bees were seen flying around the field while not attempting to visit individual flowers, was observed in 15.3 and 28.0% of the observations for 2018 and 2019 respectively. This activity was observed for *Xylocopa* and *Apis*.

Table 3.1: Bee species and their activities observed in tomato fields at four farms in 2019 and 2020.

Family	Genus	Activity			Total (%)*
		Buzzing	Scouting	Flying low	
2018					
Apidae	<i>Xylocopa</i>	93	61	23	177 (82.3)
	<i>Apis</i>	0	17	10	27 (12.6)
	<i>Amegilla</i>	5	2	0	7 (3.2)
Megachilidae	<i>Megachile</i>	2	2	0	4 (1.8)
Total (%)**		100 (46.5)	82 (38.1)	33 (15.3)	
2019					
Apidae	<i>Xylocopa</i>	42	39	33	114 (72.6)
	<i>Apis</i>	0	17	16	33 (21.0)
	<i>Amegilla</i>	2	1	0	3 (1.9)
Megachilidae	<i>Megachile</i>	5	2	0	7 (4.0)
Total (%)**		49 (28.0)	59 (33.0)	49 (28.0)	

*Total number of bees observed per genus and percentage of total number of bees per year in parentheses; **Total number of bees per activity and percentage bee activity per year in parentheses.

1.2.2 Bee observations duration per study location in 2019

The total numbers of hours spent observing and counting bees in the field are provided in table 3.2. Approximately 6.12 hours were spent at Waterpoort where 123 individual

bee species were noticed. During the observations, at least 20 bees were noted per hour, of which 18 represented the group *Non-Apis* and 2 were *Apis*. At Vreedzaam, in the 8.23 hours of observations, a total number of 27 bee species were noted. During the observations, 4 species were seen per hour (2 *Apis* and 2 non-*Apis*). At Jachtpad, 8.51 hours were spent, a total number of 14 bee species were noted. During the observations, the number of bees observed per hour were 2 and all were *Apis*. Furthermore, at Dikgale, in the 6.52 hours spent, at least 7 species were observed. During the observations period, 2 bees were observed per hour and all of them were *Apis*.

Table 3.2: Total hours spent on bee observations per farm in 2019

Study Area	Total Hours	Total bees observed	Bees observed/hour	Non- <i>Apis</i> bees/hour	<i>Apis</i> bees/hour
Waterpoort	6.12	123	20	18	2
Vreedzaam	8.23	27	4	2	2
Jachtpad	8.51	14	2	0	2
Dikgale	6.52	7	2	0	2

3.2.3 Bee identification

Table 3.3 indicates the potential pollinators of tomato flowers. The majority of bee pollinators identified belong to the order Hymenoptera. The highest number of bee species were the Apidae family and the lowest number of bee species were the Megachilidae family. The highest number of species was caught at Waterpoort (23), followed by Vreedzaam (13) and Jachtpad (4) and Dikgale (3) (Table 3.2). Some of the bee specimens caught for identification are represented in Figure 3.1.

Table 3.3: Total number of bee specimens collected per genus at the four study areas.

Genera	Waterpoort	Vreedzaam	Jachtpad	Dikgale
<i>Xylocopa</i>	14	6	1	0
<i>Amegilla</i>	6	3	1	0
<i>Apis</i> (honeybee)	1	1	2	3
<i>Megachile</i>	2	3	0	0
Total number of specimens	23	13	4	3

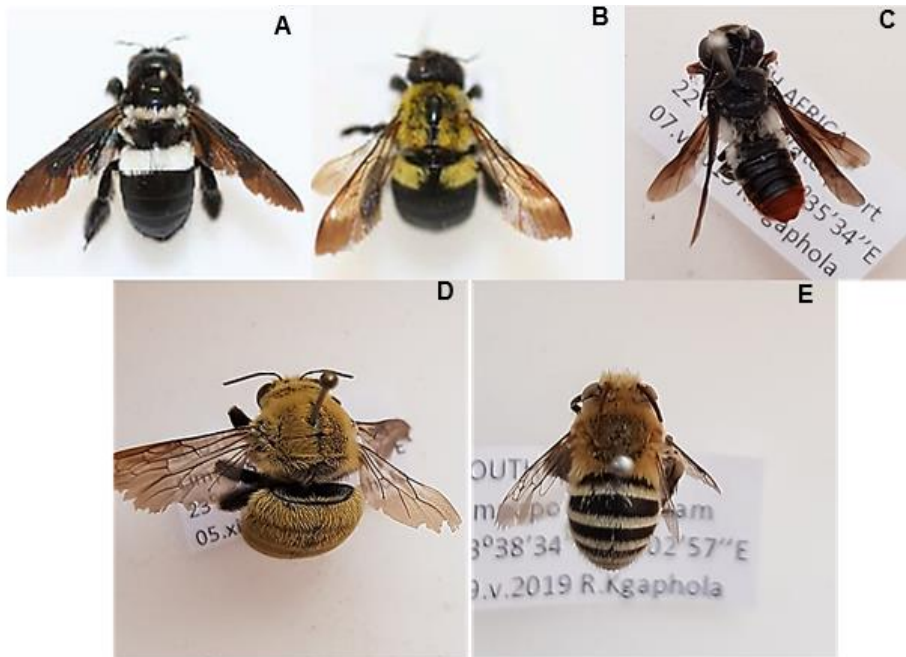


Figure 3.1: Some of the bee species that were caught in the tomato fields and identified: A, B and D (*Xylocopa*), C (*Megachile*) and E (*Amegilla*), Photo credit: Retang Kgaphola, 2019.

3.4. Discussion

The purpose of this study was to record the potential pollinators of tomato flowers, by recording bee observations activities in the tomato fields and capturing some of the specimens for identification. Melin *et al.* (2014) reported that in South Africa little knowledge on the pollination status of some of the major crops, including tomato, is available. Toni *et al.* (2020) confirmed that limited information is available in Africa in relation to the pollination status of tomato crops particularly on plant-pollinator interaction. However, the tomato bee fauna is mainly composed of bees that are capable of performing buzz pollination with a wide variety of social and nesting habits (Greenleaf and Kremen, 2006; Santos *et al.*, 2014; Barbosa *et al.*, 2019).

The results obtained in this study showed that the most observed activity in the two years of data collection in all the study areas was buzzing. Buzzing activity was observed when bees were seen gripping on the anthers of tomato flowers and vibrating them to dislodge pollen. Tomato pollen is only accessible to bees that can perform buzz pollination (King and Buchmann, 2003). This behaviour according to

Cardinal *et al.* (2018) has evolved in some bee species to access pollen grains from flowers with poricidal anthers. In addition, Cardinal *et al.* (2018) further reported that some flowering plants have evolved floral morphologies that conceal floral rewards such as pollen and nectar and this strategy is hypothesized to promote effective pollination services. This activity was observed mainly performed by the genus *Xylocopa*, *Amegilla* and *Megachile*. The genus *Apis* was not seen gripping on the flowers and shaking them, confirming observations of Toni *et al.* (2020) showing that *Apis* is incapable of performing buzz pollination. The second most observed activity was scouting whereby bees were recorded not on the flowers but flying closely to the flowers. This behaviour was mostly observed for the genus *Xylocopa* and *Apis*. Flying low which occurred when bees were flying at a lower level (approximately 30-60 cm) above ground level was also mainly observed for the genus *Xylocopa* and *Apis*.

In 2019, whereby the time parameter was recorded, it was observed that the number of bees observed per hour in the study areas varied. At Waterpoort it was noticed that most bees observed per hour were non-*Apis* and few were *Apis*. At Vreedzaam, it was noticed that half of the bees observed per hour were *Apis* and half were non-*Apis*. While at Jachtpad and Dikgale, all the bees were *Apis*. The difference in bee species observed per hour in the four study areas might be because of different factors for example planting season and different vegetations (discussed in chapter 2). *Apis* bees were constant in all the study areas while Non-*Apis* varied between the study area. Furthermore, the bee specimens identified indicated that the Hymenopterans were the most common visitors/pollinators of tomato flowers. The family Apidae was the most abundant visitor. The Megachilidae family was less abundant. Within the Apidae, several *Xylocopa* spp. frequented tomato flowers. This can probably be attributed to their ability to access pollen from poricidal tomato anthers using buzz pollination.

This genus was observed and caught in all the study areas excluding Dikgale. The area with the highest abundance of *Xylocopa* was Waterpoort which might be related to its more diverse landscape structure and composition (refer to Chapter 2) when compared to the other study areas. The second-most frequent genus was *Apis*, which was observed in all the study areas. Their high abundance may be related to their characteristic as generalists and possibly also because of the absence of other food

sources in the study areas. However, because of their inability to perform buzz pollination, which is required for tomato pollination, they were usually observed scouting in the field or flying low. In a study conducted by Dos Santos *et al.* (2009), they also observed a high abundance of honeybees visiting tomato flowers where they obtained pollen by inserting the proboscis into the anther cone, a behaviour known as milking, which was not observed in this study.

The genus *Amegilla*, also belonging to the family Apidae, was observed on the tomato flowers also gripping them while shaking their bodies to access pollen. However, these species did not make a buzz noise such as observed for *Xylocopa*. Furthermore, no marks were observed after these species had visited the tomatoes. The majority of *Amegilla* was observed and caught at Waterpoort, followed by Vreedzaam and only one specimen was caught at Jachtpad. At Dikgale, no specimens belonging to this genus were observed. The low abundance of these species in the tomato fields may be due to other factors for example the quality of the vegetation cover, presence of other flowering plants which were available during the observation period and probably other factors that were not taken into account. Amala and Shivalingaswamy (2017), conducted a study whereby species of the genus *Amegilla* were explored for tomato pollination, and indicated that the yield and quality of tomato fruits increased as a result of the pollination services provided by blue banded *Amegilla* (Bell *et al.*, 2006; Amala and Shivalingaswamy, 2017). In the same study, the authors reported that although these species may be potential pollinators of tomatoes, it will be difficult to use them for commercial production due to their nesting strategy.

The other family observed in this study was the Megachilidae which was represented by one genus i.e. *Megachile*. According to Toni *et al.* (2020), only one genus in the family Megachilidae is a potential pollinator of tomatoes. In addition, the behaviour of *Megachile* was similar to those of *Amegilla* whereby the bees were observed mainly buzzing or scouting in the field, however, no marks were left after visiting the flowers. In numerous international studies, common managed tomato pollinators are species of the genus *Bombus*, particularly in closed production systems. However, in countries where these species do not occur naturally, farmers are searching for alternatives. For example, Silva-Neto *et al.* (2013) reported that field tomatoes in Brazil are visited by native bees such as *Exomalopsis analis* and *Centris tarsata* which are currently being

explored for commercial tomato production. In Benin, *Xylocopa* spp. and *Apis mellifera* were identified as potential pollinators of tomato during an inventory of tomato field entomofauna. This contrasts with Santos *et al.* (2014) who found that *A. mellifera* was not a sufficient pollinator of tomatoes especially when other floral resources are available in the area. Several bee species can effectively pollinate tomatoes worldwide, but the unavailability of rearing strategies still hinders their use for managed pollination.

3.5 Conclusion

The family Apidae, specifically the genus *Xylocopa* was the most abundant pollinator of tomatoes in this study and because of their ability to perform buzz pollination, tomato farmers and growers should investigate the potential of developing strategies for their management and ways to produce them in large quantities. Although the genus *Apis* was also frequently observed, their inability to perform buzz pollination makes them not an ideal candidate for tomato pollination. Findings from this study have helped in identifying indigenous pollinators of tomatoes in South Africa and will enable growers and farmers to develop breeding strategies and management strategies which can improve the pollination of status of tomatoes.

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Chapter 4

Determining the Impact of Pollination Services Provided by Bees on Yield and Quality of Tomatoes

Abstract

Tomato flowers are known to be self-fertile, however, they require agitation of the anthers for pollen grains to be transferred to the stigma, which might result in fertilization and improved fruit set and quality of tomatoes. In this study, an experiment was conducted to evaluate different pollination methods for tomato production i.e. exposed flowers (open pollination) and enclosed flowers (self-pollination). The study was conducted over a period of two years in two tomato fields (Drukbreek and Pakhuis) located at Vreedzaam, in the Limpopo Province of South Africa. Twenty-four tomato flower clusters were selected for each pollination method. To encourage self-pollination, the flower clusters were enclosed with a packhouse bag while the other flower clusters remained open to allow insect pollination. In all the treatments fruit set was evaluated by counting the number of flowers that developed into fruits. At fruit maturity, approximately sixty fruits were harvested from each treatment and taken to the laboratory to evaluate fruit quality. Exposed flower clusters (open/insect-pollination) had a higher fruit set (yield) and the quality was superior compared to fruit obtained from enclosed flower clusters (closed/self-pollination). Results indicated the importance of insect pollinators as they were the main visitors to the tomato flowers.

Keywords: exposed-pollination, enclosed-pollination, fruit set, fruit quality and yield quantity

4.1 Introduction

Approximately 75% of crop species that are used for food production depend on insects, particularly bee species, for yield and quality (Klein *et al.*, 2007; Melin *et al.*, 2014). This includes crops such as apples, avocados, berries, cucumbers, peaches and watermelons (Winfree *et al.*, 2008; Klatt *et al.*, 2014). While some crops depend entirely on pollination for fruit set and development, others can produce more than 90% of the fruits without pollination (Greenleaf and Kremen, 2006). For crops that are dependent on pollination, efficient pollination influences fruit set, seed quality and nutrient content (Morandin *et al.*, 2001; Winfree *et al.*, 2008; Deprá *et al.*, 2014; Toni *et al.*, 2020). Therefore, improved pollination services may contribute to the farmers' income through increased yield and crop quality (Geslin *et al.*, 2017; Bashir *et al.*, 2018) as was shown in a study by Geslin *et al.* (2017) on apples and pears.

In South Africa, tomatoes (*Solanum lycopersicum* L.) are the second most consumed crop after potato (*Solanum tuberosum* L.) (Moodley *et al.*, 2019). Tomato fruits play an important nutritional role since they contain many nutrients such as essential amino acids, vitamin A, vitamin C, lycopene and β -carotene (Slavin and Lloyd, 2012). Tomato is also an important source of income for both small scale and commercial farmers (Geslin *et al.*, 2017; Bashir *et al.*, 2018). Despite being able to self-pollinate, pollination services provided by bees have been shown to improve yield and quality of tomato fruits (Morandin *et al.*, 2001; Deprá *et al.*, 2014; Bashir *et al.*, 2018; Toni *et al.*, 2020). The importance of pollination in improving tomato production has already been recognised in the 1890's (Toni *et al.*, 2020). Toni *et al.* (2020) found that several studies have been conducted to improve yield and quality of tomatoes using pollinators and this has resulted in the inventory of natural pollinators, particularly for green house production (Toni *et al.*, 2020). The importance of pollinators of tomato crops grown under greenhouse conditions has been recognised worldwide because of the total absence of natural pollinators and wind in these production systems (Morandin *et al.*, 2001; Hogendoorn *et al.*, 2000; Silva-Neto *et al.*, 2013).

Tomato flowers are naturally self-pollinating, suggesting pollen grains are shed within the individual flower during anthesis when there are strong vibrating forces caused by either wind or buzz pollinators (Hanna, 1999; Deprá *et al.*, 2014; Ahmad *et al.*, 2015; Silva-Neto *et al.*, 2017). Some tomato farmers are of the opinion that wind is sufficient

to vibrate the tomato flowers and release enough pollen grains for maximum self-pollination and optimal yield (Macias-Macias *et al.*, 2009; Morandin and Kremen, 2012). However, studies have shown that wind in open fields may not always be strong enough to vibrate the anthers and release the amount of pollen needed for maximum crop production (Bashir *et al.*, 2018; Toni *et al.*, 2020).

Previous studies have shown that buzz pollinating bees are potential pollinators of tomato flowers because they grip onto the flowers using their mandibles and move their flight muscles rapidly (Figures 4.1A and B) causing anthers to vibrate and subsequently dislodge pollen grains (Bell *et al.*, 2006; Vergara and Fonseca-Buendia, 2012; Silva-Neto *et al.*, 2015; Bashir *et al.*, 2018). The tight grip on the flower causes bruises also called bee kisses. These bee kisses can be used as an indicator of buzz pollinating bees that have visited the tomato flowers (Figure 4.1C).

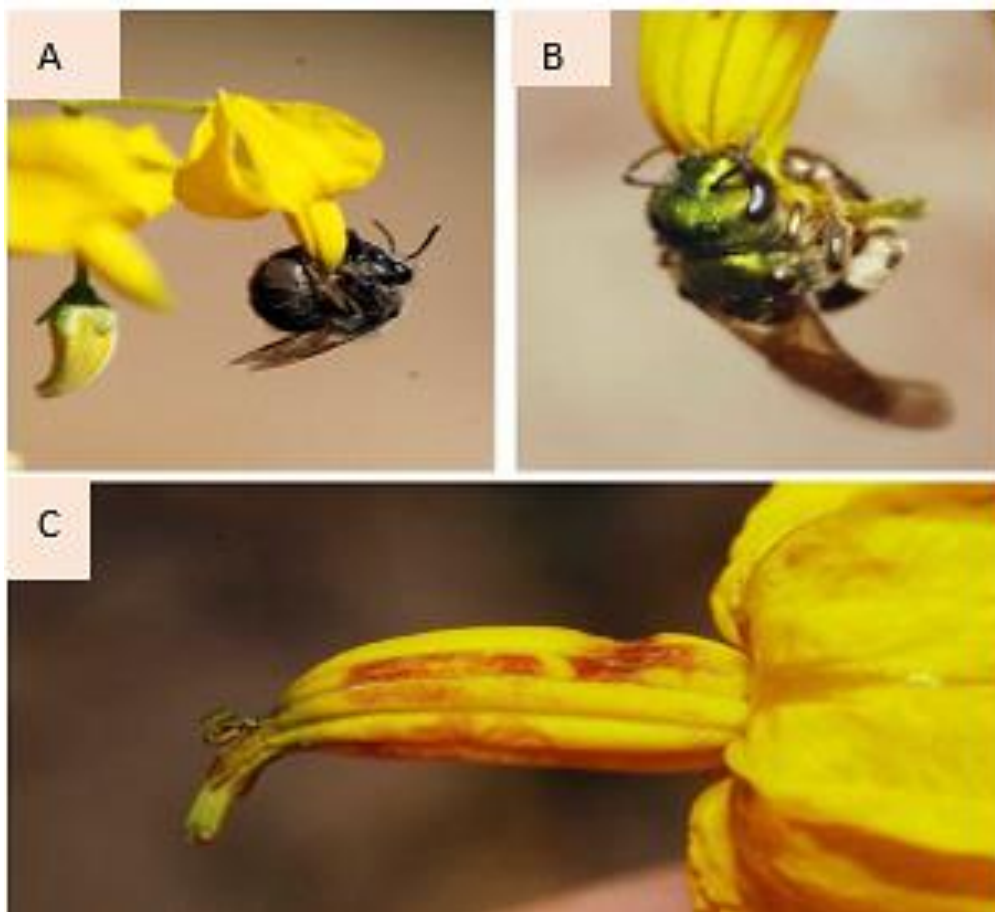


Figure 4.1: (A) and (B) indicate bees tightly gripping tomato flowers and (C) shows bee kisses left by buzz pollinating bees (brown distinct marks). (<https://permies.com/t/47793/Promiscuously-Pollinated-Tomatoes-Bees>).

Hanna (1999) reported that insufficient pollination is a major cause of decreased tomato production in terms of yield and quality while Greenleaf and Morandin (2006) confirmed that pollination provided by bees enhanced fruit set and improved physiochemical properties of field-grown tomatoes. Fruit set, which is the proportion of the number of flowers developing into fruit, is commonly used to determine yield quantity of a crop which is influenced by pollination success (Geslin *et al.*, 2017). Poor pollination may result in some tomato flowers not developing into well-developed fruits (Figure 4.2) or growing into smaller fruits (Figure 4.3). Deprá *et al.* (2014) reported higher percentages of fruit set in tomato crops that were highly visited by bee pollinators compared to fruit where bees were excluded.

Other parameters such as fruit weight, height, circumference and number of seeds (Figure 4.4) in the fruit have also been reported to be influenced by pollination (Vergara and Fonseca-Buendia, 2012; Deprá *et al.*, 2014; Bashir *et al.*, 2018).



Figure 4.2: Poor fruit set (circled tomatoes) as a result of insufficient pollination. Photo credit: Wiam Haddad, 2018



Figure 4.3: Poor pollination in tomatoes may result in larger fruits (left) and smaller fruits (right). Photo credit: Retang Kgaphola, 2019.



Figure 4.4: No seed development as a result of insufficient pollination in the tomato on the left compared to numerous seeds that developed in the tomato on the right. Photo credit: Retang Kgaphola, 2019.

The majority of studies on tomato pollination were conducted in Brazil, Chile, Mexico, United States of America, Chile and Oceania (Toni *et al.*, 2020). In Africa, there is little data available on tomato pollination especially on plant-pollinator relationships (Rodger *et al.*, 2004). Therefore, the aim of this study was to evaluate the effect of different pollination methods on the yield (fruit set) and quality (fruit weight, fruit height and diameter, seed weight, firmness and shape) of tomato crops in the Limpopo Province, South Africa.

4.2. Materials and methods

4.2.1 Study site

The study was carried out in two tomato fields at Vreedzaam. The experiments were conducted in consecutive years using two fields in the same area. Because tomato fields at ZZ2 are only planted every five years, it was not possible to use the same field but the selected fields were subjected to the same management practices and were located in the same bio-climatic area (approximately 5 km apart). The tomato fields were Drukbreek ($23^{\circ} 37'40.15''$ S - $30^{\circ} 02' 42.67''$ E) in 2018 and Pakhuis ($23^{\circ} 39'54.69''$ S - $29^{\circ} 59' 46.47''$ E) in 2019 (Figure 4.5).



Figure 4.5: Map indicating the areas where the study was conducted at Vreedzaam, Limpopo Province.

4.2.2 Experimental layout

An open tomato field covering at least 120 hectares was used at each site. The field was subdivided into 10–15 blocks, of approximately 10–12 hectares. Each block was divided into approximately 200 rows while each row consisted generally of at least 50 chambers. The distance between two poles, 5 m apart, is called a chamber. Each chamber has at least 15 tomato plants.

In this experiment, 24 data points were selected based on their proximity to the natural vegetation because, according to Greenleaf and Morandin (2006), the natural vegetation serves as a habitat for the majority of tomato pollinators. To reduce the

effect of other factors such as wind and temperature, the data points were close to one another. The data points were located in every second row at the 2nd chamber (from the beginning of the row) (Figure 4.6).

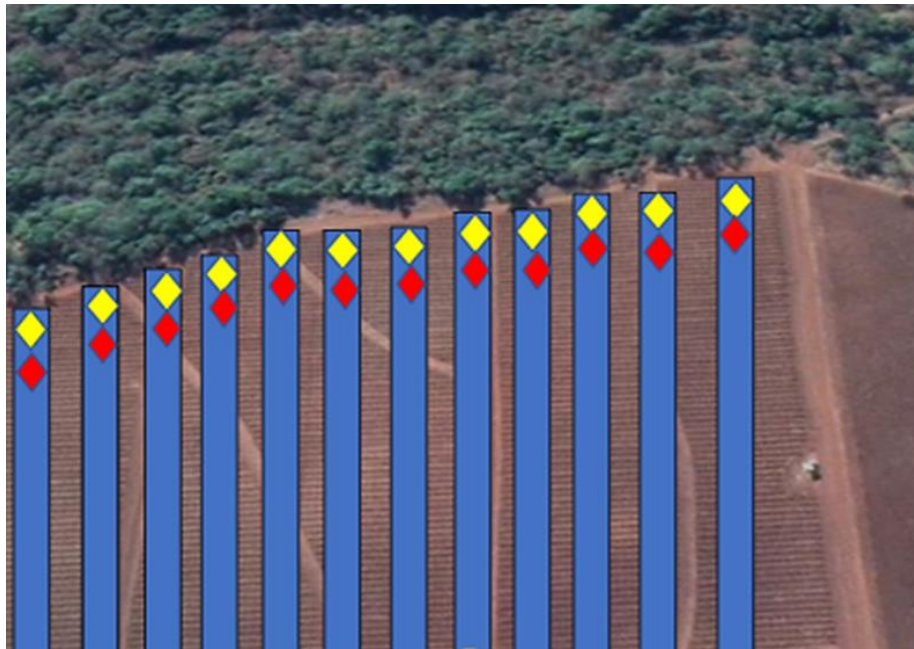


Figure 4.6: Data points in proximity to natural vegetation. The yellow diamonds represent the enclosed pollination treatment and red diamonds represents the exposed pollination treatment.

In each chamber, two plants at the same developmental stage (same number of clusters, clusters at same age, same height) close to one another were identified. On one of these plants, a partially opened tomato cluster was covered with a packhouse cap to exclude buzz pollinators while on the other plant the selected flower cluster was left exposed (open) (Figure 4.7). Both these clusters were marked with a string of wool for easy identification. This was repeated in 24 rows and resulted in 24 clusters of flowers being marked for each treatment. In total, 24 flower clusters were left open (Treatment 1) to allow bee pollination while another 24 flower clusters were closed (Treatments 2) to promote self-pollination.



Figure 4.7: Two selected tomato flower clusters. The cluster on the left was covered with a packhouse bag (closed flower cluster) to exclude pollinators and the cluster on the right, indicated with a red circle (open flower cluster), was left open to allow buzz pollinators to pollinate the flowers. Photo credit: Retang Kgaphola, 2019.

4.2.3 Experimental procedure to determine the effect of different pollination methods

In each tomato field, weekly observations were made to record the number of flowers with bee kisses, number of flowers developing into fruits (fruit to flower ratio), and to confirm that the packhouse bags were still in place. During these observations, insects that were visitors to the tomato flowers were caught and identified in the laboratory. Observations continued for approximately 2–3 weeks until flowers had set and fruit set was observed.

After fruit set, the packhouse caps used in Treatment 2 were removed to allow the fruits to develop without mechanical damage. At ripeness, determined according to commercial guidelines, all fruits on each flower cluster were harvested. This implied that a cluster was harvested over a period of approximately 3 weeks since the fruit in a single cluster does not ripen at the same time. A cluster consists on average of about 4–8 fruits.

4.2.4 Data collection

In the laboratory, the following physiochemical parameters were recorded: fruit number, fruit size (diameter and height), fruit weight, firmness, number of locules and pericarp thickness. In 2019, seed weight was also determined as an additional parameter. Although fruits were only harvested once ripe, these fruits were kept until a sufficient number of fruits were harvested before parameters were determined.

4.2.4.1 Fruit weight (g)

Fifty tomato fruits were randomly selected from each treatment and individually weighed at the ZZ2 laboratories using an electric scale.

4.2.4.2 Fruit size (mm)

The fruit size was determined by measuring the length and diameter of individual tomato fruits using a Vernier calliper.

4.2.4.3 Fruit firmness

A Bareiss HP-Fff mechanical fruit firmness tester was used to determine fruit firmness. The equipment is represented in Figure 4.8.



Figure 4.8: A Bareiss HP-Fff mechanical fruit firmness tester. Photo credit: Retang Kgaphola, 2019.

4.2.4.4 Fruit shape

The fruit shape was determined by observing whether the fruit were well rounded or malformed as represented in Figure 4.9. Twenty-five fruits were selected per treatment.



Figure 4.9: Well-rounded tomato fruit (left) and malformed tomato fruit (right). Photo credit: Retang Kgaphola, 2019.

4.2.4.5 Pericarp thickness (mm)

The pericarp thickness was measured by cutting tomato fruits into two halves. A Vernier calliper was used to measure the thickness of the pericarp in the cut tomato as shown in Figure 4.10.



Figure 4.10: Determining pericarp thickness using a Vernier calliper. Photo credit: Retang Kgaphola, 2020.

4.2.4.6 Seed weight (g)

Fruits were cut and the pulp and seed mixture were extracted into a petri dish (Figure 4.11). The petri dish was placed in a microwave to separate the pulp and the seeds, for about 2 minutes after which the petri dish was removed from the microwave and the weight was recorded.

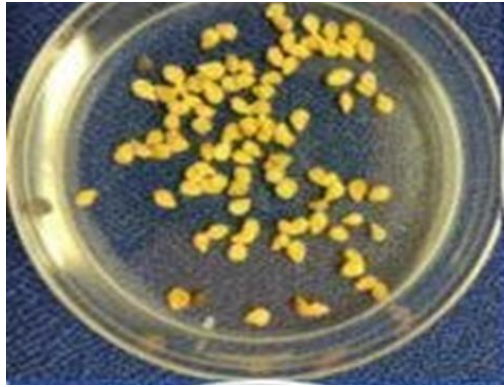


Figure 4.11: Seed of tomato fruit. Photo credit: Retang Kgaphola, 2019.

4.2.5 Data analysis

The data were analysed by means of T-tests, assuming all variances were equal.

4.3 Results

4.3.1 Effect of different pollination methods on fruit set (yield)

The results obtained showed a significant difference ($P \leq 0.05$) between the two treatments with the highest fruit number obtained from the exposed flower clusters (open pollination) at both tomato fields compared to a lower fruit set (yield) in closed flower clusters. The results are represented in Figures 4.12 and 4.13.

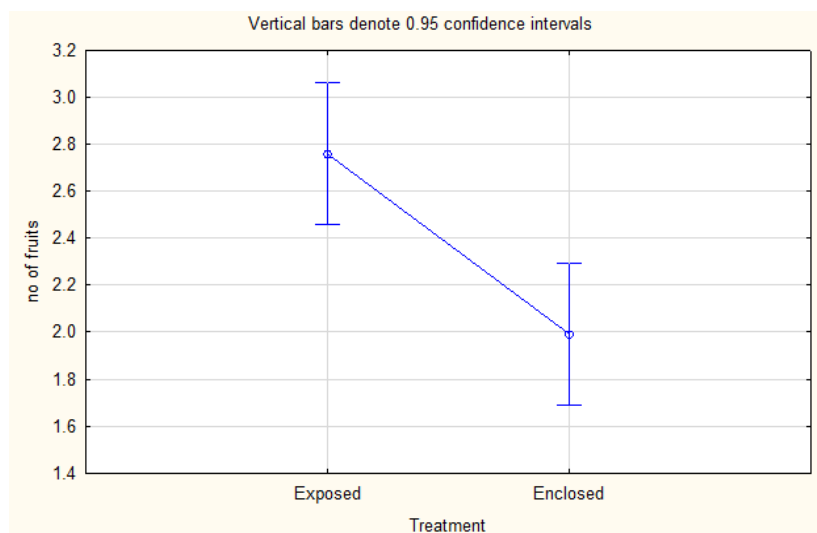


Figure 4.12: The mean number of fruits per flower cluster exposed and unexposed to pollinators at the Drukbreek tomato field in 2019.

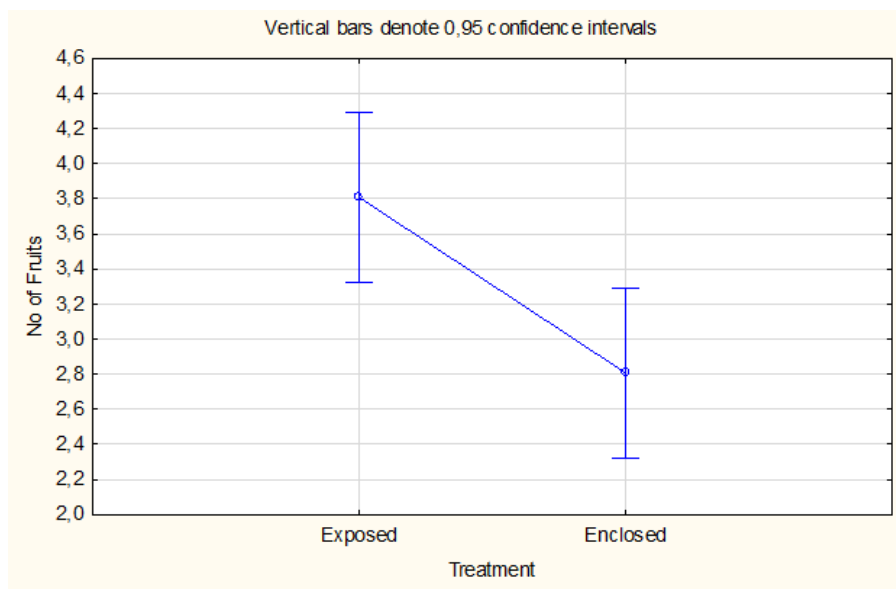


Figure 4.13: The mean number of fruits per flower cluster exposed and unexposed to pollinators at the Pakhuis tomato field in 2020.

4.3.2 Effect of different pollination methods on fruit quality

4.3.2.1 Fruit weight (g)

The results indicated that flower clusters exposed to pollinators had a significantly ($P \leq 0.05$) higher fruit weight than those that were enclosed during both years. The mean weight of tomato fruits obtained from exposed flower clusters was 107.19 g at Drukbrek and 132.90 g at Pakhuis while those that were enclosed had a mean weight of 92.70 g at Drukbrek and 108.23 g at Pakhuis (Table 4.1).

Table 4.1: Mean fruit weight, fruit length, fruit diameter, fruit firmness, pericarp thickness and dry seed weight recorded at Drukbrek and Pakhuis fields.

Treatment	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Fruit firmness	Pericarp thickness (mm)	Dry seed weight (g)
Drukbrek (2018)						
T1	107.19 ± 3.19*a	47.48 ± 0.54a	56.72 ± 0.80a	37.02 ± 0.69a	7.29 ± 0.07a	NA
T2	92.70 ± 2.41b	43.20 ± 0.58b	50.12 ± 0.84b	32.63 ± 1.02b	6.80 ± 0.09b	NA
T-test	0.0002	<0.0001	<0.0001	0.00026	<0.0001	NA
Pakhuis (2019)						
T1	132.43 ± 1.73a	51.50 ± 0.48a	64.58 ± 0.41a	34.24 ± 0.51a	7.38 ± 0.10a	2.02 ± 0.01a
T2	108.23 ± 2.17b	48.66 ± 0.58b	59.84 ± 0.50b	32.10 ± 0.51b	6.79 ± 0.11b	1.99 ± 0.00a
T-test	<0.0001	0.0002	<0.0001	0.0018	0.0068	0.19

*SE = standard error; different letters per column indicate significant differences according to $P \leq 0.05$; NA = not determined in 2018; T1 = open flower cluster; T2 = closed flower cluster

4.3.2.2 Fruit size (length and diameter)

Open flower clusters had a significantly larger fruit size (length and diameter) than closed flower clusters at both locations (Table 4.1). The fruits harvested in Treatment 1 were larger with an average length of 47.5 and 51.5 mm and a diameter of 56.7 and 64.6 mm at Drukbrek and Pakhuis fields respectively compared to fruit of Treatment 2 with an average length of 43.20 and 48.66 mm and diameter of 50.12 and 59.84 mm at Drukbrek and Pakhuis fields respectively.

4.3.2.3 Fruit Firmness

Treatment 1 (exposed flower clusters) had a significantly higher fruit firmness than Treatment 2 (enclosed flower clusters) at both the locations. The highest fruit firmness found in Treatment 1 was 37.02 at Drukbrek and 34.24 at Pakhuis, while the fruit firmness observed in Treatment 2 was 32.63 at Drukbrek and 32.10 at Pakhuis (Table 4.1).

4.3.2.4 Dry seed weight (only 2019)

The results obtained showed no significant difference in dry seed weight between Treatment 1 and Treatment 2 (Table 4.1).

4.3.2.5 Fruit shape

The results indicated a significant difference between Treatment 1 and Treatment 2 in terms of the fruit shape at both the study locations. At Drukbrek the percentage of well-rounded fruits was 34% for Treatment 1 and 26% for Treatment 2, while the percentage of malformed fruits in both treatments were 16% and 24% respectively (Figure 4.14). At Pakhuis, the percentage for well-rounded fruits was 40% for Treatment 1 compared to 30% for Treatment 2 while the percentage of malformed fruits was 10% in Treatment 1 and 20% for Treatment 2.

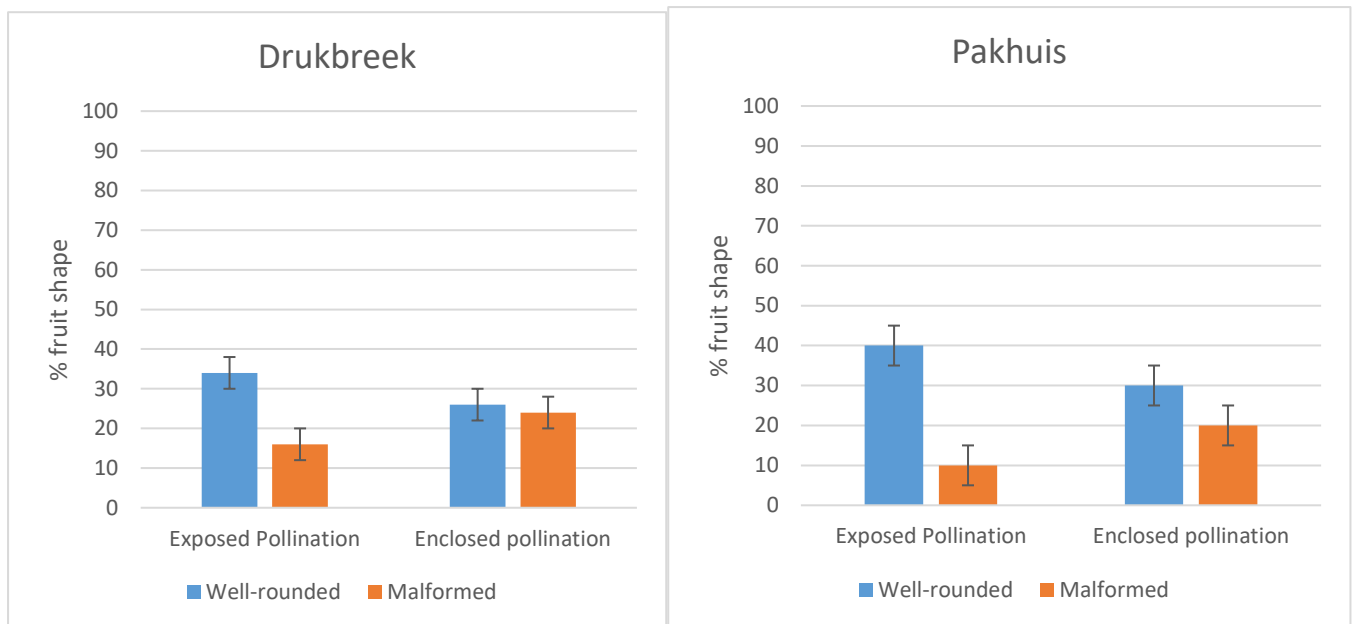


Figure 4.14: The incidence of well-rounded and malformed fruit harvested from flower clusters that were either exposed (open) to insect pollination or enclosed (self-pollination). Bars indicate standard errors.

4.4 Discussion

Pollination ecosystem service is an essential contributor to crop production. It is widely recognised that approximately 35% of crops are dependent on pollinators for improved yield and quality (Melin *et al.*, 2014). Pollinators play a key role in transporting pollen grains from the anther to the stigma between or within crops of the same species. Bees are the main and most common pollinators of many crops globally (Klein *et al.*, 2007). Efficient pollination may result in fertilization, which will result in fruit formation. Lack of efficient pollination was reported to negatively influence fruit characteristics such as fruit weight, fruit size, sugar content, shape and the number of seeds per fruit (Isaacs and Kirk, 2010; Klatt *et al.*, 2014; Toni *et al.*, 2020).

Although cultivated tomatoes are considered self-compatible, Morandin *et al.* (2001) reported that the quantity of pollen grains released by flowers may not be enough to ensure efficient pollination. The results obtained from this study indicated the importance of pollinators in open fields in terms of fruit yield and quality.

In both the study locations it was observed that fruit formation took place in both exposed and enclosed flower clusters. However, in the exposed pollination method

the majority of the flowers developed into fruits while in the enclosed pollination some of the flowers aborted before they could develop into fruits. It has been reported that the amount of pollen grains deposited on the stigma of flowers plays a role in determining fruit formation (Toni *et al.*, 2020). In a study of tomato crops in Brazil, Vinícius-Silva *et al.* (2017) indicated that insufficient pollination may result in a considerable percentage of flowers not forming fruits. Our experiments indicated that tomato production could be lower when pollinators are excluded from visiting/pollinating tomato flowers. Klatt *et al.* (2014) found similar results and reported that yield of strawberries was improved by the presence of pollinators and that strawberry flowers formed fewer fruits when pollinators were excluded. Several other studies conducted on tomatoes indicated that pollination services provided by pollinators enhanced fruit set percentage (Greenleaf and Kremen, 2006; Macias-Macias *et al.*, 2009; Al-Abbadi, 2010; Silva-Neto *et al.*, 2013; Deprá *et al.*, 2014; Amala and Shivalingaswamy, 2017).

Furthermore, bee pollination had a positive impact on the quality of the tomato fruit particularly fruit weight, height and diameter. According to Bashir *et al.* (2018) insufficient pollination may lead to fruit malformation or not properly developed tomato fruits. In this study, a similar observation was made with a higher percentage of the tomato fruits from the enclosed flowers being malformed compared to the exposed tomato flowers. It was also observed that the majority of the fruits harvested from the exposed pollination treatment had a significantly larger size when compared to fruits obtained from the enclosed pollination treatment. The improvement of the fruit characteristics by pollinators should increase the commercial value of tomato fruits (Klatt *et al.*, 2014). Moreover, the combined positive effect of pollinators on fruit characteristics and fruit set percentage increased tomato yield as was also observed by Amala and Shivalingaswamy (2017). As a result, pollinators can contribute to improved income by tomato growers.

4.5 Conclusions

This study indicated the importance of bee pollination in the production of tomatoes. When bees were allowed to pollinate flowers, yield and quality of tomato fruits were significantly better compared to flowers that were subjected to self-pollination. This highlights the importance of pollinators in tomato production. However, more research

has to be done in this regard. Future research should address the comparative efficiency of different pollination methods including hand pollination and mechanical pollination.

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Chapter 5

Conclusions and recommendations

Pollination is one of the essential ecosystem services and it is important for the production of approximately one third of crops globally (Klein *et al.*, 2012). Insects, particularly bees, are the most common and effective pollinators globally while flowering plants vary in terms of their reliance on bee species for sufficient pollination (Greenleaf and Kremen, 2006; Klein *et al.*, 2012). Tomato flowers are self-pollinating, however, because of the morphology of the anthers it has been reported that pollination services, provided by bee species, improve production (Deprá *et al.*, 2014; Silva-Neto *et al.*, 2017).

The main aim of this study was to quantify the pollination ecosystem service provided by bee species on tomato production in the Limpopo Province, South Africa through studying the pollination ecology of this crop. The objectives were to determine the effect of field proximity to natural vegetation on bee visitation rates on tomato flowers in four bio-climatic areas (Chapter 2), describe bee species that provide pollination ecosystem service to tomato flowers (Chapter 3), determine the effect of the pollination provided by bees on the yield quantity and quality of tomatoes (Chapter 4). In Chapter 5, general discussions, conclusion and recommendations are provided.

Main conclusions

- Bee visitation rates during the observation period differed between the four locations since at Waterpoort visitation rates decreased while at Vreedzaam and Jachtpad they increased during the observation period. It would seem as if temperature and humidity have played a role in bee visitation rates, because tomatoes are planted in different times of the year in the four localities, the highest bee visitation rates were observed at different times in the planting cycle.
- When the average of bee visitation rate was determined for the four localities visitation rates were significantly higher at < 50 m away from the natural vegetation compared to > 200 m away from the natural vegetation. The

presence of natural habitats and the availability of floral resources are important determinants of pollinator diversity and abundance in tomato fields (Greenleaf and Kremen, 2006; Toni *et al.*, 2020).

- Findings from this study indicated that potential pollinators of tomato flowers belonged to the Hymenoptera. The Apidae family, with three genera i.e. *Xylocopa*, *Apis* and *Amegilla*, was the most represented family while Megachilidae with one genus, i.e. *Megachile*, was the second most observed family. Other authors have previously observed similar species in the tomato fields (Hogendoorn *et al.*, 2000; Bell *et al.*, 2006; Amala and Shivalingaswamy, 2017; Silva-Neto *et al.*, 2017).
- This study suggests that the genus *Xylocopa* is the most important pollinators of tomatoes in the study area.
- The results of the exclusion experiment indicated that, although tomato flowers did produce fruits without the aid of bees, allowing bees to pollinate improved the quantity of the fruit, which confirmed observations by Vinícius-Silva *et al.* (2017).
- Bee pollination had a positive impact on the quality of the tomato fruit particularly in fruit weight, height and diameter, while the majority of the fruits harvested from the exposed flowers had a significantly larger size when compared to fruits obtained from the enclosed flowers.
- The combined positive effect of pollinators on fruit characteristics and fruit set percentage increased tomato yield as was also observed by Amala and Shivalingaswamy (2017) and as a result, pollinators can contribute to improved income of tomato growers.
- In this study bee pollination, compared to self-pollination, did not have a significant influence on the number of seeds per fruit. However, Bell *et al.* (2006) found that the fruits obtained from flowers pollinated by bees had a significantly higher number of seeds than when bees were not involved.
- This study has proven the importance of bee species on tomato production, it is therefore, important to recognise management practices that will aid in improving bee visitation rates, abundance and diversity in tomato fields for improved tomato production.

Anecdotal conclusions

- At Waterpoort, the presence of wood poles used as a framework to support tomato plants tend to have increased the abundance of *Xylocopa* species in the tomato fields because they use these frameworks as habitat.
- Because of the rich diversity of the vegetation of the two bushvelds at Waterpoort, this might have contributed to the abundance of bee species in the area moving from one area to the next. Waterpoort is found at the transition between the Soutpansberg Mountain Bushveld and the Musina Mopane Bushveld, according to Mucina and Rutherford (2011).
- The abundance of bees is usually lower in tomato fields that are surrounded by a lot of wildflowers. This is ascribed to the presence of alternative sources of nectar (tomato flowers do not produce nectar). This was observed at Dikgale.
- This study has indicated the importance of planting season on bee visitation rates however, at Dikgale which is planted in the summer season we expected to observe more pollinators in the area but there was an overall lack of pollinators in the area and the bee visitation rate was also low. This might be because of other factors that were not discussed in this study, for example, natural vegetation quality and the use of pesticides

Recommendations and further studies

- It was observed that the planting period has an influence on bee visitation rates it may therefore be recommended to farmers to adjust the planting schedule for improved pollination ecosystem services.
- The adverse effect of certain climatic conditions on bee visitation rates was not studied on a broad scale and it is further recommended to conduct studies on the influence of climatic conditions on bee abundance, activity and diversity. For example, exact daily temperatures, humidity and wind speed should be taken into consideration.
- Although it was observed that bee visitation rates were influenced by the proximity of the tomato plants to the natural vegetation, further studies should be conducted to perform a vegetation survey in the four study areas to

determine how the type of vegetation influences bee visitation rates, abundance and diversity.

- It is further suggested that during winter months, when food (nectar and pollen) is scarce, sugar water is put inside tomato fields to attract more pollinators.
- Although the ground nesting *Amegilla* was observed in this study only few species were noted and caught. There is a lot of soil cover in the tomato fields which may be suitable habitats for this species, but it seems like there are other factors that influence the abundance of this species inside tomato fields.
- It is also recommended that plant pots filled with suitable soil be put in the fields to create a habitat for ground nesting bees. Further research should be conducted into such practices and to observe if it is something tomato farmers can adopt.
- The availability of nesting material is important for bees, and making this resource available in tomato fields may help improve the abundance of these species (Kremen *et al.*, 2002). For example, some species in the genus *Xylocopa* nest in deadwood and twigs, and ensuring that the materials are available in the field may result in better abundance of these pollinators.
- Further studies may also include, conducting field surveys for bee species collection not only inside the tomato fields but also the neighbouring natural vegetation to determine if there are potential pollinators that do not visit tomato fields.
- The exclusion experiment in this study focused on some of the quality parameters to determine the effect of bee pollination on tomato fruits. Further studies may include physiochemical properties such as moisture content, colour tonality of tomatoes, total soluble solids, total ash and seed weight (g)/100 seeds.

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Addendum

Appendix A: Ethics Approval



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ETHICS APPROVAL LETTER OF STUDY

Based on approval by the Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC), the Faculty of Natural and Agricultural Sciences Ethics Committee hereby approves your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Quantifying the pollination ecosystem service provided by non-Apis bees in tomato production			
Study Leader/Supervisor: Prof J Van Den Berg			
Student: RN Kgaphola			
Ethics number:	N	W	U
	-	0	1
		4	2
		3	-
		2	0
		-	A
			9
	Institution	Study Number	Year
			Status
Status: S – Submission; R – Re-Submission; P – Provisional Authorisation; A – Authorisation			
Application type: Single	Risk Category:	Minimal	
Commencement date: 01/02/2020			
Expiry date: 01/04/2021			
Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.			

Special in process conditions of the research for approval (if applicable):

- The following documentation are archived by FNASREC and should be complete and kept up to date:
 - Research proposal
 - Signed approval from the scientific committee indicating the proposed risk category
- All researchers involved in the study should submit signed NWU code of conduct statements annually.
- All researchers of low risk studies should submit proof of relevant ethics training every two years.
- All researchers that take part in activities that pose a safety and security threat to the researchers or the environment should submit a risk assessment form annually.
- All research involving human interaction should follow best ethical practise and keep documents as proof. This includes informed consent, questionnaires, incorporation of risk-benefit, and responsible data management.
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the FNASREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the FNASREC:*
 - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
 - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the FNASREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and FNASREC reserves the right to:*
 - *request access to any information or data at any time during the course or after completion of the study;*
 - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
 - *withdraw or postpone approval if:*
 - *any unethical principles or practices of the study are revealed or suspected;*
 - *it becomes apparent that any relevant information was withheld from the FNASREC or that information has been false or misrepresented;*
 - *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
 - *new institutional rules, national legislation or international conventions deem it necessary.*
- *FNAS-REC can be contacted for further information or any report templates via Roelof.Burger@nwu.ac.za 018 299 4269*

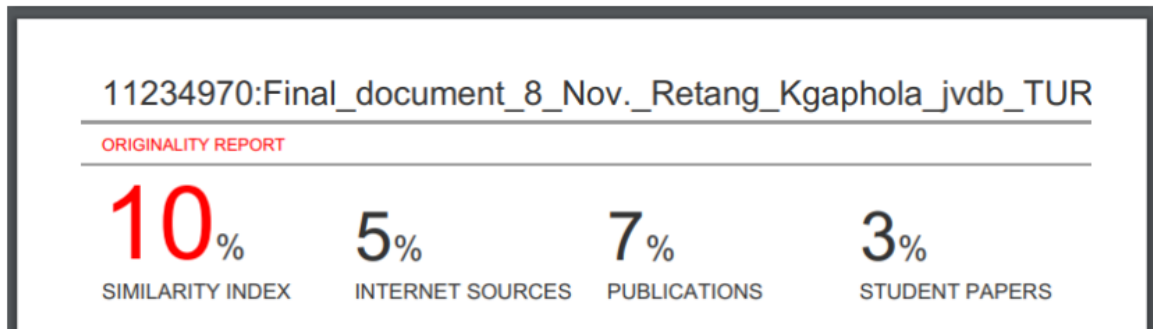
The FNASREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the FNASREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely,



Prof Roelof Burger
Chairperson Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)

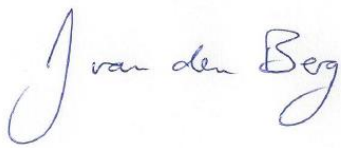
Appendix B: Plagiarism Report



Appendix C: Declaration of Language Editing

To whom this may concern,

I, Prof. Johnnie Van den Berg, hereby declare that the thesis titled: “Quantifying the pollination ecosystem service provided by non-*Apis* bees in tomato crops in the Limpopo Province” by Retang Nkakole Kgaphola has been edited for language correctness and spelling by some of the supervisors. No changes were made to the academic content or structure of this work.

A handwritten signature in blue ink that reads "Johnnie Van den Berg". The signature is written in a cursive style with a large initial 'J'.

Prof. Johnnie Van den Berg

Date: 18 November 2020