Cape Peninsula University of Technology

Influence of water application rates on visitation by the South African honeybee (*Apis mellifera capensis*) and seed yield of Texas Grano onions

By

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A thesis submitted in fulfilment of the requirements for the degree

Master of Agriculture

In the Faculty of Applied Sciences

At the Cape Peninsula University of Technology

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Wellington

December 2022

DECLARATION

I, Charles Salmon, declare that the contents of this dissertation/thesis represent my unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

16 December 2022

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ABSTRACT

South Africa is one of the major producers of onion seed in the world, with onions being the most valuable vegetable seed crop in the country. Grown mostly in the Klein Karoo, onion seed production in South Africa has suffered from historic and periodic problems in crop pollination. That results in honey bees being used for commercial pollination sometimes not working on the flowering onions, leading to poor pollination and poor seed set. Losses can be as much as 44% of annual production. Problems in onion pollination are a global problem with no clear explanation, why honey bees find onion flowers unattractiveness. Best supported theory is that irrigation resulting in high levels of repellent phytochemicals and /or lowered levels of nectar sugars best-supported,In this study the relationship between water irrigation levels, visitation rates of Cape honey bees (*Apis mellifera capensis*) and other insects, and seed production was studied using Texas Grano onions in Ladismith in the Klein Karoo. A mini-plot design was used with a field of onions being divided into 56 mini-plots, comprising of seven treatments and eight replicates of each treatment. Plots were provided with variable amounts of water during the flowering period, with insect visitation rates being recorded, together with resultant seed production. Water application rates during flowering were found to have no effect on the foraging rates of honey bees during the morning, or on the presence of other insects, and a small but significant effect on the afternoon and overall honey bee foraging rates. Seed production was highly correlated with insect visitation rates, as expected. It was concluded that water irrigation levels during the flowering of onions is not responsible for the periodic unattractiveness of seed producing onions, and water application rates during the earlier growth phases of the onion plants should be assessed in terms of their impact on the attractiveness of the onions to insects during the flowering period. More research must be done to determine the attractiveness of different onion cultivars, with regard to the nectar and pollen compositions. When a baseline of cultivar attractiveness has been established, it could be possible to breed cultivars with more attractive pollen and nectar to honeybees. Additionally, it is extremely important to establish pollen compatibility among hybrid cultivars, and the emphasis must be put on the investigation of non-viable pollen in Hybrid onion cultivars. Lastly, it is crucial to assess the foraging behaviour of honeybees with regard to the natural factors, namely temperature, humidity, rainfall, hive activity and insect activity. This data will enable the industry to use it as an indication of possible complications regarding honeybee pollination on onion cultivars.

ACKNOWLEDGEMENTS

I offer my heartfelt thanks to Prof M. Fanadzo for his unwavering support, care and dedication throughout the supervision of this project. Special thanks to my co-supervisor, Mr Mike Allsopp from the Agriculture Research Council (ARC-Stellenbosch) for his guidance in the design and execution of trials and overall support with thesis.

I am also grateful to Klein Karoo Seed for their financial support and inputs towards this study, without their cooperation and support, this study would not have been possible. Appreciation to Johan Immik, Irrigation Engineer for his considerable input towards the water management and manipulation during the trials.

In addition, a special thanks to my wife who supported me with the thesis.

DEDICATION

To my wife who had to sacrifice a lot, during the time that I was busy with my studies. May our good LORD richly bless you!

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GLOSSARY

CMS	Cytoplasmic-genic male sterility
DALRRD	Department of Agriculture, Land Reform and Rural Development
FAOSTAT	Food and Agriculture Organisation of the United Nations
MSL	Male sterile line
OP	Open pollinated
SANSOR	South African National Seed Organisation
µL	Microliter
MAD	Management allowable depletion
ETc	Evapotranspiration
NPK	Nitrogen, Phosphorus, and Potassium
KNO_3	IUPAC name for Potassium nitrate
kPa	Kilopascal
ANOVA	Analysis of variance
PROC GLM	General Linear Models Procedure
PCA	Principal component analysis

CHAPTER ONE: INTRODUCTION

1.1 Background

Onions (*Allium cepa* L.) are one of the oldest cultivated vegetables in the history of the world. Onions originated in Central Asia and spread across the world. Onions have been cultivated for approximately 5000 years (National Onion Association of USA, 2019) and are a very important food crop globally, used largely in the food industry for flavouring dishes as well as a staple vegetable. Onions are a biennial crop that forms a bulb during the first growing season and then flowers in the second growing season, producing seeds. Seed onions are currently grown in many parts of the world, particularly in the USA, Australia, India and South Africa (Gabai et al., 2018). In general, onion seed is produced in areas with warm, dry summers and a low humidity.

In South Africa, onions were one of the earliest vegetables to be produced. The first recorded onions to be produced in South Africa were by survivors of the shipwreck Haarlem in 1647 (prior to the arrival of Van Riebeeck in the Cape), with two survivors named Jansz and Proot reporting to Holland that onions flourish in the Cape area (Van Rooyen and Comrie, 1995). In recent times, the vegetable seed industry has become very important in South Africa, producing a wide range of seeds, with onion seed being one of the major crops. The industry is steered by the South African National Seed Organisation (SANSOR) that was established in 1989. Onion seeds have been produced in South Africa since the 1950's and production is mainly in the Klein Karoo, a semi-arid area with low humidity and mild temperatures in winter, with seed production a critical part of the economy of the area. Hybrid onion seeds, in particular, are an important commercial crop and are largely exported (SANSOR, 2019).

Pollination regulates plant reproduction through the movement of pollen, and while onion plants are normally self-fertile, insects are needed to transfer the pollen from the anthers to the stigmas. Insect pollination is especially crucial in the pollination of F1 hybrid onion cultivars, to carry the pollen from the male-fertile cultivar to the male-sterile flowers (Johannsmeier, 2001). A diverse range of insects visit onion flowers (Cook et al., 2020), including many species of flies and bees, with most important pollinating insect globally being honey bees (Nye et al., 1973). Throughout the world, managed and commercial honeybees are used to pollinate commercial onion fields. In South Africa, the insect pollinators in seed onions in the Klein Karoo were overwhelmingly honeybees, whether or not the onion fields were close to natural vegetation, and even when no managed honeybee colonies were introduced to the fields (Brand, 2013).

Notwithstanding the dependence on commercial honey bees for crop pollination, there has been a long history of pollination problems in onions in most parts of the world, including in South Africa, reaching back at least to the 1970’s, with the pollination problems invariably resulting in poor seed yields (Mayer and Lunden, 2001). Periodically, insect pollinators, including honeybees, appear reluctant to work in the onion fields, resulting in poor pollination and poor seed yields. These pollination problems have been widely reported in the USA, Australia and South Africa and seem to occur in all onion types and cultivars, to a lesser or greater extent (Mayer and Lunden, 2001; Long and Morandin, 2011). The poor pollination seasons in South Africa extend back to at least the mid-1990’s (Allsopp pers. comm., 2020), with very poor seed set obtained in some years in comparison to normal years. Year 2012 was reportedly the worst in history, with losses of at least R70 million being reported by growers in South Africa (Salmon, 2013). 2021 was an even bigger challenge with bees not wanting to work on the onion flowers and approximately R250 million of losses for the industry (Malan pers. comm., 2021).

Many explanations have been advanced for the periodic and unpredictable poor pollination seasons experienced in seed onion production. These include ‘bad bees’ or ‘lazy bees’, the impact of pesticides, the effect of better alternative forage recruiting pollinating insects away from the onion fields and the result of poor rainfall. Most commonly, the poor pollination and poor seed yield has been ascribed to a lack of attraction to the flowering onions by honeybees and other pollinators; and with lack of attraction being ascribed to high nectar potassium levels, or low sugar concentration levels, or low sugar volume (Hagler, 1990; Silva and Dean, 2000; Nicolson and Thornburg, 2007). Additionally, these factors are commonly considered to be caused by water imbalances in the plant, with too little water suggested to result in nectar that is too thick and viscous to collect and too much potassium in the nectar, and too much water resulting in nectar with inadequate sugar concentrations necessary to attract pollinators (Waller et al., 1972; Hagler, 1990). Alternative explanations have been that floral traits of the different cultivars are responsible, with some cultivars being very unattractive to pollinators (Soto et al., 2018), or that better alternative forage is responsible for pollinators not being active on the onions.

Various attempts have been made to overcome the low attraction of pollinators to onions and the seed set problems, typically by spraying carbohydrate solutions on the flowers to try to attract insects (Waller, 1972; Malan pers. comm., 2021), or by conditioning bees to onion flower scents (Silva et al., 2003), but neither method has been successful in addressing the attraction and yield problems. Internationally, there remains no clear explanation for the repeated and periodic pollination problems in seed onion production, nor solutions for growers to prevent this from happening.

The issue under investigation is as follow sometimes onion flowers are not adequately visited by honey bees and other pollinators, resulting in poor seed set and economic losses. Similarly, the question being asked is, why does this happen? The occurrence is widespread, being reported wherever commercial seed onions are grown, and persistent, with reports of pollination problems stretching back decades. Similar problems are now being reported in other crops, such as carrots (Gaffney et al., 2019), and such pollination problems are only expected to worsen with climate change when higher or lower rain fall received (Broussard et al., 2017).

The purpose of this study was to investigate potential pollination problems in the production of seed in Texas Grano onions in the Ladismith area of the Klein Karoo, South Africa with a focus on whether water application rates during the flowering was responsible for any observed pollination problems. The study intended to investigate the following issues: (1) the relative importance of commercial honey bees in the pollination of Texas Grano onions, and the relationship between honey bee numbers and seed production and quality, (2) the relative importance of other pollinators in the pollination of Texas Grano onions, and the relationship between other pollinators numbers and seed production and quality, (3) the impact of environmental factors on pollinator activity, and (4) the effect of different water application regimes during the flowering period of Texas Grano onions on the numbers of pollinators present, the type of pollinators present, and on seed production.

1.2 Aim and Objectives

The specific objectives were to:

- To determine the effects of variable water application rates during flowering of Texas Grano onion seed plants on honeybee visits, seed yield and quality.
- To determine the relationship between bee visitation, seed yield and seed quality of Texas Grano onions.
- To determine whether variations in water application rates, during the flowering period of Texas Grano onions, have an influence on seed yield and quality.
- Establish if there is a correlation between bee visits, seed yield and seed quality of Texas Grano onions.
- Determine the impact of high or low temperature and humidity on bee visits during flowering period of Texas Grano onions.

1.3 Research questions

- Do variable water application rates have an influence on the attractiveness of Texas Grano onion flowers to honeybees, seed yield and quality?
- Do variations in water application rate during the flowering period of Texas Grano onions, have an influence on seed yield and quality?
- Is there a collaboration between bee visits, seed yield and seed quality of Texas Grano onions?
- Do high or low tempters and humidity have any effect on honeybee activities and visits during flowering period of Texas Grano onions?

CHAPTER TWO: LITERATURE REVIEW

2.1 Onion

Onion (*Allium cepa L.)* is a member of the *Alliaceae* family. It is a biennial plant, requiring two growing seasons to complete the cycle from seed to seed. Bulbs are produced in the first growing season, and then flowers (and seeds) in the second season. The onion flower head, or umbel, is a single elongated inflorescence with 200-600 small flowers. Flowers are open for a two to three-week period and a crop will flower for four to five weeks. Onion seeds are small and black, and weigh about 1/300 of a gram. Onions take 64-67 days to grow flower stems with flowers, and then flowers bloom for 40-45 days (Kavitha and Reddy, 2018).

Onions no longer occur naturally in the wild and it is uncertain where they originated, but Central Asia is considered the most likely origin (Vavilov, 1951). In general, onion seed is produced in areas with warm, dry summers and a low humidity. The cytoplasmic-genic male sterility (CMS) system is used to produce hybrid onions (Brewster, 1994), which are much favoured as they result in better bulb yields and more uniform bulbs. F1 Hybrid onion seeds are produced by crossing a male sterile line (MSL) with a male fertile line. Seeds for both normal (open pollinated [OP]) and F1 hybrid onions are now grown across the globe, with hybrid lines typically producing less seeds than do OP cultivars. Hybrid vegetable seed production is common and growing in the industry, in crops such as carrots, cauliflower, onions, cabbage and radish, and all require the transfer of pollen from the anthers of male fertile flowers to the stigma of male sterile (female) flowers (Gabai et al., 2018). Such pollen transfer is usually performed by honeybees, which are crucial for viable commercial seed production. Hybrids are grown more because they have more vigour, more uniformity, and provide breeders rights.

Onion is one of the major vegetable crops of the world (Soto et al., 2018). On average, each person consumes 6 kg of onions per year across the world (Yara, 2011). The USA National Onion Association (2019) revealed that the consumption per capita in the U.S. has risen by 70% in the last two decades, from 5.5 kg of onions per person in 1982 to 9 kg per person in 2018. Onions are the third largest fresh vegetable crop that is produced globally (Statista, 2014). Annually, about 60 million tonnes of dry onions are produced globally on three million hectares in 134 countries across the world, yielding an average of 17 tons/ha (Yara, 2011). The biggest growers of onions are China, India and the USA (Yara, 2011). The worldwide hectarage of shallots and green onions production increased by 259% between 1961 (80 212 ha) to 2020 (208 347 ha), with global yields now 196% (FAOSTAT, 2020) higher per hectare. Overall, the world production of dry onions increased 1014.83% between 1961 (total 66 000 tons) and 2020 (735 786 tons) (Figure 2.1; FAOSTAT, 2020).

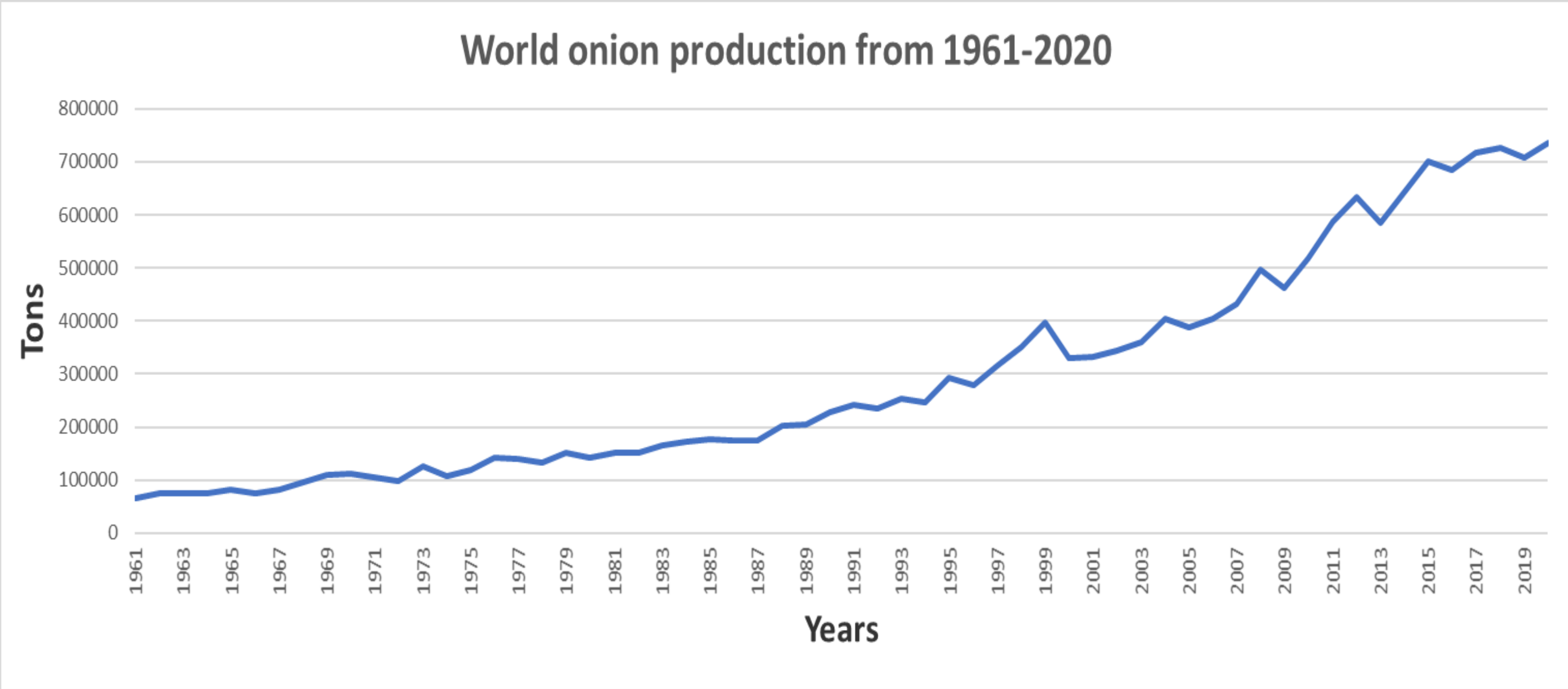

Figure 2. 1: World onion production from 1961-2020 (FAOSTAT, 2020).

To meet the demand for fresh onions, the onion seed production has become a global enterprise and plays a critical role in ensuring that the production of onions can keep up with the global daily demand. About 8-10 kg of onion seeds are required for transplanting to produce one hectare of dry onions (Rajput and Patel, 2006). Out of the 16 major vegetable crops listed in the global seed market in 2011, onion seed has the biggest production (McBride, 2011), with onion seed production having increased by 48% between 1961 and 2013 (Figure 2.2; FAOSTAT, 2015).

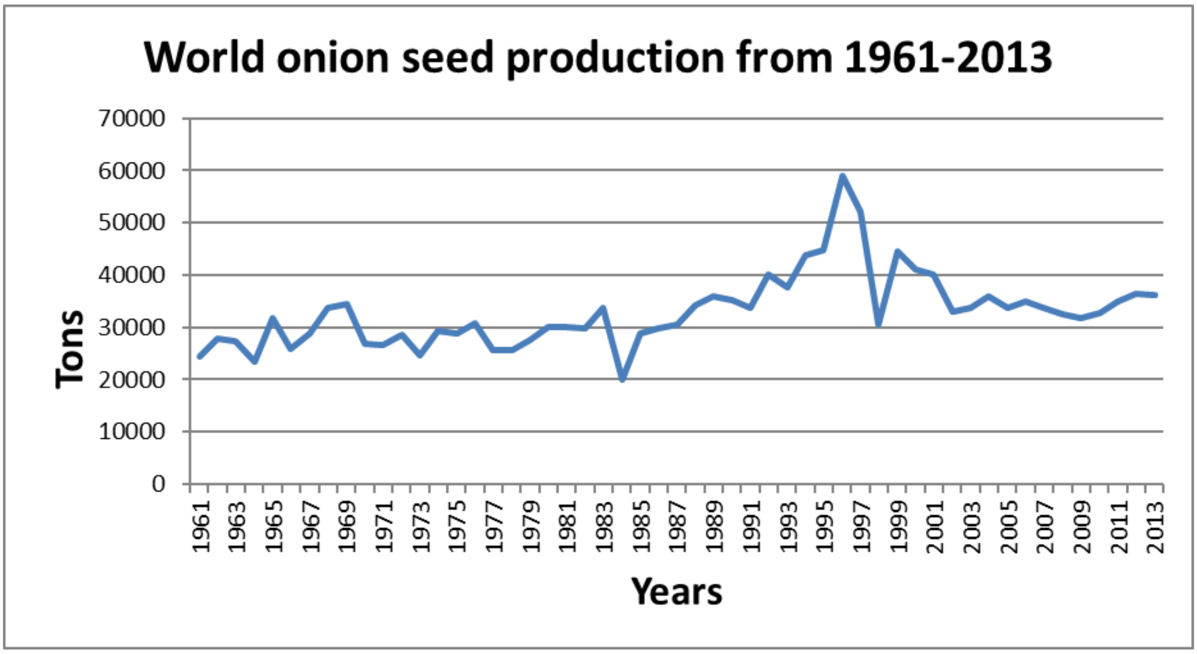

Figure 2.2: Worldwide onion seed production over the past 52 years (FAOSTAT, 2015).

2.2 Onions in South Africa

In South Africa, onions are the third most popular vegetable, after potatoes and tomatoes (DALRRD, 2020). The production and consumption of onions in South Africa increased dramatically from 2010 to 2019 by about 20% (DALRRD, 2020). Production has been slightly higher than the consumption from 2010 to 2019 (Figure 2.3, DALRRD, 2020), which means that South Africa is self-sufficient with regards to onion production, and production has increased gradually since 1961 (Figure 2.4, FAOSTAT, 2015). The average onion consumption in South Africa is about 600 000 tonnes per annum with surplus onions exported to the neighbouring countries, chiefly to Mozambique (DALRRD, 2020).

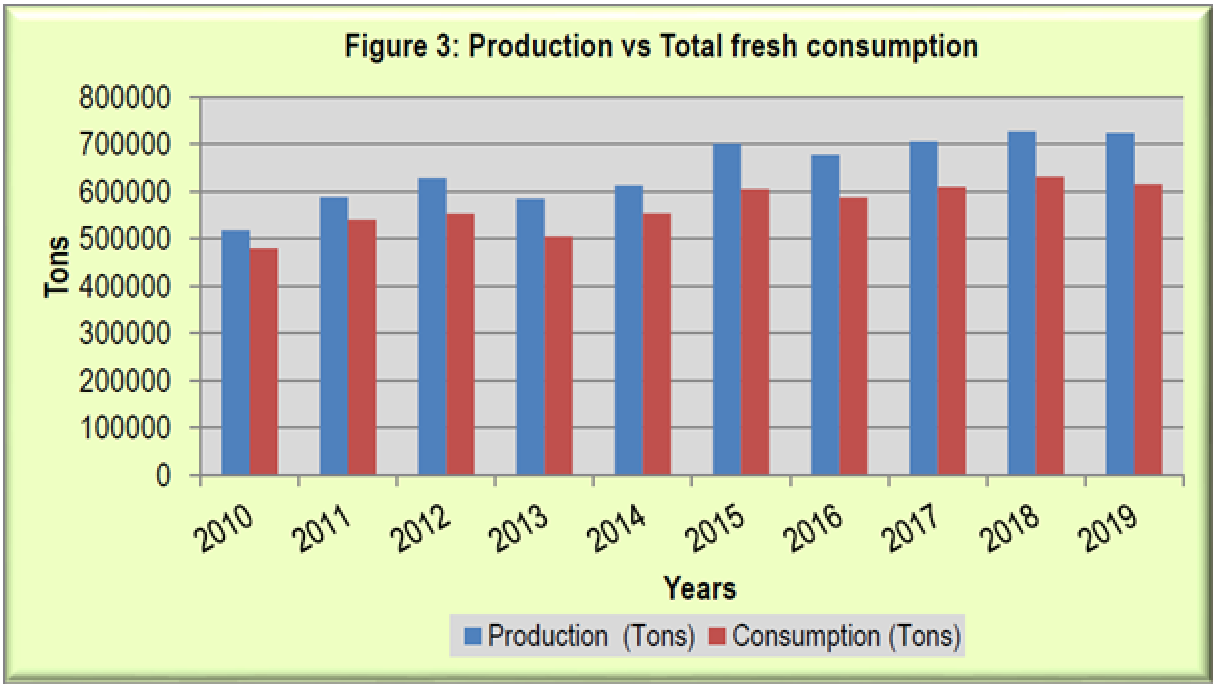

Figure 2.3: South Africa onion production and consumption from 2010 - 2019 (DALRRD, 2020).

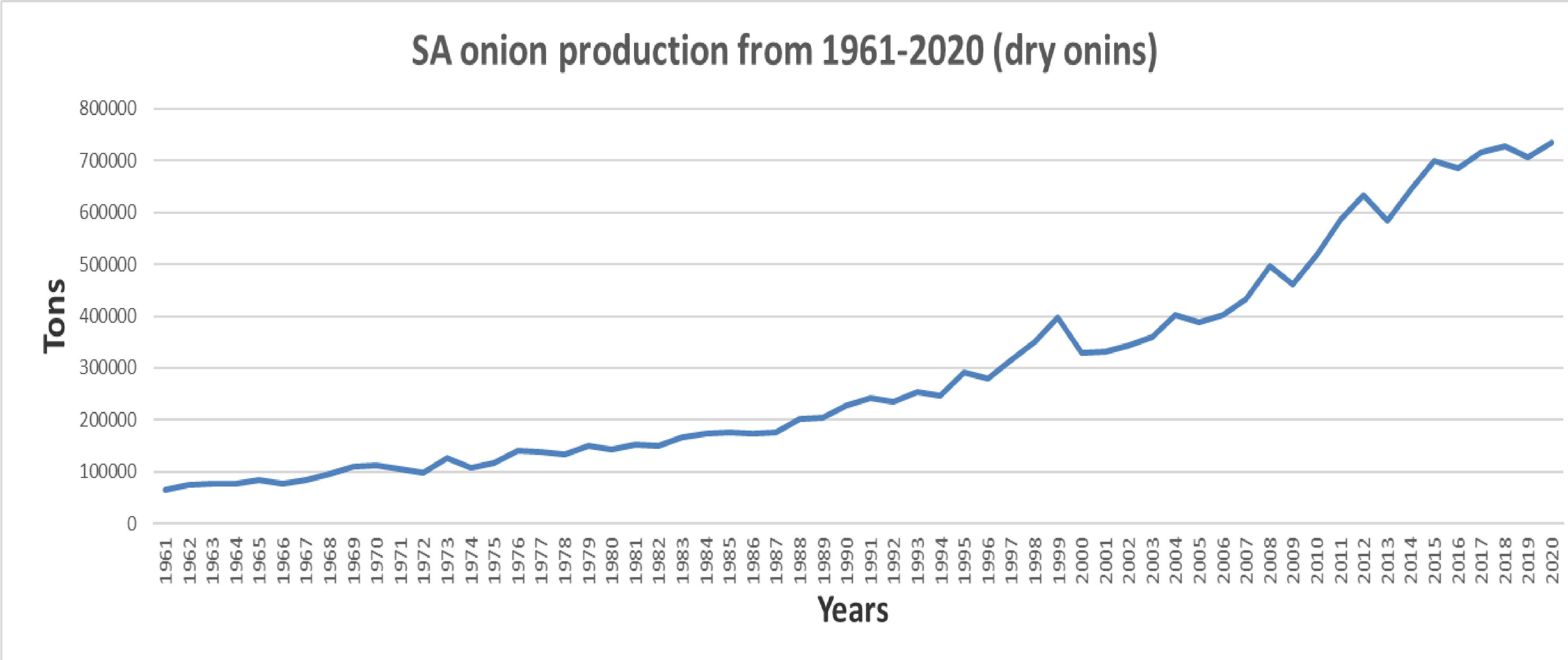

Figure 2.4: South Africa dry onion production from 1961-2020 (FAOSTAT, 2020).

The vegetable seed industry is very important in South Africa, producing a wide range of seeds. The industry is steered by the South African National Seed Organisation (SANSOR), which was established in 1989. Onion seeds have been produced in South Africa since the 1950's and production is mainly in the Klein Karoo, a semi-arid area with low humidity and mild temperatures in winter. The value of seed production in South Africa in 2018/19 was over R2-billion (SANSOR, 2019), of which onion seed value was R420-million. Onion seed production is thus of critical importance to the Klein Karoo region, but also nationally, and its importance extends beyond the financial and commercial markets to the labour force and community level. Vegetable seed production is now fundamental to the economic and agricultural sustainability of the Klein Karoo, and onion seed is the most valuable vegetable seed crop produced, making up 20% of the total horticultural seed crop produced in South Africa annually (SANSOR, 2015). Out of the five major horticultural seed crops that are produced, onion seed is significantly the most important, and the value of onion seeds continues to increase annually (Figure 2.5; SANSOR, 2016-2018). Seed production value has increased by a massive 68% since 2015 to 2019 (SANSOR, 2020).

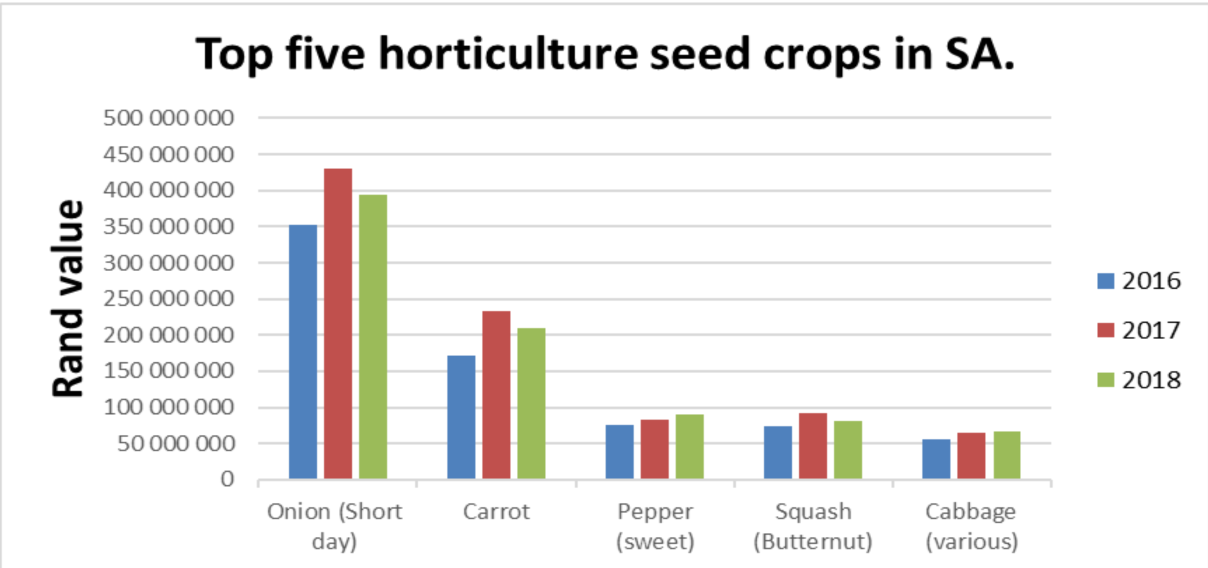

Figure 2.5: Top five horticulture seed crops in SA (SANSOR, 2016-2018)

Onion seed production in South Africa has more than doubled since 2004 with a dramatic increase since 2010 (Figure 2.6; SANSOR, 2004-2016). By 2013, the onion seed production had reached a monumental high of 1.865 million kg produced in South Africa, which is four times the norm for the previous years (SANSOR, 2016). A sharp decline in production occurred after 2013, however, with only 657 531 kg of onion seed produced in South Africa. The onion seed crop produced in South Africa consists of 62% OP onion and 38% consists of Hybrid varieties. Of the total onion seed produced in South Africa, only 9% is used internally and the other 91% is exported to international markets (SANSOR, 2016). Currently onions are still the leading vegetable seeds sales crop in SA, with an increase both in volume and value (SANSOR, 2021).

Figure 2.6: South Africa onion seed production from 2004 to 2016 (SANSOR, 2004-2016).

2.3 Pollination of onions

Nearly 75% of the world`s flowering plants are dependent on insects for pollination, with pollination regulating plant reproduction through the movement of pollen (Klein et al., 2007). Onions are generally self-fertile, but they do require insect pollination. Insect pollinators are especially crucial in the pollination of F1 hybrid cultivars, to carry the pollen from the male-fertile cultivar to the male-sterile flowers (Gabai et al., 2018). Onion flowers produce nectar to attract insect pollinators, which are typically rich in sugars with a concentration of more than 40% (Free, 1993; Hagler et al., 1990). Many insects visit onions, mostly flies and bees (Free, 1993; Sajjad et al., 2008; Howlett et al., 2005; Cook et al., 2020), with bees viewed as the more important pollinators. In India 98% of visitors to onion flowers were hymenopterans, with 96% of the total visitors being honeybees (Karuppaiah et al., 2018) and with peak activity being in the afternoon. In a similar study, also in India, Hosamani et al. (2019) found that 88% of insect visitors were hymenopterans with 9% being Diptera and 2% Lepidoptera. Again, the vast majority of the hymenopteran visitors were honeybees. In general, and throughout the world, the most common insect pollinator of onions is honeybees (Nye et al., 1973).

In South Africa, Brand (2013) also found that honeybees were by far the most abundant insect visitor. Other insects included ladybirds, flies, milkweed bugs, non-Apis bees, wasps, butterflies and other beetles. Honeybees were shown to collect a significant amount of pollen, as well as onion nectar, and were very effective pollinators. This was the case whether or not the onion fields were close to natural vegetation, and even when no managed honeybee colonies were introduced to the fields (Brand, 2013). In high intensity agriculture, with many hectares of cropland, and few natural pollinators, pollination and yield always depends on introduced managed pollinators, and this is usually honeybees.

In South Africa, onion crops are pollinated by the Cape honeybee (*Apis mellifera capensis)* in the Western Cape and by the savannah bee (*Apis mellifera scutellata)* in the Northern Cape, typically from October to late November. Bee activity is strongly correlated with temperature, sugar availability and relative humidity (Abrol, 2010), with maximum bee activity between 28-32 °C (Kavitha and Reddy, 2018). A substantial number of commercial colonies are needed to ensure good pollination, with 5-12 colonies per hectare being recommended in South Africa (Johannsmeier, 2001). It has been widely found that there is a clear correlation between pollination by bees and onion seed yield, more bees meaning more pollination and better seed yield (McGregor, 1976; Hagler and Wailer, 1991; Soto et al., 2013; Gillespie et al., 2015; Soto et al., 2018). In fact, insect pollination is as important in crop yields as is plant quality (Fijen et al., 2018), and a more significant factor in seed production than either fertilisers or irrigation (Fijen et al., 2020). In South Africa, honeybee numbers directly affected crop yield, but the presence of other pollinators had no effect on crop yield (Brand, 2013).

Onions are but one of 87 global food crops or 70% of the world's crops, which are dependent on animal pollination (Klein et al., 2007) for quality and yield, with the total economic value of pollination globally valued at 158 billion Euros (Gallai et al., 2009). Honeybees are the most important animal pollinators (Williams et al., 2001), and there is a global concern that sufficient honeybee colonies will be able to be maintained to service ever-increasing pollination demands (Aizen et al., 2009). An inability to sustain the necessary honeybee colony numbers would threaten the viability of all insect-dependent crop pollination, including onion seed production.

While there is no good scientific or other data on pollinator decline in South Africa, or on the different contributions of the various pollinators (Melin et al., 2014), and only a very limited beekeeper survey in 2012 (Pirk et al., 2014), which found a high degree of colony losses in the country, these same concerns exist in South Africa. Academics, growers and government increasingly worry that there will be insufficient honeybees for our commercial needs (Conradie and Nortjé, 2008; Allsopp and Masehela, 2017; BIS, 2018). Of the 130 000 managed colonies in South Africa, at least half are in the Western Cape, but this number will need to double in the next five years to service the increasing pollination demands on cherries, blueberries, macadamias, almonds, and seed crops. Notwithstanding the increased pollination demands and a pollination tariff that has increased every year, there have not been enough colonies available to service all the pollination requirements in the past couple of years.

With natural habitat and vegetation cover for pollinators declining worldwide, of which agriculture plays a huge role, through the modification and elimination of pollinator habitats and the use of agricultural chemicals like pesticides, herbicides, and fertilisers (Bezabih and Gebretsadikan, 2014), there are reasons to be concerned. To try to maximize the sustainable use of honeybee colonies for commercial pollination, the Western Cape Bee Organisation (2021) has put standards in place to which all colonies used for pollination in the fruit industry must comply. These standards are also used for the pollination of vegetable seeds. More research is needed to see if the swarm standards are adequate for vegetable seed pollination.

2.4 Pollination Problems in Onions

From the beginning of global onion seed production, there have been sporadic problems with poor pollination, resulting in poor seed yields. Franklin (1970) reported that the decline in onion seed yields started around 1961 with several unexplained instances of complete or nearly complete seed failures. Before 1961, low seed yields were attributed to poor husbandry, disease or inbred parents known to be reproductively weak, but after that, the causes for poor yield have been much more difficult to identify (Franklin, 1970). Yield problems have been widely reported in North America (Waller, 1972, 1974; Nye et al., 1971; Hagler, 1990; Mayer and Lunden, 2001), with the yield in onion seeds in California steadily decreasing from 2003-2008 (Long and Morandin, 2011). Increasingly, the low yields have been viewed as resulting from poor insect pollination (Campbell et al., 1968; Carlson, 1974; Waller, 1983) and continue to present day.

There have also been historical problems with onion seed production in South Africa from at least the 1990's (Allsopp pers. comm., 2020), where in some years very poor seed set is obtained in comparison to normal years. Several factors have been considered to be the cause for these problems, ranging from 'bad bees' or 'lazy bees' (colony strength not sufficient for pollination), to pesticides that is use on onions before and during pollination period and poor rainfall leading to very dry conditions. The year 2012 in South Africa was particularly bad, reportedly the worst in history, with losses of R70 million reported (Salmon, 2013).

Various attempts have been made to overcome the low attraction in onions and the seed set problems, typically by spraying carbohydrate solutions on the flowers to try to attract insects (Waller, 1972) this was also done in 2012 by producers in SA, with no results (Malan pers. comm., 2021), or by conditioning bees to onion flower scents (Silva et al., 2003), but neither method has been successful in addressing the attraction and yield problems. Hence, the periodic problems in onion seed production persist in both South Africa as well as in Australia and the USA (Silva et al., 2003; Silva and Dean, 2000), and none of these countries appear to have identified the exact problem or found a solution for the weak pollination.

While the direct cause of the periodic poor pollination of onions has not yet been elucidated, there are a number of possible explanations, all of which could be responsible, in whole or in part, and much research has been conducted in recent years to identify the cause of the problem. These explanations can be separated into a number of categories, each of which could be partially or wholly the explanation for the pollination problems.

2.4.1 Varieties and Nectar Levels

The attraction of honeybees to flowers is due to flower attributes such as size, colour, flower organs, amount of pollen, chemical components contributing to fragrance, nectar volume and nectar composition, and a combination of these factors will determine the attraction of the flower to honey bees and honey bee visitation rate (Stashenko and Martnez, 2008). In particular, the concentration and composition of the nectar is crucial in determining which insects are attracted, and how many. Plants must attract and retain pollinators to ensure fertilisation, and this is achieved by offering sufficient nectar rewards. The nutritional value of nectar comes from glucose, fructose and sucrose. Nectar sugar concentrations can vary from 10% to 70%, with honeybees preferring nectar values from 30-50% (Wright et al., 2018). Above 60%, nectar is too viscous to collect efficiently. The proportions of the various sugars are not critical to bees in terms of the attractiveness of the nectar (Wright et al., 2018). Nectar also has amino acids, lipids, phenols and anti-oxidants. The amino acids in the nectar affect the taste of the nectar, and affect how bees learn floral traits. All amino acids can be present in nectar (Nicolson and Thornburg, 2007) and some are clearly attractive to foraging bees. Minerals in nectar are also important, with low concentrations of magnesium and sodium being attractive to bees, and high potassium levels being aversive (Wright et al., 2018).

While onion flowers in general are relatively unattractive to pollinators (Gary et al., 1972; Gary et al., 1977), onion cultivars are very variable in terms of all the above factors (floral colour, floral size, fragrance, nectar content). Hence, they are very variable in terms of their attraction to pollinators (Lederhouse et al., 1972; Hagler and Wailer, 1991; Caselles et al., 2019; Silva et al., 2003), and this may be responsible for the pollination and yield problems in the less attractive cultivars. Some hybrid cultivars, in particular, have poor seed yields and are largely unattractive to pollinators (Hagler, 1990; Hagler et al., 1990; Silva and Dean, 2000; Silva et al., 2003; Silva et al., 2004; Marino et al., 2013). The most important factors in insect pollinator numbers are considered floral structure of the different cultivars, and nectar volume (Silva et al., 2004).

Onion nectar is generally highly concentrated with sugar concentrations typically over 40% (Free, 1970; Hagler et al., 1990; Lederhouse et al., 1972). Sugar concentrations of onion nectar can even be above 60% (Brown et al., 1977; Waller, 1974), although bees in onion fields reject flowers containing such concentrated nectar (Lederhouse et al., 1972; Brown et al., 1977; Waters, 1972). Onion nectar is 53-56% fructose, 40-43% glucose and 4% sucrose (Waller, 1974), which is a fairly typical nectar profile.

Nicolson and Thornburg (2007) reported that the correlation between sugar composition and nectar concentration may arise very early in floral development. Nectar originates from sucrose-rich phloem sap or from sucrose synthesised in the nectary tissue and the proportion of monosaccharides depends on the presence and activity of various nectary enzyme systems, including invertase (Nicolson and Thornburg, 2007). The variation in water component of nectar occurs by nectary activity (secretion or reabsorption), removal by pollinators and may be affected by equilibration with ambient humidity (Corbet, 2003).

Typically, an average of 1.80μl of nectar per floret is produced (Kavitha and Reddy, 2018) and in simple terms, the onion cultivars with the greatest nectar volumes and concentrations attract the most insect visitors (Kavitha and Reddy, 2019). There are often large differences in nectar levels between different cultivars (Silva and Dean, 2000; Soto et al., 2013; Brand, 2013) with MSL (male sterile lines) generally producing less nectar (Wilkaniec et al., 2004) and male-fertile lines being preferred (Williams and Free, 1974; McGregor, 1976; Woyke, 1981; Silva, 1998; Mayer and Lunden, 2001). There remains, however, insufficient information on the nectar quality of most onion cultivars (Benedek, 1976; Sajjad et al., 2008).

It is probably unlikely that the sugar concentrations in nectar alone is responsible for the lack of pollinator attraction and problems in seed production, and more likely that it is only part of the problem. It is worth noting, however, that in carrots, sugar concentrations in nectar are believed to be responsible for growing pollination problems. Carrot pollination was not previously regarded as a problem, but increased variability between cultivars has developed in recent years, with some cultivars now hardly visited by pollinators at all, and carrot pollination is now a substantial and growing problem (Gaffney et al., 2019).

2.4.2 Environmental conditions, rainfall and irrigation

Climatic conditions can also be a major factor in seed crop pollination, especially onion seed. Nectar production is influenced by many factors including temperature, humidity, soil moisture and genetics, and pollinator activity is influenced more by temperature and relative humidity than it is by nectar composition (Caselles et al., 2019). As in South Africa, onion pollination for seed production occurs in October to late November, a period of low rainfall. This could result in water stress to the plants which results in reduced nectar quality or quantity, even though onion plants are constantly irrigated. Brand (2013) for example, found that rainfall was the only factor that significantly correlated with seed yield in onion seed production in the Klein Karoo.

Onions are a shallow-root crop and have been reported to have little tolerance for water stress (El Balla et al., 2013; Kumar et al., 2007; Kadayifci et al., 2005; Al-Jamal et al., 1999; Shock et al., 1998). The water balance of the onion umbel is different to that of most agronomic crops and there is little evaporative cooling by transpiration (Brown et al., 1977). Millar et al. (1971) reported that the lowest water potentials in the onion plant existed in the flower and pedicels, indicating there is considerable resistance to the flow of water from the soil to the floret and the onion stomata are very sensitive to water deficit.

The effects of high temperature and water stress during pollination and seed development can affect the pollen viability and stigma receptivity, leading to poor fertilisation or abortion of developing seed (Nye et al., 1971). In early studies regarding soil moisture, the highest seed yield of yellow ‘Sweet Spanish’ onion was with high soil moisture (Hawthorn, 1951; MacGillivray, 1948). Water stress at any stage of reproductive growth significantly reduces seed yield and its effects are variable depending on the plant growth stage (El Balla et al., 2013; Alqudah et al., 2011; Mermoud et al., 2005; Abdul-Jabbar et al., 1983). Water stress at the bulbification and ripening stage of dry onions also leads to significant differences in the yields achieved (Pelter et al., 2004; De Santa Olalla et al., 2004).

Onions therefore require frequent and light irrigation to maintain a high soil-water potential (Shock et al., 2000), to avoid water deficiency and to adequately recharge the plant's root zone (Koriem et al., 1994). The methods of irrigation are very important in terms of the attractiveness of crops to pollinating insects. Sprinkler irrigation, for example, can influence the availability of pollen by making it wet and sticky. Irrigation can also result in changes in nectar viscosity (Brown et al., 1977; Mayer and Lunden, 2001), influencing the availability of nectar and the attractiveness of the onion flowers. To avoid water stress in onion production, drip irrigation is preferred, because of high water-application efficiency and reduced losses, surface evaporation and deep percolation (Rajput and Patel, 2006; Mmolawa and Or, 2000; Rolston et al., 1979). This enhances the management allowable depletion (MAD), the point below which the soil available water should not be depleted to avoid excessive water stress and, therefore, a reduction in production (Enciso et al., 2006). Drip irrigation also improves fertigation (Mmolawa and Or, 2000; Rolston et al., 1979) and is very important in obtaining optimal use of fertiliser, by applying regularly and timely in small amounts (Rajput and Patel, 2006; Neeraja et al., 1999), to increase the amount of fertiliser used by the plant and reduce the amount lost by leaching (Shock et al., 1995).

In order to ensure sufficient irrigation, the water retention capacity of the soil needs to be considered (Kirda, 2002). Scheduling of irrigation can be done by either using the ETc formulae or making use of probes and tension meters (Enciso et al., 2006; Enciso et al., 2008). Irrigation scheduling is one of the most important tools for developing best management practices for irrigation areas (Al-Jamal et al., 1999) and of vital importance to preserve water resources, quantitatively and qualitatively, and to produce more food with the available water, particularly as water is becoming a very scare natural resource throughout the Western Cape.

Pollen and nectar production levels in flowers can vary with soil moisture content (Gillespie et al., 2015; Waser and Price, 2016) and bee visitation rates correlate with soil moisture (Gillespie et al., 2015). If the plant is water-stressed, the nectar becomes thick and the trace elements and sugar concentrations will increase, causing the bees to avoid these onion flowers. Alternatively, if there is too much water, it can reduce the sugar concentration in the nectar below a level that is attractive to pollinators. Most significantly, Gillespie et al. (2015) found that the relationship between nectar production and soil moisture content was non-linear, with the highest nectar production occurring at moderate moisture levels. Changes in soil moisture, therefore, whether resulting from reduced rainfall or reduced irrigation may therefore reduce nectar production, and thus pollinator activity and seed production. Fijen et al. (2020), however, found that reducing irrigation to leeks (*Allium porrum*) reduced the amount of nectar available, but that this did not affect the pollinator visitation rates, and had no effect on seed yields.

2.4.3 Trace Elements, Phenolic and Repellency

In addition to sugars, onion nectar has trace elements that contribute to the aroma and taste (Free, 1970), with the potassium levels being particularly high (Waller et al., 1972; Hagler, 1990), as well as plant phenolics and alkaloids. Phenolic compounds or polyphenols are a product of secondary plant metabolism (Cianciosi et al., 2018) and are divided into two categories – flavonoids and phenolic acids. These products are largely responsible for the antioxidant activity of honey, and are associated with the antibiotic activity found in honey. Flavonoids occur widely in the nectar of plants, and may be either attractants or deterrents. They are typically viewed as a resistance to herbivory but their presence in nectar is not well understood; it is generally believed that they are there to deter nectar robbers and to favour specialist pollinators (Feinsinger and Swarm, 1978; Nicolson and Thornburg, 2007; Soto et al., 2016). The presence of both trace elements and phenolics in onion nectar have been proposed to be responsible for the periodic lack of attraction (repellency) of pollinators to onion nectar, and the resultant poor yield of onion seeds.

Concerning trace elements and high potassium levels in onion nectar, it has been reported to reduce foraging, with potassium levels in onion nectar typically 10 times higher than is the case in most other flowers (Waller et al., 1972; Waller et al., 1974; Hagler, 1990), and this has often been considered the most likely explanation for onion seed production problems. Hagler (1990) found that the relationship between potassium levels and carbohydrate levels in the nectar dictated attraction to pollinators. He reported that honeybee foragers avoided cultivars with the highest potassium levels but that this could be compensated for by higher carbohydrate rewards in the nectar (Hagler, 1990). Potassium and other trace elements find their way into the nectar through the fertiliser that growers apply to the plant and through natural processes through the roots of the plant. Silva and Dean (2000) and Soto et al. (2013) however, did not find that potassium levels influenced bee foraging, and it was unrelated to poor seed yields.

As regards phenolics, Soto et al. (2013) suggested that a flavonoid such as Luteolin, Naringenin or Quercetin might be involved in the repellency of pollinators by onion nectar, and later that alkaloid compounds such as nicotine, theophylline, theobromide, caffeine, harmaline and piperine might be involved (Soto et al., 2016). While marked differences were found in the amounts of different alkaloid and phenolic compounds in different cultivars, no evidence has been found that any of the compounds in onion nectar is acting as a deterrent to pollinators (Liao et al., 2017; Soto et al., 2018). In carrots, however, intrinsic differences in phenolics in carrot nectar have been shown to affect the attractiveness of different cultivars to bees (Broussard et al., 2017), with the phenolics reported to be directly responsible for differences in seed set between carrot cultivars.

In onions, results have been contradictory and inconclusive, even though a number of studies have directly looked at the various characteristics of onion cultivars, their sugar concentrations and trace elements and phenolic compounds, to try to determine which factors were important in determining insect attraction and seed yield (Soto et al., 2013; Soto et al., 2016). In South Africa, Brand (2013) recommended a comprehensive study to measure the nectar levels and odour levels of the various onion cultivars, and how these relate to pollinator activity, but this work has yet to be done. Most recently, Hernández et al. (2019) concluded that, it is an interaction between the phenolics and potassium in the nectar that influences attractiveness, but this remains to be verified.

2.4.4 Alternative Forage

The availability of food is fundamental to survival in honeybee colonies, and food sources need to be optimally exploited. Nectar and pollen are collected from flowers to feed both adult and juvenile bees, with nectar being the carbohydrate resource and pollen providing protein, fats, minerals and vitamins. Sources of food for bees are ephemeral and unpredictable, and colonies constantly adjust their foraging to the availability of forage, prevailing conditions and the demand within the hive. Food resource quality is the primary determinant in where bees forage, and when a target commercial crop is relatively poor in food quality, it should not be near to better competing crops, or high quality natural forage. Carrots, avocadoes, pears and plums are crops that are often affected by bees being attracted to alternative forage (Afik et al., 2006; Gaffney et al., 2019; Allsopp pers. comm., 2020). When crops are nectar-poor or repellent in some other way, foraging insects can easily be lured away to other plants that produce better quality forage, including weeds, other crops or wild flowers.

Onions are normally less attractive than competing plants (Waller et al., 1972; Gary et al., 1972; Gary et al., 1977; Nye et al., 1973), and foragers tend to favour other plants when available (Hagler and Wailer, 1991). This too is a common explanation as to why onion fields are periodically unattractive to pollinators, and for the periodic problems in onion seed production (Waller et al., 1972; Hagler and Wailer, 1991). In the Klein Karoo in South Africa where seed onions are principally grown, in seasons after good winter and early spring rains, there is an abundance of flower reserves (Hepburn and Guillarmod, 1991), most of which are more attractive to bees than are onion flowers. In one season of her study, Brand (2013) reported a negative correlation between hive stocking rates and the numbers of bees active in the fields, and interpreted these data as a combination of extreme competition on an unfavourable crop and that the vast majority of the foraging bees were not attracted to the onions and were rather foraging elsewhere.

2.4.5 Pesticides

A final explanation for the lack of attraction of onion flowers to pollinators, resulting in a poor seed set, is the timing and nature of insecticide or fungicide applications applied to the crop. The major pest and disease problems associated with the cultivation of onions are thrips (*Thrips tabci)* and black mould (*Aspergillus niger*) (Downes et al., 2008), for which growers need to spray. It is self-evident that such applications have the potential to reduce seed yield by direct means such as by affecting pollen germination or pollen tube growth (Yi et al., 2003; Long and Morandin, 2011), as well as by reducing the pollinator attraction or retention to a crop by rendering the flower or the nectar repellent to foragers (Long and Morandin, 2011; Gillespie et al., 2014). Either situation would have the effect of reducing effective pollination, or reducing seed yield.

The decrease in onion yield in California from 2003-2008 was suspected to be pesticide related (Long and Morandin, 2011), and Gillespie et al. (2015) investigated if pesticide applications were responsible for reduced pollinator activity in the USA, and found no correlation between insecticide use and insect visitation rates. Rather, insect visitation rates were positively correlated with temperature, nectar levels and soil moisture. Beyond this study, no work has been done on the possible impact of spray applications as regards its impact on pollinator attraction to onions, and clearly more research is needed.

2.5 The South African Situation

The 2012 season in South Africa was particularly bad with respect to onion seed yield, reportedly the worst in history, with losses of R70 million reported (Salmon, 2013). In 2012, Salmon conducted a questionnaire-based survey in order to gather information from onion seed growers in the Northern Cape, Eastern Cape and the Western Cape Provinces of South Africa. These areas are the main areas in South Africa where onion seeds are produced. All 160 known producers of onion seed throughout South Africa were asked to participate in the survey, with 66 responses. Seed producers were asked to give facts and opinions on causes for poor pollination and seed production and their experiences during their time in seed production. Analyses of the survey results (Salmon, 2013) indicate that in total, the 66 growers planted 506 ha of onion varieties in 2012, and of this total 263 ha (52%) produced low seed yield or poor seed quality, ranging from 7% to 100% of losses on their onion seed crop, with an average loss of 44%. Furthermore, pollination problems were found all over the country, on all types of onions, and with all types of irrigation systems being used (Salmon, 2013).

The growers in the survey identified six possible causes for weak pollination (Salmon, 2013):

- weak swarm standards,
- fertiliser programmes,
- water and irrigation methods,
- type of cultivar,
- insecticides and fungicides and
- distance to alternative foraging.

After thorough discussion of the findings of the survey and the six identified possible causes for weak pollination with market leaders of onion seed production, producers, farmers and various knowledgeable individuals with insights into pollination, it was decided that water and irrigation techniques were the main binding factor among all possible causes. Therefore, it was decided that this study would investigate the effects of water application on bee visits and seed yield of Texas Grano onion seeds in the Klein Karoo area.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction

Water availability and irrigation have long been considered as prime candidates to explain the observed occasional unattractiveness of onions (Mayer and Lunden, 2001), but this link has yet to be unequivocally demonstrated, or its specifics elucidated (Gillespie et al., 2015). There are two obvious methods with which this question may be approached. The first is that followed by Silva et al. (2004) and others; namely, to collect and collate as much information as possible from commercial onion plantings as possible, and to try to correlate insect activity and seed production with environmental conditions and/or farming methods. The second method is that followed by Soto et al. (2018), namely controlled experiments looking at specific variables, typically in a randomised plot design.

The second methodology is utilised in this study to investigate potential pollination problems in the production of seed in Texas Grano onions in the Ladismith area of the Klein Karoo, South Africa with a focus on whether water application rates during the flowering was responsible for any observed pollination problems. Seven different water regimes were applied to a field of Texas Grano onions, which was then assessed for honey bee activity, activity of other pollinators, and the impact on onion seed production. The structure of the project was a series of mini-plots within a single field with different water treatments between plots, with pollinator activity between plots being assessed.

3.2 Site Location

The study site was situated 15 km outside Ladismith, in the Little Karoo, Western Cape. The Little Karoo is very well suited for the production of onion (*Allium species*) as it has the ideal climate, with hot and dry conditions in the summer months. This area receives rainfall mostly during autumn and spring, with moderately cold winters. The average rainfall for the study site area is between 250 and 300 mm per annum. There is considerable commercial onion seed production in the near vicinity of the study site. The field site selected was appropriate for seed onion production, with Lucerne produced to the south and east, and with natural vegetation to the north and west. This site is typical of that used by commercial growers in the region for onion seed production, and the distance to the nearest commercial producer was also typical, to prevent cross pollination of cultivars.

3.3 Study Site

A single piece of land was used, approximately one hectare in size. This was to allow for the control of as many variables as possible. All of the climate, sunlight period, soil type and structure, water quality, alternative forage, disease control, bee colony strength and overall field management may be regarded as constants within this experimental design, as all were applied equally to all plots used in the trial. A randomised mini-plot design was used with all treatment plots treated the same with regards to irrigation, fertiliser, spray programme and all other treatments until the flowers started to open, when differential water manipulation for the various plots began. Each trial plot was 3 m x 3 m with an average of seven meters in between the trial plots. There were seven treatments (A – G) with eight replications of each, resulting in 56 treatment combinations. The plots were randomly scattered on the one-hectare piece of land, by using a randomisation design. Each plot was clearly marked with a number from one to 56 (Figure 3.1 and all the plots of the same treatments had the same colour as shown in Figure 3.2.)

Figure 3. 1: Coloured signs with numbers in each plot to help with distinguish between the different plots and treatments.

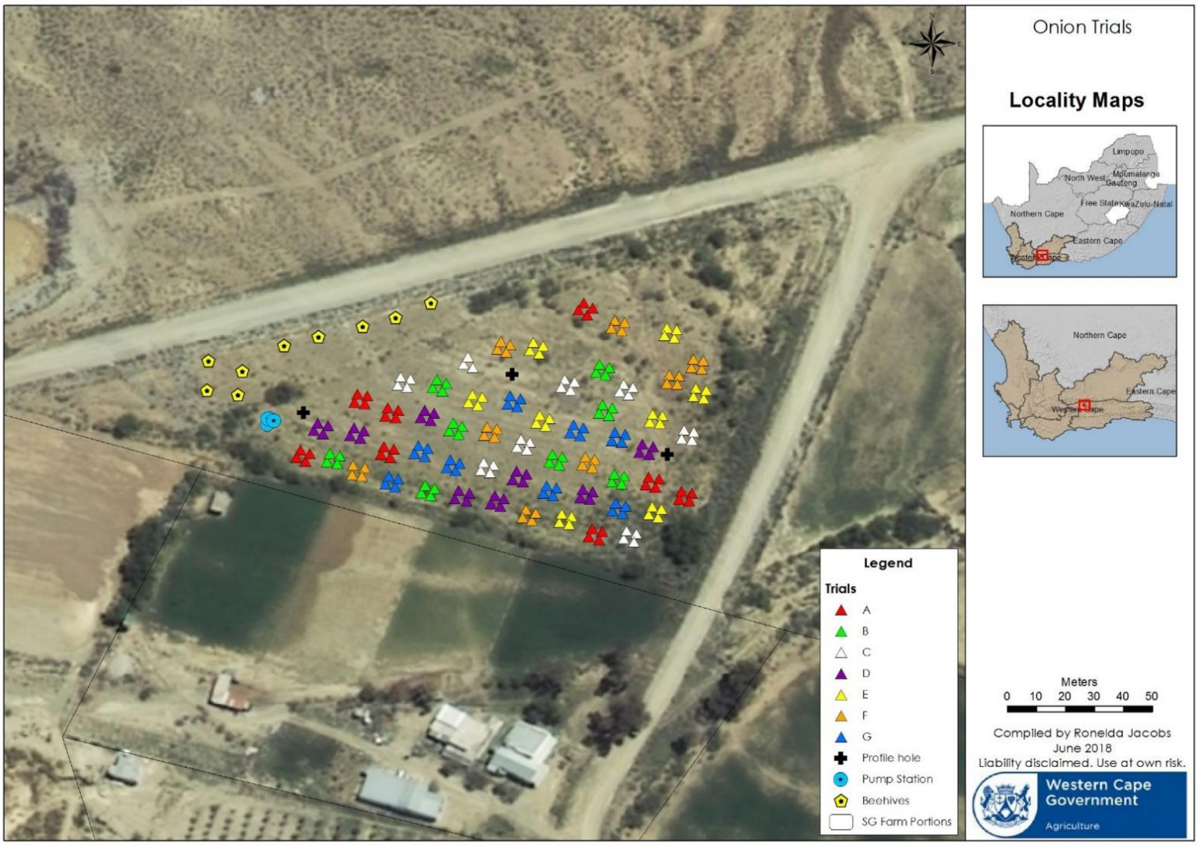

Figure 3. 2: Map of the study site in Ladismith area. Each treatment group is indicated in a different colour, and the positions were chosen randomly. Honey bee colonies are indicated by yellow pentagons.

The colour coding of plots helped to distinguish between the different treatments and ease the installation of the irrigation system, because each treatments plot was connected to the same mainline. When the water manipulation started, the coloured signs helped to ensure that all the plots from a certain treatment were irrigated correctly and simultaneously. The only difference between treatments was the amount of irrigation to be received during flowering; all other variables were kept constant. Soil classification of the site was conducted by a soil scientist (Grobler, 2015) from the Western Cape Department of Agriculture (Table 3.1). Three areas on the study site were identified where profile holes were dug. The piece of land has a moderate slope from west to east, and the analysis showed that the study site was fairly uniform with a sandy loam to sandy clay loam texture and a depth of 650 mm up to 950 mm, with a shale bank underneath (Table 3.1).

Table 3. 1: Soil classification of the study site

PROFILE HOLE	**1**	**2**	**3**
Soil Form	OAKLEAF	OAKLEAF	AUGRABIES
Diagnostic Horizon 1	Orthic A	Orthic A	Orthic A
Horizon 2	Neocutanic B	Neocutanic B	Neocutanic B
Horizon 3	Shale bank	Shale bank	Shale bank
Depth (mm) Horizon 1	350	200	300
Horizon 2	300 – 950	200 – 650	300 - 900
Horizon 3	950+	650+	900+
Clay % estimate Horizon 1	10 – 15	15 – 20	15
Horizon 2	20 – 25	30	25
Sand grade	Fine	Fine	Fine
Texture class Horizon 1	Sandy loam	Sandy loam	Sandy loam
Horizon 2	Sandy clay loam	Sandy clay loam	Sandy clay loam
Structure class Horizon 1	Weak, med, block	Structure less	Structure less
Horizon 2	Massive block	Coarse, strong block	Massive block
Consistency Horizon 1	Friable	Loose	Loose
Horizon 2	Slightly firm	Firm	Firm
Coarse fragments type and (%) Horizon 1	Gravel, angular 20-50%	Few, round, <20%	Gravel, angular, 20 – 50%
Horizon 2	Gravel, angular, >50%	Gravel, angular, 20 – 50%	Gravel, angular, < 20 %
Absorption (sec) Horizon 1	3	<1	<1
Horizon 2	1 to 5	7	1 to 5
Transition	Clear	Gradual	Gradual
Diagnostic Horizon 1	Orthic A	Orthic A	Orthic A
Horizon 2	Neocutanic B	Neocutanic B	Neocutanic B
Horizon 3	Shale bank	Shale bank	Shale bank

3.4 Land preparation

The land was cleared with a bush cutter towed behind a tractor, by chopping down the bushes and weeds that covered the land. This piece of land had not been cultivated for the previous five years and some natural vegetation had overgrown the land. The soil was very hard and dry, which resulted in the soil not being suitable for preparation. The top layer of the soil was loosened with a seven tines tiller to a depth of about 10 cm in a north-south direction, to prevent runoff of the irrigation water that was applied and to improve water absorption. A moveable irrigation system consisting of draglines with impact sprinklers were used to irrigate the piece of land. The entire land was irrigated by irrigating sections for six hours each, over a continuous period including through the nights, to ensure uniformity of soil moisture across the whole field. After the irrigation was completed, the land was allowed to rest for 48 hours in order to reach field water capacity, only thereafter did soil preparation begin.

The land was then ripped to a depth of 40-60 cm and a width of approximately 70-90 cm in an east-west direction and repeated with a cross rip at a 60° angle. The ripping of the soil was implemented to ensure that the soil was deeply loosened and to ensure that the soil was well drained. After the soil was ripped with a single ripper, a seven tines tiller was used to prepare a fine seed bed (Figure 3.3). The soil was then worked cross directions and all the bigger plant material that was left on the surface was gathered with a rake and removed by hand, this eased the planting process. Klein Karoo Seed Company recommended that fertiliser must be applied to the land just before planting. The fertiliser was spread by hand onto the land and worked in with the tiller.

Figure 3. 3: Tractor with tiller preparing seed bed and collection of plant residues.

Due to thunderstorms that occur during the summer months, a digger loader was used to dig a trench on three sides of the land to channel away flood water, to ensure that the trial would not be compromised by heavy rains. The digger loader was also used to dig three profile holes, as deep as possible, for soil analysis. Soil preparation was done according to Klein Karoo Seed company recommendations and a no leakage drip irrigation system was installed that was designed by Irricor, an irrigation company in Oudtshoorn. A standard fertiliser and spraying programme from the industry, which was recommended by Klein Karoo Seed representatives, was followed.

3.5 Planting material

Texas Grano onion was the cultivar that was planted, as this was historically the cultivar with the greatest pollination problems in the Little Karoo (Salmon, 2013). Klein Karoo Seed Company delivered 360 bags of onion bulbs, of which only 130 were used. The bags were emptied out onto the floor, the onions bulbs were sorted by size and all the rotten or small bulbs were discarded. Only the large, healthy onion bulbs were used for planting in the trial plots. Random samples of six crates of onion bulbs were taken and out of each crate a random sample of 20 onion bulbs were measured with a caliper to determine their size (Table 3.2). Bulb sizes ranged from 32 mm to 74 mm in width, with an average size of 48 mm (Table 3.2). All the bulbs smaller than 32 mm were removed to ensure uniformity of planting material. Six random crates of onion bulbs were weighed to determine the average weight per crate. Out of those six crates, a random selection of 100 bulbs were also weighed to establish the average weight per bulb. The average weight of the six crates was 17.3 kg. The average weight of 100 bulbs was found to be 7.23 kg which results in an average weight of 72.26 g per bulb that was planted.

Table 3. 2: Onion bulb size in millimetre (mm) for plant material comparison

Bulbs	Crate 1	Crate 2	Crate 3	Crate 4	Crate 5	Crate 6
1	38	42	50	57	43	41
2	44	42	53	78	47	45
3	55	52	48	43	50	48
4	41	64	49	56	45	46
5	46	48	42	56	49	52
6	45	58	52	45	61	51
7	49	48	45	46	55	38
8	40	46	62	46	52	39
9	44	44	52	44	50	47
10	43	42	48	54	56	52
11	52	50	46	45	53	54
12	49	32	46	44	50	47
13	36	36	50	54	61	48
14	46	42	52	45	60	43
15	49	48	46	44	51	47
16	46	58	42	51	52	44
17	52	58	45	50	50	45
18	42	38	48	74	45	42
19	36	36	44	47	49	41
20	52	58	45	41	46	41
Average bulb size per group:	45.25	47.1	48.25	51	51.25	45.55

3.6 Planting

The onion bulbs were planted on 11th April 2015. A metal frame was used to ensure uniformity throughout all the trial plots and to ensure that the spacing between rows was the same (Figure 3.4). After soil preparation was completed, the soil was levelled using a garden rake before the metal frame was placed on the ground and the trenches were made with a spade in which the bulbs were planted. The rows faced in an east to west direction to allow the wind to blow through the rows and expel the humid air built up between the rows that can cause diseases.

Figure 3. 4: A metal template that was used when making the planting trenches to ensure all the plots were the same.

As recommended by Klein Karoo Seed Company, the row spacing was 40 cm apart, with an opening of 60 cm between two rows for walking and with an inner row spacing of 15 cm between the bulbs (Figure 3.5). As soon as the planting furrows were made, a crate of bulbs was moved to the side of the plot and bulbs were planted 15 cm apart by hand. All the trial plots were planted before the bulbs were covered with soil, ensuring that the growing tip is above the soil surface. After establishment, the bulbs were irrigated once with the draglines system consisting of impact sprinklers for four hours per section over a continuous period including through the night, until the whole piece of land was irrigated to ensure the uniform development of roots. This was repeated 14 days later.

Figure 3. 5: The row spacing between bulbs for optimal growth.

3.7 Installation of the irrigation system

In mid-May, a non-leakage dripper system was installed. A concrete slab was constructed for the pump station and tank to stand on. A roof was erected over the pump to protect it from the rain. The pump station consisted of a 10000-litre tank, a small pump, a 130-micron filter, a 100-litre fertigation tank, and seven taps for the seven treatments. One main line trench was dug in the middle of the land, in which all seven treatments mainlines were positioned. From the main trench the lateral line trenches were dug to all the different treatments plots as subdivisions of the mainline. The established plots` irrigation lines were sub-divisions branching off from the main line. Every treatment was done individually by first installing the main line, for that particular treatment and then installing its lateral lines. The main and subdivision lines consisted of 25 mm black poly pipe class three; the lateral lines were connected to the main lines using T-joints and clamps. All the trenches were dug by hand and it took five general workers seven hours a day for four days to install the system. The inline, none-leakage dripper lines that were used had a dripper spacing of 30 mm apart which delivered one litre per hour. To install the dripper lines connectors, 9 mm holes were drilled into the lateral lines using a cordless drill with a 9 mm drill bit. The holes were drilled in the middle of the two rows, to ensure that the spacing from the dripper line to the onion bulbs was 20 cm (Figure 3.6).

Figure 3. 6: Drilling holes in sub-line to connect the dripper pipes.

The dripper line connection coupling was installed by hand into the lateral lines (Figure 3.7) and then the dripper lines were connected. The end of the dripper line that was connected to the coupling had to be heated slightly in order to fit it over the coupling on the lateral line. Each treatment plot was completed before moving to the next plot to prevent any confusion.

Figure 3. 7: Installing the dripper lines.

Wire clips (marked in yellow circles, Figure 3.8) were made by hand, and pressed over the dripper lines into the soil to keep dripper lines from shifting and ensure even spacing of drippers in relation to the position of the onion bulbs.

Figure 3. 8: Wire clips holding dripper lines in place.

The irrigation system was flushed before the line ends were closed, to ensure that any sand or other debris that could plot the drippers was removed, after which the system was tested to see if it was functioning correctly. All the drippers worked properly though it was realised later that some of the clamps had to be tightened.

3.8 Fertiliser management

A representative soil sample was taken of the study site and analysed. Post analysis, Klein Karoo Seed Company recommended that 2.3.4(30)+Zn fertiliser must be applied at planting time, at an application rate of 300 kg/ha. In order to comply with the deficiency of the soil, 300 kg of N.P.K fertiliser was spread over the entire study site which was one hectare and worked in with the tiller. After this process planting could commence. In addition, on the 4th of August 2015 one litre of Gip flow was applied through the dripper system, to counter the increase of salts in the soil as indicated by soil analysis report.

Table 3.3 shows the foliar fertiliser program that Klein Karoo Seed Company recommended be followed during the growing period of the onion plants. A total of 120 litres of water was needed to spray all the plots and insure a good coverage of all plants with a knapsack sprayer. The foliar fertiliser used consisted of an exceptional variety of different trace elements and minerals that the plants need (Table 3.3). Four applications were done during the growth period of the onion plants.

Table 3. 3: **Leaf fertiliser application products, the volume per 120 Litres of water and dates that it was sprayed.**

Leaf fertiliser name and compilation	Mix per 120 Litres	Dates of spraying:
Combi pro Nutech (fertiliser group 2) -Zinc 19g/kg (25g/l) -Iron 29g/kg (38g/l) -Copper 10/kg (13g/l) -Manganese 17g/kg (22g/l) -Boron 4g/kg (5g/l) -Molybdenum 136mg/kg (178mg/l) -Sulphur 41g/kg (54g/l -Amino-Acids 145g/kg (190g/l) -Nitrogen derived from amino acids 28g/kg (37g/l) -Di-amino charged SG=1.31 ±0.02	600ml	29 July 2015 22 August 2015 28 September 2015 13 October 2015
Voema Starter NT(fertiliser group 1)2:1:2 water soluble (40) -Nitrogen 107g/kg (160g/l) -Phosphorus 53g/kg (80g/l) -Potassium 108g/kg (162g/l) -Sulphur 13.5g/kg (20g/l) -Boron 1030mg/kg (1545mg/l) -Copper 210mg/kg (315mg/l) -Iron 507mg/kg (761mg/l) -Manganese 208mg/kg (312mg/l) -Molybdenum 55mg/kg (82mg/l) -Zinc 510mg/kg (765mg/l) -Cytokinis 13mg/kg (58mg/l) -Auxins 20mg/kg (91mg/l) -Amino acids 8g/kg (12g/l) -SG=1.43 ±0.02@ 20°C	1.2 litres	
Liquid Boron 9 (fertiliser group 2) Boron 100g/kg (130g/l) SG=1.30 ±0.02	360ml	

As recommended by Klein Karoo Seed Company, two cycles of KNO_3 (Ultraso K 25 kg) were applied to the trial plot by diluting it with water and administering it through the dripper system. The original recommendation from Klein Karoo was that 7.5 kg of KNO_3 be administered twice at three-week intervals. However, after the first application, Immik (2015) recommended that the second application should be divided, because it is better to provide smaller quantities of fertiliser over a longer period of time, rather than over-fertilising at one stage resulting in fertiliser leaching form the soil.

3.9 Spray program

A recommended spraying program from Klein Karoo Seed Company was followed. A total of 120 litres water was needed to cover the 56 plots properly. The amount of pesticide needed was re-calculated by dividing the pesticide amount by the original water amount and multiplying with needed amount. Representatives of Klein Karoo visited the trial plots on a regular basis to inspect the plants for any pests or diseases. The spraying program was administered using a knapsack sprayer. Masta 900 SP was only sprayed once for thrips, as the recommendation was only to spray it when there are more than 10 thrips per plant. The spray program consisted of insecticides, fungicides and bactericides covering a wide variety of pests and diseases. A buffer was also used to improve and enhance the absorption through the products.

3.10 Water application

Tensiometers and probes were used to gather soil moisture data. The probes were used as a backup and secondary data source to verify the tensiometer data. The probes provided graphs of the water applications and absorption for the whole season. A tensiometer was installed in each plot to measure the soil moisture. A probe was also installed in two random plots for each treatment. The correct installation and reading of the equipment is critical for accurate data, thus Immik (2015) provided training in the use of both. The tensiometers were calibrated (Figure 3.9) and the probe software was activated by the representative of Irricor. The representative also facilitated training on the correct method of reading and collection of data from tensiometers and probes. As water manipulation was the main focus of the study, it was recommended by Immik (2015) that dividing the treatments by tensiometer readings would give the most accurate means of managing the water application to each treatment. The seven categories of treatment (water application rates) are as shown in Table 3.4.

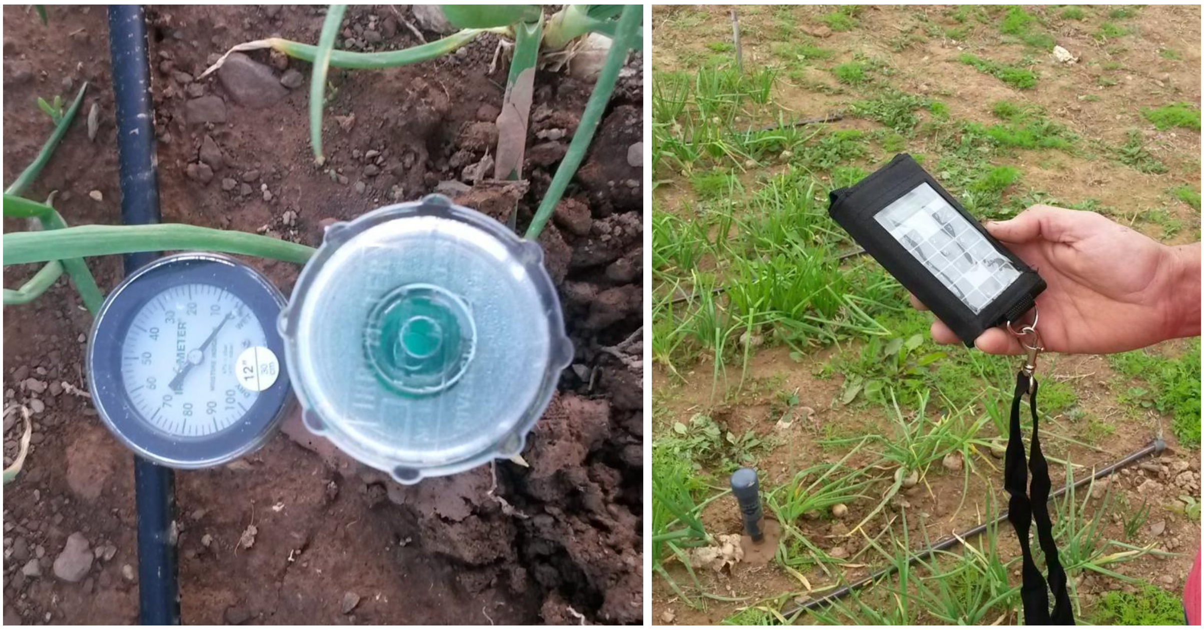

Figure 3. 9: Tensiometer calibration and setup of data collection software for Probes.

Table 3.4 Different treatments used in the study

Category	Tensiometer reading	Treatment
A (Control)	15 kPa	According to requirement
B	0 kPa	over irrigate
C	5 kPa	over irrigate
D	10 kPa	over irrigate
E	40 kPa	under irrigate
F	50 kPa	under irrigate
G	Never irrigate	under irrigate

The probes and tensiometers were installed with the assistance of a representative from Irricor, the irrigation company (Figure 3.10). The tensiometers were installed on the 19th of June 2015 in each treatment plot, after which data collection began. The probes were installed on the 20th of July 2015 in the second row to the south in randomly ordered plots.

Figure 3. 10: Installation of the Probes and Tensiometers.

The soil moisture was measured with a tensiometer in each plot. After planting, up to the end of July, the tensiometers were read weekly on Mondays at 7:00 am. From August, when flower stems started to grow, until harvesting on the 16^{th} of December the tensiometers were read daily at 7:00 am in the morning and tensiometer readings were recorded.

Prior to the first flowering, only a single tensiometer was used as a 'marker' and used for determining the irrigation schedule. As soon as the marker tensiometer reading was at 15 kPa all the plots would be simultaneously irrigated for two hours per cycle and then normally the tensiometer reading would go down to zero kPa, which indicated that sufficient irrigation was provided. When the onions began to flower on the 20^{th} of October 2015, the water manipulation started, with some plots over irrigated, some under irrigated and some plots were irrigated normally. These were the control plots. From each treatment category a specific plot was selected and the tensiometer monitored to determine the irrigation scheduling for the water manipulation. Readings were taken from these selected plots numerous times a day. These readings were only to indicate times during the day for irrigation and the data was not recorded. Irrigation cycle was always for two hours per treatment, due to higher temperature in the flowering period this resulted sometimes that tensiometer readings was still high and not at zero, thus a second or third cycle of irrigation was needed for the tensiometer to rich zero. This manipulation continued until the umbels were harvested.

All the treatments (category) tensiometer readings were the same at 15 kPa prior to blossom, during which times all plots and all treatments received the same water application of two hours. At the onset of blossom on the 20^{th} of October, the differential water manipulation started. This is illustrated in Figure 3.11, which shows the difference in the water application per treatments according to the tensiometer readings. Treatment A was the control plot and it was irrigated when the tensiometer was at 15 kPa, Plot B was irrigated as soon as the tensiometer lifted from 0 kPa, Plot C was irrigated when reading was 5 kPa, Plot D at 10 kPa, Plot E only at 30 kPa, Plot F only at 50 kPa and Plot G never received irrigation water during the flowering period.

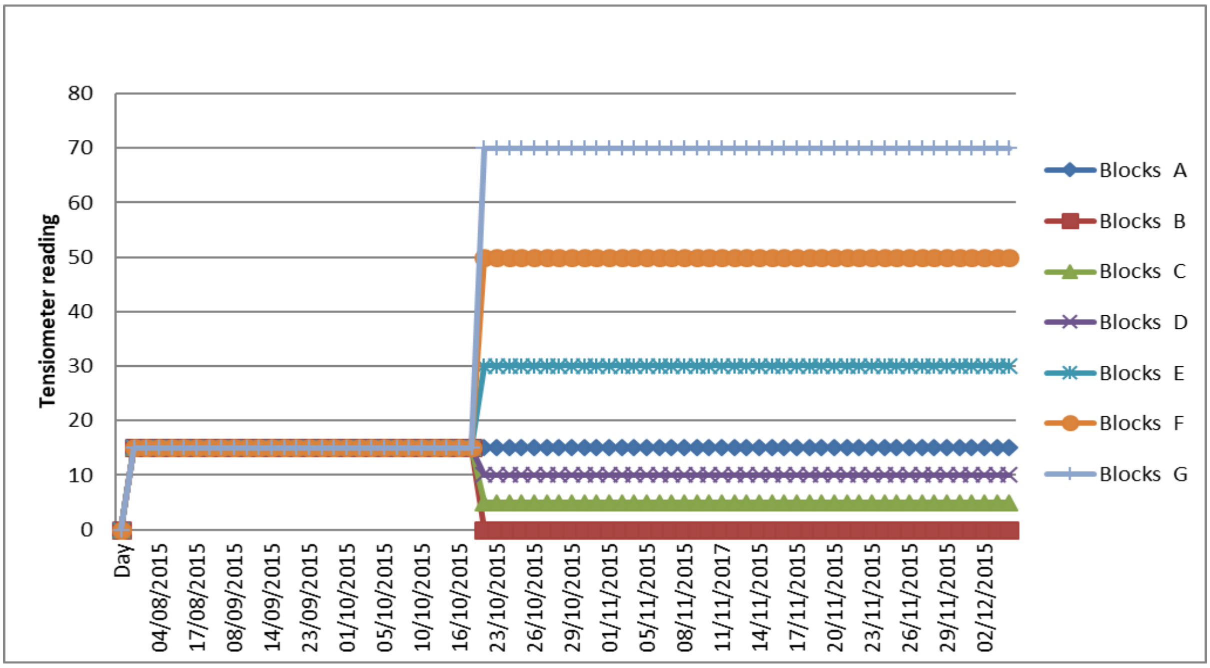

Figure 3. 11: Water application according to tensiometer readings, from the planting of the onions until the harvest of the seeds.

3.11 Continuous site management

During the entire period that the site was used, there were unscheduled activities that were conducted. The site had to be constantly managed to ensure that research could be conducted optimally and that there are no interferences with the data that could be obtained. After the site was identified and the use of the land approved by all role players, it was decided to improve and repair the existing fencing around the land. This was done as a preventative measure to keep out wild animals that might damage trials, for example porcupines that might dig up onion bulbs. It had also been found on farms in the area that porcupines will chew on the irrigation pipes in order to get to water. After unknown footprints were found in the trial plots it was decided to put locks on the gate to prevent vandalism and theft of irrigation equipment. Constant management of the site was necessary to ensure the faultless progress of the trials. The irrigation main lines and lateral lines were inspected frequently to ensure no leakages were present and that all drippers were functioning correctly. Onion plants were also inspected regularly to safeguard against any diseases or pest infestations. Tensiometers and probes were inspected and tensiometers were recalibrated when needed. On a daily basis, the water tank had to be refilled to make sure that water for irrigation was available at all times. Two months after planting it became clear that manual weed control had to be implemented. Thereafter weeds were controlled by pulling them out by hand, after irrigation on a monthly basis, to prevent any competition for the onion plants.

3.12 Bees for Pollination

At 10% flowering, bees were brought in for pollination, and positioned on the north side of the field. Nine colonies of bees were introduced, as this was the normal stocking rates indicated by Klein Karoo onion growers in the questionnaire of Salmon (2013). The colonies were placed 20 - 50 meter away from the first treatment plots, on one side of the field. Care was taken to ensure that the colonies that were used for pollination met the Western Cape pollination standards, as Salmon (2013) questionnaire found that 12 out of the 58 producers believed that ‘bad bees’, meaning sub-standard colonies, were the reason for poor pollination.

The hives brought in were checked and evaluated according to the Western Cape Pollination standards, namely:

- The minimum colony strength is the equivalent of three frames with brood with an average 75% of cells filled with brood in all stages.
- Average colony strength must be at least the equivalent of four frames with brood with an average of 75% of cells filled with brood in all stages.

To assist in the navigation of the foraging honey bees in the field, and to potentially allow the foragers to distinguish the different treatment plots, various coloured signs and objects were distributed around the field (Johannsmeier, 2001; Figure 3.12 and Figure 3.13). Wild flowers could be seen flowering next the treatment plots (Figure 3.12), testimony to the good rainfall (23 mm) received during the onion flowering period.

Figure 3. 12: Signage erected to help bees navigate between treatment plots.

Figure 3. 13: Signposts to help bees to navigate between plots, and flowering wild flowers adjacent to the onion plots.

3.13 Data collection

Honey bee colony strength was determined using standard methods (Deleplane et al., 2013a), namely the counting of the number of frames with bees and the number of frames with brood on the 28th of October, and again on the 5th of December. Honey production was also measured through the number of frames that were harvested in the period. Bee counts from the hive were also measured by standing at the side of the hive and counting all the bees that were leaving the hive for 60 seconds, to measure the strength of the hive as well as the level of foraging activity (Deleplane et al., 2013a).

The foraging behaviour of honey bees and other insects were monitored daily in each of the treatment plots, again using standard methods (Delaplane et al., 2013b). Plot counting began at 8:00 am in the morning, and again at 15:00 pm in the afternoon. Plots were counted in a random sequence in each observation period. Counts of honey bees and other insects in each plot were made by standing ±1.5 m from the plot and looking down the rows, counting all visible foraging insects, and then slowly circling the plots (thus a point-in-time, snapshot count of the honey bee and other insect activity in each plot). Daily weather data was collected on an hourly bases, with a data-logger that was situated in the middle of the field, to determine how temperature and humidity influenced bee and other insect activities.

3.14 Harvesting and drying of umbels

When the onion umbels started to show signs of drying out and the seeds became visible to the eye from above, the onion umbels were harvested (16th of December). The onion umbels were cut with clippers (Figure 3.14) and all the umbels from a specific plot were packed into crates. The umbels of every plot were kept separate and each crate was numbered clearly with the number of the plot to ensure no confusion during transport.

The onion umbels were grouped by trial plot number and stored in a closed shed for storage and drying. During the first week, umbels were turned once a day and there after every second day until thoroughly dried. Turning was conducted to prevent the umbels from rotting or from mould forming between umbels. The umbels were packed into clean 50 kg bags for transporting. Each bag had a sheet of paper with the trial plot number on it, placed inside the bag as well as a clearly marked label with details attached to the outside. These measures were put in place to ensure no confusion will occur when the seeds are processed at Klein Karoo. The bags were loaded on a pickup truck and delivered to Klein Karoo`s facilities in Oudtshoorn. There they have the necessary machines for rubbing out the seed out of the umbels and cleaning it, and determining the amount of seed produced in each treatment plot.

Figure 3. 14: The harvesting of Onion umbels.

The cleaned seed of each plot was weighed separately before the seed was sent to the Klein Karoo laboratory to test the seed germination percentage of each plot. They test for normal seed development, abnormal seed, which is seed with growth deficiencies and did not produce a healthy plant, and dead seed which is seed that did not germinate at all.

3.15 Statistical analysis

Levene's test for homogeneity of variance was performed to test if the treatment variability in observations was of comparable magnitude (Levene, 1960). Shapiro-Wilk test was performed on the standardised residuals from the model to test for normality (Shapiro and Wilk, 1965). The data were continuous and subjected to analysis of variance (ANOVA) using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott and Longnecker, 2001) this was also done for this study.

Pearson's Product Moment Correlation Coefficients ($p \leq 0.05$) were used to test the relationship between variables (correlations), using the PROC CORR of SAS Software Version 9.2; SAS Institute Inc, Cary, USA. Principal component analysis (PCA) was conducted to investigate the relationship between treatments and variables using XYSTAT (Version 2015, 1.03, 15485, Addinsoft, Paris). PCA is a multivariate technique statistical method to identify data patterns as well as similarities and dissimilarities among observations and variables. It uses orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. The number of principal components is less than or equal to the number of original variables. This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it be orthogonal to (i.e. uncorrelated with) the preceding components.

Principal components are guaranteed to be independent if the data set is jointly normally distributed. PCA is sensitive to the relative scaling of the original variables. Rencher (2002) recommended that a correlation matrix should be used to standardise the data. PCA is the simplest of the true eigenvector-based multivariate analyses. Often, its operation can be thought of as revealing the internal structure of the data in a way that best explains the variance in the data. If a multivariate dataset is visualised as a set of coordinates in a high-dimensional data space, PCA can supply the user with a lower-dimensional picture, a "shadow" of this object when viewed from its most informative viewpoint. This is done by using only the first few principal components so that the dimensionality of the transformed data is reduced.

CHAPTER FOUR: THE EFFECT OF WATER APPLICATION DURING ONION FLOWERING ON INSECT VISITATION AND SEED PRODUCTION

4.1 Application of water to treatment groups

The Texas Grano onions used in the trial were planted on the 11th of April 2015 and thereafter were given uniform water application throughout their growing phase until the 20th of October when they began to flower, and they were then watered according to tensiometer readings from the indicator plots. The actual amount of water applied to each treatment during the 36 days from the start of differentiated irrigation (20th of October) until the harvesting of the onion seeds (16th of December) are indicated in Appendix 4.1 and in Table 4.1. Water application may be indicated either by the total volume of water applied to a plot, or by the number of hours during which a plot was watered. The latter is chosen for simplicity reasons, with the maximum hours that a plot could be watered being 7 hours a day or 252 hours for the 36-day period. Each of the seven treatments had eight plots and treatment B received the most water (232 hours of water application), followed by treatment C (100 hours of water application). Treatment G received no water application for the duration of the flowering period. Different treatments received significantly different levels of water application (Table 4.1; Students t-test, $p \leq 0.05$).

Table 4. 1: **Water application for the different treatment groups**

Treatments	Water application (hours irrigated)	t Grouping
A	20.00	c
B	232.00	a
C	100.00	b
D	20.00	c
E	18.00	d
F	18.00	d
G	0.00	e

4.2 Honey bee foraging activity

The number of foraging honey bees was monitored twice daily in each of the treatment plots, using standard methods (Delaplane et al., 2013b). Honey bee counting in plots began at 08:00 in the morning, and again at 15:00 in the afternoon. Plots were counted in a random sequence in each observation period. The complete honey foraging data is presented in Appendix 4.2, and the average honey bee foraging activity for the various treatments are presented in Table 4.2 and Figure 4.1. It is apparent that there was a great deal more honey bee foraging activity in the afternoon, in comparison to the morning, with afternoon counts being three times greater than morning counts. This has previously been reported in onions (Karuppaiah et al., 2018), and indicates that nectar levels in onion umbels are greater in the afternoon, indicating afternoon secretion of nectar in onions (Nicolson and Thornburg, 2007).

There were no significant differences between treatments for morning bee counts (Table 4.2; p=0.74), although it can be noted that the foraging activity was lowest in Treatment group G, which received no irrigation, and that foraging levels are the highest in treatment group B, which received maximum irrigation. With regard to afternoon honey bee foraging levels, however, there were highly significant differences (p=0.004) between the different treatments (Table 4.2). Once again, the treatment group G had the lowest level of foraging activity, and treatment B had the highest level, together with treatment C. While there were significant differences between treatments groups when comparing bee counts PM, these are not consistent with the water application levels for the groups, and it must be concluded that there is no discernible relationship between water application levels during the flowering period and afternoon honey bee activity in Texas Grano onions.

Table 4. 2: Honey bee foraging activity across the water application treatments, indicating morning (AM) activity, afternoon activity (PM) and overall activity.

Treatments	Bee Count AM	Bee Count PM	Total Bee Count
A	306.88 ± 35.25 a	907.50 ± 18.54 b & c	1214.38 ± 47.66 a & b
B	350.25 ± 43.21 a	1018.88 ± 49.54 a	1369.13 ± 84.71 a
C	358.25 ± 43.28 a	963.88 ± 35.51 a & b	1322.13 ± 74.49 a
D	321.63 ± 29.26 a	902.00 ± 20.61 b & c	1223.63 ± 48.85 a & b
E	346.88 ± 31.41 a	884.25 ± 28.05 b & c	1231.13 ± 57.43 a & b
F	348.38 ± 40.22 a	877.50 ± 24.92 c	1225.88 ± 58.19 a & b
G	282.13 ± 30.44 a	852.00 ± 14.88 c	1134.13 ± 39.46 b
LSD (p=0.05)	103.98	83.98	171.92
Means depicted by the same letter do not differ at p=0.05			

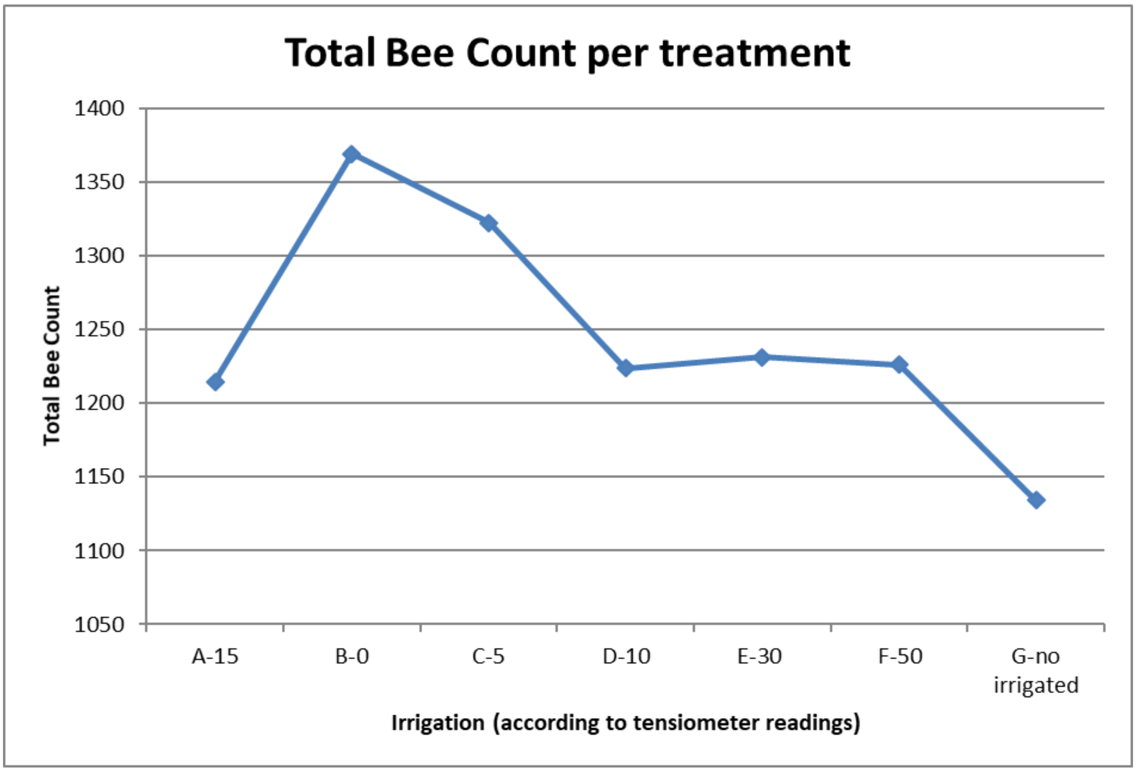

Figure 4.1: Overall honey bee foraging activity across water application treatment groups.

The same pattern was repeated when overall honey bee foraging activity on the onions was investigated (Table 4.2). There were significant differences between treatment groups, but once again these did not correspond with the water application levels of the groups, although group G (no irrigation) again had the lowest levels of overall honey foraging activity. The best that can be said in regard to the relationship between water application rates during onion flowering and its effect on honey bee foraging activity is that the results are indicative and suggest that a positive relationship with irrigation levels and foraging activity, particularly with respect to afternoon foraging.

When looking at the overall linear correlation between water applied to treatment groups, no significant difference was found for honey bee count AM ($r=0.13$, $p=0.32$, $n=56$). Water application had a highly significant correlation with honey bee count PM ($r=0.54$, $p<0.001$, $n=56$) and total honey bee count ($r=0.37$, $p=0.006$, $n=56$). These data show that water application has no influence on the bee visits to onion umbels in the morning, but that water application has significant influence on bee counts in the afternoon.

A more thorough investigation of a possible linear relationship (correlation) between the water application with honey bee counts at AM, PM and overall was conducted for all days that data was collected ($n=36$). This showed that there was no direct correlation between water application treatments and AM bee counts ($r=0.03$, $p=0.66$, $n=196$) or PM bee counts ($r=0.14$, $p=0.13$, $n=119$). These analyses suggest that there is no significant difference in honey bee visitations to onion umbels AM or PM, irrespective of water application. The relationship between water application and total bee counts (regardless the treatments), however, showed that there was a positive significant correlation (r=0.14, p=0.04, n=203). This confirms the previous conclusion that there is a positive trend between water application rates and honey bee foraging activity, even if the relationship is not highly significant.

When the linear relationship between water application and honey bee count AM, PM and overall was interrogated by looking at the interaction per day, then there were only a few days that showed significant correlations for honey bee count AM. These were day 10 (09 Nov) (r=0.75 p=0.05), day 12 (11 Nov) (r=0.76 p=0.05), day 31 (30 Nov) (r=0.91 p=0.004), and day 35 (04 Dec) (r=0.91 p=0.005). When the relationship between honey bee count PM and water application is considered on an individual day basis, again only a few days revealed a significant relationship. These are day 18 (17 Nov) (r=0.89 p=0.008), day 19 (18 Nov) (r=0.84 p=0.02), day 20 (19 Nov) (r=0.87 p=0.01), day 32 (01 Dec) (r=0.90 p=0.006), and day 34 (03 Dec) (r=0.92 p=0.004). A similar pattern was repeated with the interaction between water application treatments and total bee count with only a few days being positively correlated: day 18 (17 Nov) (r=0.88 p=0.01), day 19 (18 Nov) (r=0.84 p=0.02), day 20 (19 Nov) (r=0.81 p=0.03), day 31 (30 Nov) (r=0.91 p=0.004), day 32 (1 Dec) (r=0.88 p=0.008) and day 35 (04 Dec) (r=0.91 p=0.005).

These results show that water application has a slight influence on the bee activity on onion umbels, but additional research is needed. Gillespie et al. (2015) found that the relationship between nectar production and soil moisture content was non-linear, with the highest nectar production occurring at moderate moisture levels, so it is perhaps not surprising that there is not a clear and obvious relationship between water application levels and honey bee foraging activity. The linear correlation between water applied per treatment showed that only treatment A showed a positive strong significant relationship between water application comparing to honey bee counts PM (r=0.51, p=0.04, and n=17) and total honey bee counts (r=0.40, p=0.03, and n=29). Honey bee count AM for treatment A, as well as all the other treatments B, C, D, E, F and G`s bee counts for AM, PM and total, show no significant correlation.

4.3 Insect foraging activity

Insects other than honey bees were also found to be foraging on the flowering onions, as was expected. The actual numbers of the different types of insects were not recorded, just the total numbers of non-Apis visitors to the onion flowers. These were consistent with the insect visitors to onions in the Klein Karoo reported by Brand (2013), with non-Apis bees, ladybirds, flies, beetles, butterflies and wasps being the most represented insect groups in her study. Other insect visitors to the onions were recorded during the same morning and afternoon observation periods of the 56 treatment plots in which honey bee visitors were recorded, and the numbers of other insect visitors is recorded in Appendix 2 and Table 4.3.

The numbers of non-Apis visitors was significantly less than that of honey bee visitors, with total numbers being only a little more than 10% of the honey bee visitors. This corresponds with the numerous and global accounts of honey bees being the primary visitor and pollinator of flowering onions (Nye et al., 1973; Howlett et al., 2005; Brand, 2013; Karuppaiah et al., 2018; Cook et al., 2020), even in a region as species diverse and bio-rich as the Klein Karoo. It is also apparent that the morning and afternoon foraging activity levels found for other insects on the onions did not reflect those of the honey bee foraging, where afternoon foraging activity was significantly more than morning foraging activity. For insects other than honey bees, there was more activity in the morning than there was in afternoon (Table 4.3). These results are surprising if there are higher afternoon nectar levels, as must be expected due to the increased honey bee activity, and probably reflect that active competition of honey bees in afternoon limits the numbers of other insects present.

Table 4. 3: Insect other than honey bees foraging activity across the water application treatments, indicating morning (AM) activity, afternoon activity (PM) and overall activity.

Treatments	Insect count AM	Insect count PM	Total Insect count
A	94.88 ± 8.54 a	69.13 ± 5.82 a	164.00 ± 11.45 a
B	88.50 ± 7.25 a	66.63 ± 8.69 a	155.13 ± 15.29 a
C	85.13 ± 4.34 a	73.13 ± 4.60 a	158.25 ± 7.76 a
D	83.75 ± 7.43 a	64.50 ± 4.96 a	148.25 ± 9.87 a
E	91.75 ± 10.88 a	64.00 ± 3.53 a	155.75 ± 13.81 a
F	86.13 ± 5.31 a	62.25 ± 4.91 a	148.38 ± 8.57 a
G	87.75 ± 6.79 a	66.13 ± 4.01 a	153.88 ± 9.79 a
LSD (p=0.05)	21.28	15.48	31.91
Means depicted by the same letter do not differ at p=0.05			

The non-honey bee insect foraging activity across the water application groups also does not follow the pattern of the honey bee foraging activity, where there limited correlations indicating some relationship between water application (irrigation) and honey foraging activity. In the case of the foraging activity of other insects, there was no relationship between water application and insect visitation across any of the treatment groups (Table 4.3; Figure 4.2). This holds true for the interaction between treatments groups and AM insect counts (p=0.95), PM insect counts (p=0.85) and total insect counts (p=0.96). This means that no difference between treatments occurs for insect count.

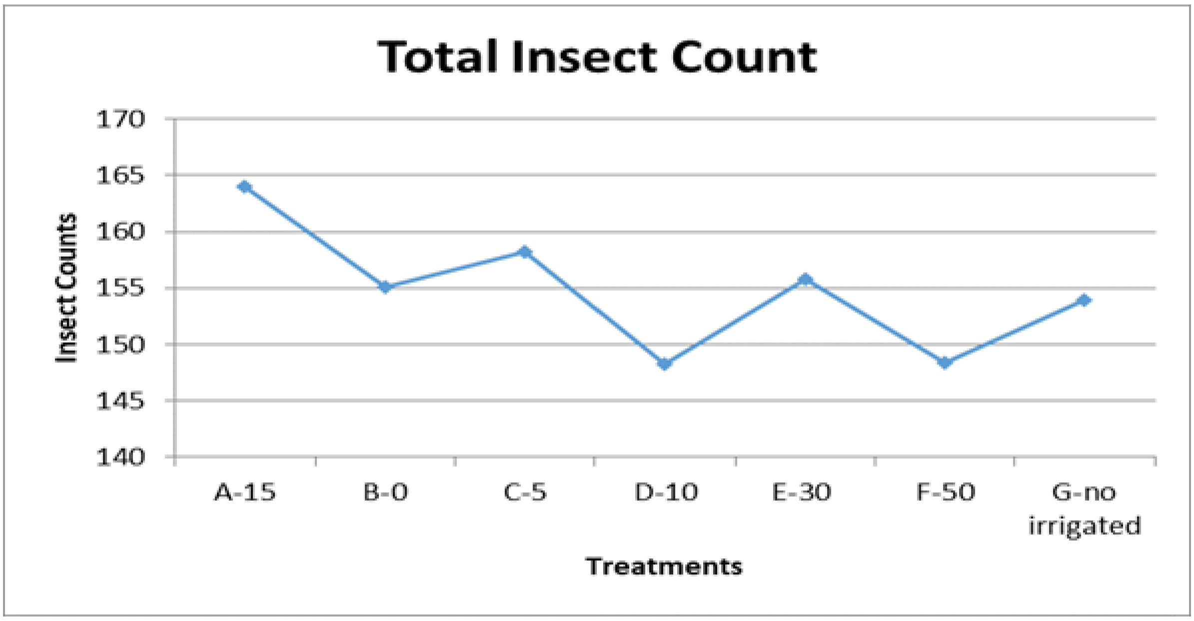

Figure 4. 2: Overall insect other than honey bees foraging activity across water application treatment groups

When the relationship between water application and non-Apis foraging activity were examined on an individual day basis, no noteworthy correlations could be found. Across the total days that data was collected (n=36), irrespective of treatments, there were no significant overall correlations between water application and insect counts AM, PM or total insect count (Insect count AM r=-0.002m p=0.98, n=182; insect count PM r=0.006,p=0.95,n=105; total insect count r=0.06, p=0.40,n=182). This reflects that the more or less water that was applied to the onions had no impact on non-honey bee insect visitations to onion umbels. When comparing the treatment overall correlation between water application and insect counts AM, PM and total, it indicates that insect counts AM (r=-0.02, p=0.90); PM (r=0.071, p=0.60); total insect counts (r=0.02, p=0.87) has no connection. There is no correlation between water application and insect counts AM, PM and total.

4.4 Water application and seed production

The onion umbels from all of the 56 treatment plots were harvested at the end of the flowering period (16th December), and the seeds from the umbels harvested, dried and assessed. This assessment involved weighing the harvested seed (yield), determining the germination rate, the percentage dead seed, and the percentage abnormal seed. These raw data are presented in Appendix 2, and the relationship between the seed criteria and the various treatment groups in illustrated in Table 4.4 and Figure 4.3.

With respect to seed yield, there is no significant difference between the water application treatments (Table 4.4; p=0.20), although the t-test does show a difference between treatments (Table 4.1). As shown in Table 4.4 below, the seed yield was grouped into three different groups. The first group with the highest seed yield was treatment B marked with the letter a. The second group consisted of treatments A, C, D, and G marked with the letter a & b. Treatment E and F which had the lowest seed yield mark with letter b. The overall lack of significance indicates, however, that the differences between the treatments is only marginally significant (Figure 4.3). There are no significant relationships between water application treatments and any of the percentage of dead seed, the percentage of abnormal seed, or the percentage of seed germination (Table 4.4).

Table 4. 4: Effects of water application treatment on seed yield, seed germination, dead seed and abnormal seed.

Treatments	Seed yield	P. Germination	P. Abnormal	P. Dead
A	1.32 ± 0.08 a & b	92.63 ±0.84 a	4.00 ±0.57 a	3.86 ±0.40 a
B	1.40 ± 0.10 a	91.38 ±1.07 a	4.75 ±0.56 a	3.88 ±0.77 a
C	1.34 ± 0.08 a & b	91.13 ±1.01 a	5.00 ±0.53 a	3.88 ±0.61 a
D	1.24 ± 0.04 a & b	92.75 ±0.84 a	4.88 ±0.79 a	2.71 ±0.18 a
E	1.16 ± 0.06 b	91.00 ±1.16 a	5.00 ±0.60 a	4.00 ±0.89 a
F	1.19 ± 0.08 b	93.38 ±0.42 a	3.63 ±0.26 a	3.43 ±0.43 a
G	1.23 ± 0.04 a & b	92.38 ±0.98 a	4.38 ±0.53 a	3.25 ±0.53 a
LSD (p=0.05)	0.20	2.65	1.60	7.54
Means depicted by the same letter do not differ at p=0.05				

If the water application and seed yield are compared, throughout all the treatment plots (n=56), it shows a slightly positive(r=0.31) and highly significant (p=0.02) relationship. This indicates that the more water applied, the higher the seed yield or the less water applied, the less the seed yield. Increasing water application therefore has a slight positive significant impact on the seed yield of onions, but insufficient data are available to look at individual treatments and more research needs to be done regards water application and seed yield. When looking at the overall linear correlation between water application and seed quality, which is measured by seed germination, it shows no significant correlation (r=-0.17, p=0.21). This means that water application has no influence on the quality (germination) of onion seeds.

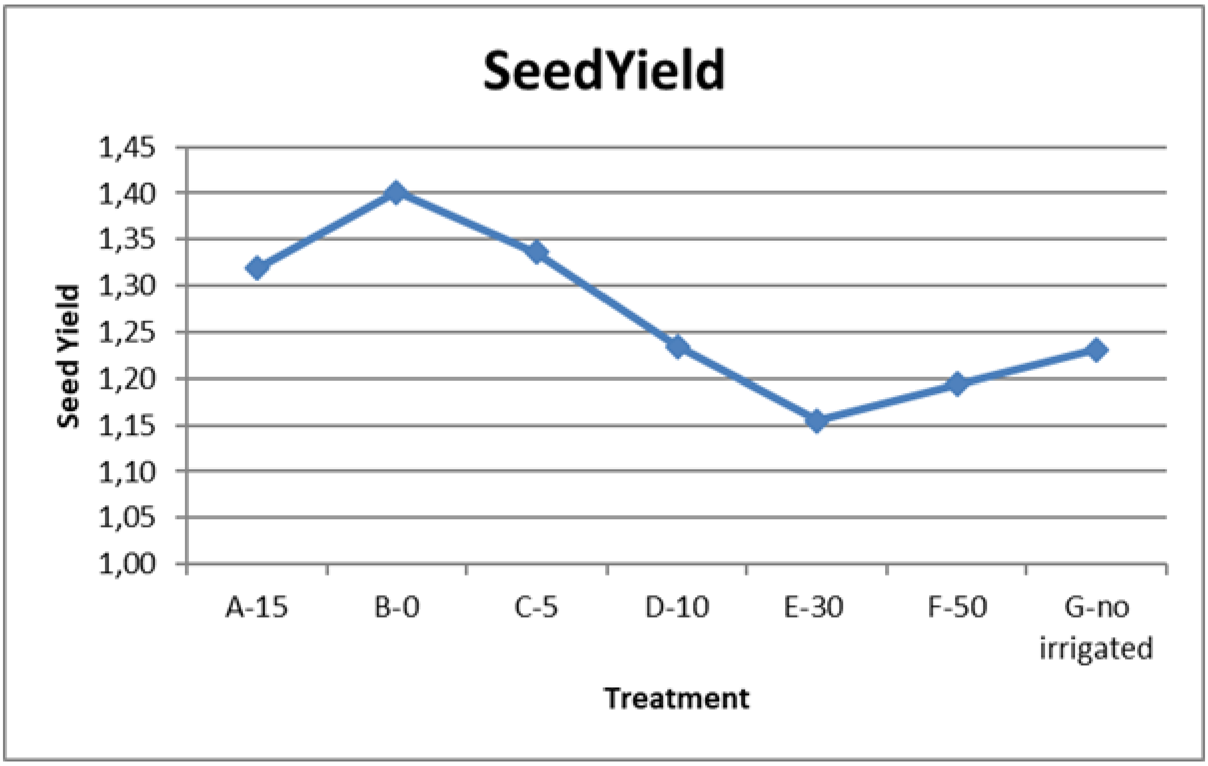

Figure 4. 3: Interaction between water application treatment and seed yield, seed germination, dead seed and abnormal seed

Investigating the relationship between honey bee activity and other insect activity, and seed yield, also delivers interesting and valuable results. The overall relationship between total honey bee counts of all the treatments (n=56), and seed yield has a highly significant positive correlation(r=0.70, p>0.0001), demonstrating the importance of honey bees in the pollination of onions and in the production of onion seed. Multiple studies have shown that more honey bees mean more onion seed (McGregor, 1976; Hagler and Waller, 1991; Soto et al., 2013; Gillespie et al., 2015; Soto et al., 2018). When loo king the correlation between total bee count and seed yield per treatment, treatment A (r=0.05, p=0.91) and treatment G (r=035, p=0.40) do not have any significant correlation. Treatment B (r=0.76, p=0.03) and E (r=0.79, p=0.02) has a strong positive significant correlation. There was positive significant correlation between treatments C (r=0.96, p=0.0001), D (r=0.84, p=0.009) and

F(r=0.92, p=0.001) and seed yield. This shows that as the bee counts increased, the seed yield also increased and that when the bee counts declined, the seed yield also declined. This demonstrates that bees are important for pollination of onions and play a crucial role in the pollination of onion seeds, as numerous studies have shown.

An interesting finding is that when looking at the overall correlation between total bee counts and seed quality that is given as a percentage of germination, the data shows a slight (r=-0.34) significant (p=0.01) negative correlation. This means that as the bee counts increase, the seed quality decreases. This confirms a previous finding by Parker (1982) which revealed that the percentage of aborted seed was significantly higher in onion flowers pollinated by honey bees compared to similar flowers pollinated by Halictus bees, and that perhaps the deposition of non-viable pollen by honey bees is one of the causes of seed abortion commonly found in hybrid onion seed production.

The overall correlation between total insect count and seed yield (n=56) shows a strong (r=0.41) significant (p=0.002) positive correlation, which means that if the insect visits increase, the seed yield increases also, and if the insect visits decrease, the seed yield also decreases. This indicates that other insects are also important for onion seed pollination, but as non-Apis insects are very difficult to manage for pollination, this may have limited economic value to growers. Nonetheless, growers should be encouraged to support natural vegetation around their fields and to plant verges to support the natural insect populations, as this will improve crop pollination and seed yield.

CHAPTER FIVE: FACTORS IMPACTING ON HONEY BEE POLLINATION OF ONION SEED

5.1 Introduction

The principal focus of this study was to assess the impact of differential water application regimes during the flowering period of onions on the attractiveness of the onion flowers to honey bees and other insect visitors, and onion seed production resulting from this insect pollination. It was found that different water regimes during the flowering period had no effect on the level of attraction to insects other than honey bees, and a limited effect on the attraction of honey bees (Chapter Four). Findings of the study indicated that water application had a highly significant correlation with honey bee count in the afternoon ($r=0.54$, $p<0.001$, $n=56$) and the total honey bee count ($r=0.37$, $p=0.006$, $n=56$), but that water application has no influence on the bee visits to onion umbels in the morning ($r=0.13$, $p=0.32$, $n=56$).

In addition, and notwithstanding the limited relationship between irrigation water levels during flowering and honey bees' activity, it was found that there was a highly significant positive correlation between honey bee foraging activity and seed production in onions ($r=0.70$, $p<0.0001$), demonstrating the importance of honey bees in the pollination of onions and in the production of onion seed. This result was not unexpected as multiple studies have shown how crucial honey bees are in onion seed production (McGregor, 1976; Hagler and Waller, 1991; Soto et al., 2013; Gillespie et al., 2015; Soto et al., 2018), and confirm honey bees as the primary pollination agents of onions (Nye et al., 1973; Howlett et al., 2005; Brand, 2013; Karuppaiah et al., 2018; Cook et al., 2020).

In addition to water application rates during flowering, a number of other factors were found to influence honey bee foraging activity on onions, and hence seed production in onions. These factors were temperature, rainfall, relative humidity, level of foraging activity at the hive entrances and the foraging activity of other insects. The raw data for these factors, for the duration of the 36 days of honey bee foraging activity, are presented in Appendices 5.1 and 5.2. Pearson's Product Moment Correlation Coefficients ($p \leq 0.05$) were used to test the relationship between these factors and honey bee foraging activity and onion seed production, using the PROC CORR of SAS Software Version 9.2 (SAS Institute Inc., Gary, NC, USA). These results are presented in Table 5.1 (overall honey bee foraging activity), Table 5.2 (morning honey bee foraging activity) and Table 5.3 (afternoon honey bee foraging activity).

Table 5. 1: Pearson's Product Momentum Correlation Coefficients ($p \leq 0.05$) were used to test the relationship between overall honey bee foraging activity and insect foraging activity, temperature, humidity, and foraging activity at the hives.

	Overall honey bee count	**Insect count**	**Temperature**	**Humidity**	**Bee count at hives**
Overall honey bee count	r = 1.0000 n = 45	r = -0,308 p = 0,050 n = 41	r = 0.51484 p = 0,0003 n = 45	r = -0,6705 p = 0,0001 n = 45	r = 0.8272 p = 0,0017 n = 11
Insect count	r = -0,308 p = 0,050 n = 41	r = 1.0000 n = 41	r = 0.01545 p = 0,9236 n = 41	r = -0,0091 p = 0,9548 n = 41	r = -0,3340 p = 0,4641 n = 7
Temperature	r = 0.51384 p = 0,0003 n = 45	r = 0.01545 p = 0,9236 n = 41	r = 1.0000 n = 51	r = 86852 p = 0,0001 n = 51	r = 0.8668 p = 0.0006 n = 11
Humidity	r = -0,6705 p = 0,0001 n = 45	r = -0.0091 p = 0,9548 n = 41	r = 86852 p = 0,0001 n = 51	r = 1.0000 n = 51	r = -0.7198 p = 0,0125 n = 11
Bee count at hives	r = 0.82719 p = 0,0017 n = 11	r = -0,3340 p = 0,4641 n = 7	r = 0.8668 p = 0.0006 n = 11	r = -0.7198 p = 0,0125 n = 11	r = 1.0000 n = 11

Table 5. 2: Pearson's Product Momentum Correlation Coefficients ($p \leq 0.05$) were used to test the relationship between morning (AM) honey bee foraging activity and insect foraging activity, temperature, humidity, and foraging activity at the hives.

	Overall honey bee count	**Insect count**	**Temperature**	**Humidity**	**Bee count at hives**
Overall honey bee count	r = 1.0000 n = 26	r = -0.14927 p = 0.4863 n = 24	r = 0.41209 p = 0.0364 n = 26	r=-0.52822 p = 0.0055 n = 26	r = 0.86522 p = 0.0260 n = 6
Insect count	r = -0.14927 p = 0.4863 n = 24	r = 1.0000 n = 24	r = -0.21671 p = 0.3091 n = 24	r=-0.00019 p = 0.9993 n = 24	r = -0.50412 p = 0.4959 n = 4
Temperature	r = 0.41209 p = 0,0364 n = 26	r =-0.21671 p = 0.3091 n = 24	r = 1.0000 n = 30	r=-0.69938 p = <.0001 n = 30	r = 0.62873 p = 0.1812 n = 6
Humidity	r = -0.52822 p = 0.0055 n = 26	r = -0.00019 p = 0,9993 n = 24	r = -0.69938 p = <.0001 n = 30	r = 1.0000 n = 30	r = -0.36108 p = 0.4819 n = 6
Bee count at hives	r = 0.86522 p = 0.0260 n = 6	r = -0.50412 p = 0.4959 n = 4	r = 0.62873 p = 0.1812 n = 6	r=-0.36108 p = 0.4819 n = 6	r = 1.00000 n = 6

Table 5. 3: Pearson's Product Momentum Correlation Coefficients ($p \leq 0.05$) were used to test the relationship between afternoon (PM) honey bee foraging activity and insect foraging activity, temperature, humidity, and foraging activity at the hives.

	Overall honey bee count	Insect count	Temperature	Humidity	Bee count at hives
Overall honey bee count	r = 1.0000 n = 19	r = -0.70423 p = 0.0016 n = 17	r = 0.02439 p = 0.9211 n = 19	r = -0.32309 p = 0.1773 n = 19	r = 0.75735 p = 0.1381 n = 5
Insect count	r = -0.70423 p = 0.0016 n = 17	r = 1.0000 n = 17	r = 0.09744 p = 0.7099 n = 17	r = 0.14433 p = 0.5805 n = 17	r = -0.79346 p = 0.4166 n = 3
Temperature	r = 0.02439 p = 0.9211 n = 19	r = 0.09744 p = 0.7099 n = 17	r = 1.0000 n = 21	r = -0.82795 p = <.0001 n = 21	r = 0.80034 p = 0.1038 n = 5
Humidity	r = -0.32309 p = 0.1773 n = 19	r = 0.14433 p = 0.5805 n = 17	r = -0.82795 p = <.0001 n = 21	r = 1.0000 n = 51	r = -0.52791 p = 0.3605 n = 5
Bee count at hives	r =0.75735 p = 0.1381 n = 5	r = -0.79346 p = 0.4166 n = 3	r = 0.80034 p = 0.1038 n = 5	r = -0.52791 p = 0.3605 n = 5	r = 1.0000 n = 5

5.2 Relationship between honey bee foraging activity and temperature

There were significant positive relationship between bee counts on plots and temperature (the linear relationship), r=0.51 with p=0.0003 and n=45 (Table 5.1). As temperatures increased a positive increment in bee counts also followed. The relationship between bee count on trial plots and temperature in the early morning (AM) also had a significant (r=0.41 p= 0.04) positive correlation (Table 5.2), but surprisingly, the relationship between bee counts and temperature in the afternoon (PM) showed no substantial correlation (r=0.024 p=0.92, Table 5.3). The positive linear correlation between bee counts and temperature in the morning clearly demonstrates that very low temperatures will restrain crops visits and bees will remain in their hives until temperatures are favourable for them to work.

These data are not surprising as it is widely accepted that temperature is a fundamental determinant of honey bee foraging activity (Abrol, 2010) and typically the most indicative factor in honey bee foraging behaviour (McGregor, 1976). The lack of any positive correlation in the afternoon foraging activity suggests that, in the Klein Karoo region in summer, honey bees are seldom constrained by temperatures in the afternoon, and hence there is no temperature-related relationship. As maximum honey bee foraging typically occurs between 28-32 °C (Kavitha and Reddy 2018), temperatures common during the afternoon in the Klein Karoo region during onion pollination, this explains why there is no positive relationship between temperature and foraging activity in the afternoon (Table 5.3). Due to the typically warm weather, honey bee activity will normally be at a maximum in the afternoon, as witnessed by the foraging activity in the afternoons relative to the mornings (Table 5.3). Globally, there is more honey bee foraging in the afternoons than in the morning

(Karuppaiah et al., 2018), probably because of nectar secretion in the afternoon, but also because of favourable temperature

5.3 Relationship between honey bee foraging activity and humidity

Humidity plays a significant role overall in bee visits to onion umbels. It was found that overall bee counts on trial plots and humidity were highly significantly negatively correlated with each other (r=-0.67 with p<.0001 and n=45, Table 5.1). When looking at overall bee leaving the hives and humidity correlation, it also shows a strong negative linear correlation between them (r=-0.72, p=0.01, n=11) (Table 5.1). Thus, inclement weather indicated by high humidity results in bees not leaving the hives and therefore not foraging. This indicates that the higher the humidity, the fewer the bee activities on the umbels, and also the lower the humidity, the more bee activities occur on the umbels.

This showed that bees prefer lower humidity to work sufficiently. Looking at bee counts and humidity in the mornings (Table 5.2), it also shows a significant negative correlation (r=-0.53 p=0.006). There is no noteworthy (r=-0.32 p=0.18) relationship between bee count and humidity in the afternoon (Table 5.3), as might be expected in the hot and dry Klein Karoo region when afternoon rainfall is an unusual event. Again, these results are not unexpected, as every beekeeper knows that honey bees stop foraging when the humidity rises, and that it is widely known that relative humidity is negatively correlated with honey bee foraging (Abrol, 2010).

5.4 Relationship between honey bee foraging activity and hive foraging activity

When comparing overall bee foraging counts on trial plots with bees leaving the hives, there was a highly significant positive and linear relationship (correlation) between bee counts on trial plots and bee count of hives ($r=0.83$ with $p=0.002$ and $n=11$, Table 5.1). This means that there was a direct relationship between bees leaving the hives and bee counts on the umbels of trial plots at all times during the day. This clearly indicates that the onions were the primary focus of the honey bee colonies for foraging, despite an alternative food source (veld) being available within a short distance from the study site. Bee counts taken on the trial plots showed that bee visits increased with increase in bees leaving hives, and also if bee counts of bees leaving hive decreased, the bee visits on umbels also decreased.

5.5 Relationship between honey bee foraging activity and other insect activity

There was a slightly ($r=-0.31$) and significant ($p=0.05$) negative correlation between insect counts and honey bee counts. The data shows that an increase in bee activity causes a decrease in insect visits to onion umbels. This can be directly related to a number of bees around the study site. Due to the fact that bee hives are brought to the site to aid in pollination, a number of bees available are far greater than the number of wild insects that naturally occur in the area, and it suggests that honey bees are out-competing the other insects when present in high numbers, especially in the afternoons (Table 5.3). Once again, this is not a surprising revelation as Brand (2013) reports that no insects other than honey bees have a significant effect on commercial seed pollination of seed onions in the Klein Karoo.

CHAPTER SIX: DISCUSSION

In 2012 there were significant difficulties with onion pollination over much of the seed onion growing areas of South Africa, with commercial honey bees reportedly declining to work on the onions, and with very poor seed set obtained by many growers. Losses of at least R70 million were reported by growers (Salmon, 2013). This was only the latest of periodic and unpredictable poor pollination onion seasons, and similar situations have been reported in all other seed onion growing areas of the world (Silva and Dean, 2000).

Poor pollination experienced in seed onion production has been attributed to a number of factors including ‘bad bees’ or ‘lazy bees’, the impact of pesticides, the effect of better alternative forage recruiting pollinating insects away from the onion fields, and the result of poor rainfall. Most commonly, the poor pollination and poor seed yield has been ascribed to a lack of attraction to the flowering onions by honey bees and other pollinators; and with lack of attraction being ascribed to high nectar potassium levels, or low sugar concentration levels, or low sugar volume (Hagler, 1990; Silva and Dean, 2000; Nicholson and Thornburg, 2007). Additionally, these factors are commonly considered to be caused by water imbalances in the plant, with too little water suggested to result in nectar that is too thick and viscous to collect and too much potassium in the nectar and too much water resulting in nectar with inadequate sugar concentrations necessary to attract pollinators (Waller et al., 1972; Hagler, 1990). Alternative explanations have been that floral traits of the different cultivars are responsible, with some cultivars being very unattractive to pollinators (Soto et al., 2018), or that better alternative forage is responsible for pollinators not being active on the onions.

Six possible causes for weak pollination were identified by the growers in the survey conducted following the 2012 season (Salmon, 2013). After thorough discussion of the findings of the survey and the six identified possible causes for weak pollination with market leaders of onion seed production, producers, farmers and various knowledgeable individuals with insights into pollination, it was decided that water and irrigation techniques were the main contributing factors among all possible causes. Therefore it was decided that this study would investigate the effects of water application on bee visits and seed yield of Texas Grano onion seeds in the Klein Karoo area.

There are two obvious methods with which this question may be approached. The first is the one followed by Silva et al. (2004) and others; namely, to collect and collate as much information as possible from commercial onion plantings, and to try to correlate insect activity and seed production with environmental conditions and/or farming methods. The second method is the one followed by Soto et al. (2018), namely controlled experiments looking at specific variables, typically in a randomised plot design.

The choice was made to utilise the second methodology in this study to investigate potential pollination problems in the production of seed in Texas Grano onions in the Ladismith area of the Klein Karoo, South Africa with a focus on whether water application rates during flowering was responsible for any observed pollination problems. Seven different water regimes were applied to a field of Texas Grano onions, which was then assessed for honey bee activity, activity of other pollinators, and the impact on onion seed production. The structure of the field trial was a series of mini-plots within a single field with different water treatments between plots, with pollinator activity between plots being assessed.

While it was determined that there was a positive relationship between irrigation water levels and honey bee foraging activity, the correlation was relatively weak, and it must be concluded that the study did not generate conclusive evidence to explain why the onion pollination deficits occur from time to time. Changing the levels of water application to onion plants during their flowering stage did not significantly change the response of honey bees to the plot of onions, or change the number of onion seeds produced in those plants. Hence, while discriminant analysis of the data collected highlighted the crucial importance of honey bee pollination in onion seed production, it was emphatically clear that differential water application during the flowering period was not an explanation for the periodic pollination disasters in onion seed production and not the answer that we had been searching for.

To emphasise the point that the problem has not been solved, the 2021 onion season has been the worst on record, markedly worse than the 2012 season. Losses of 40-50% have been estimated for the Klein Karoo and Northern Cape regions, with a cost of approximately R250 million to the growers (Malan pers. comm., 2021). The pattern was exactly the same, with bees refusing to work in fields that they had previously worked in, and the losses extended to almost all regions and to almost all onion cultivars.

Given that the problem has not been solved, the question is: What reflections can be made resulting from this study, to guide future efforts to solve the onion pollination riddle? The first conclusion is that, even though water application levels during the flowering period did not turn out to be the solution, it is still a good bet that irrigation is crucial to the puzzle. There are multiple possible explanations for the variation in attraction of onion nectar to honey bee foragers and other pollinators. These include sugar concentration in nectar, nectar volumes, elements like potassium or magnesium, or phenolics. All will be influenced by water / irrigation. Brand (2013) found that rainfall was the only factor that dictated seed yield, and research should not look further than that. Other possible explanations such as varietal differences (Hagler and Waller, 1991; Silva and Dean, 2003), or repellent pesticides, or poor bees, will not give rise to such sporadic and extensive responses.

However, perhaps the timing of water differential application is crucial, and if so, it is suggested that the application of different water regimes during the flowering phase might not have been the best choice. As a shallow rooted crop, onions have a low tolerance for water stress (Kumar et al., 2007; El Balla et al., 2013), and water stress during the early growth phase is reportedly most important (Mermoud et al., 2005; El Balla et al., 2013). It is suggested that differences in water application much earlier in the onion growth cycle might have resulted in very different results, and may have resulted in differences in honey bee visitation and in differences in seed production. Bee visitation is reported to vary based on soil moisture levels during the early growth phase (Gillespie et al., 2015), and such a treatment regime might well have resulted in differences in nectar levels or the concentration of deterrent phenolic (Soto et al., 2013).

The relationship between water application levels, irrigation, honey bee foraging activity and seed production may also not be simple, linear interactions. Gillespie et al. (2015) found that the relationship between nectar production and soil moisture content was non-linear, with the highest nectar production occurring at moderate moisture levels, and Fijen et al. (2020) found that reducing irrigation to leeks (*Allium porrum*) reduced the amount of nectar available, but that this did not affect the pollinator visitation rates, and had no effect on seed yields.

What is relatively certain is that environmental conditions will play a major role in the honey bees' response to the onion fields in some years, with temperature, humidity and rainfall all being significant potential factors in making onions less attractive than normal (Caselles et al., 2019), and also potentially making alternative forage sources (normally natural vegetation) more attractive than normal. The large-scale abandonment of a commercial crop by honey bees is likely to occur only when there is an alternative forage source available, as the colonies need fodder, and all reports of onion pollination disasters report that the colonies are foraging very well, but not on the onions. Thus, while poor irrigation management might be the proximate cause of bees abandoning an onion field, the ultimate cause is the presence of a more attractive alternative forage.

This is a problem faced by all commercial crops (Gaffney et al., 2019), not only onions. Carrot pollination was not previously regarded as a problem, but increased variability between cultivars has developed in recent years, with some cultivars now hardly visited by pollinators at all, and carrot pollination is now a substantial and growing problem (Gaffney et al., 2019). A similar situation is developing in canola cultivars (Allsopp pers. comm., 2020), and the problem is very difficult to manage because of the vast potential foraging distance that honey bees are able to cover. The grower has limited choices as follows:

1. Grow crops under nets with 'locked-in honey bee colonies' that have no choice, but to forage on the target crop.
2. Introduce pollinator attractiveness in cultivar selection and breeding programmes (Broussard et al., 2017), so that onion cultivars can outcompete alternative forage in terms of attractiveness. To fully address the pollination problems in onions, it is necessary to collect good data on the nectar

characteristics of all cultivars and lines. The only long-term solution is selection and breeding of more attractive cultivars (Hagler and Waller, 1991).

3. Use waves of bees for pollination, for periods of 10-14 days, rather than the same bees for the duration of the pollination period, as is the case with other crops that are not particularly attractive to pollinators. This is to counter the shift in forager activity to more attractive floral opportunities, and should be particularly rigorously applied in good rain years when alternative forage issues might be expected.

Brand (2013) recommended a comprehensive study to measure the nectar and odour levels of the various onion cultivars, and how these relate to pollinator activity. Different cultivars differ markedly in terms of nectar characteristics and in scent, and offer great potential for breeding cultivars more attractive to pollinators and not subject to pollination-driven seed production problems. It also may be necessary to carefully assess foraging behaviour of honey bees on the different onion cultivars; whether they collect nectar or pollen, and when they forage in order to give standardised assessment value of different cultivars, and to have a basis for selection for the various onion cultivars. This comprehensive study has yet to begin, and the onion pollination problems will sadly probably repeatedly happen until these crucial baseline studies are done.

CHAPTER SEVEN: CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

- While it was determined that there is a positive relationship between irrigation water levels and honey bee foraging activity, the correlation was relatively weak. It therefore can be concluded that the study did not generate conclusive evidence to explain why the onion pollination deficits occur from time to time.
- Changing the levels of water application to onion plants during their flowering stage did not significantly change the response of honey bees to the plot of onions, or change the number of onion seeds produced in those plants. Therefore, while the data collected highlighted the crucial importance of honey bee pollination in onion seed production, differential water application during the flowering period was not an explanation for the periodic pollination disasters in onion seed production.
- While poor irrigation management might be the proximate cause of bees abandoning an onion field, the ultimate cause is the presence of a more attractive alternative forage.
- With regard to non-Apis pollinators, whose numbers were 10% of the honeybee visitors, water application rates had no impact on onion umbel visits. Non-Apis pollinators were more active during the morning hours and less in the afternoon. This may be due to competition with honeybees, which are more active during the afternoons.
- When considering seed yield, there was no significant relationship between water application and the percentage of dead seeds, the percentage of abnormal seeds, and the percentage of seed germination.
- There was a significant positive correlation between water application and seed yield, with regard to the data show that there is a significant positive

relationship with regard to number of seeds harvested from treatments receiving more water than from the treatments receiving less water. Thus, the more water applied, the better the seed yield was, and vice versa.

- In relation to the effect of honeybee visits on seed yield, the more the honeybees that visited the onion flowers, the better the seed yields. However, when comparing the total honeybee count and the seed germination percentage, there was a slight negative correlation. This might be due to non-viable pollen being deposited by bees causing seed abortion. Similar trends were observed in non-Apis pollinators, whereby the more insects visited the onion flowers, the more the seed yield.
- Despite the honeybee showing that it is the most active pollinator in the onion seeds, several other factors were found that influenced their foraging activity. These factors were temperature, rainfall, humidity, foraging activity at the hive, and foraging activity of other insects.
- Temperature had a significant effect on foraging activity of honeybees. Honeybees remained in the hives when temperatures were low (cool) and only started foraging when the temperature became favourable (warm).
- Greater honeybee numbers were observed on days with lower humidity, compared to days with high humidity.
- Comparing overall bee counts on the trial plots to the bees leaving the hive, it was found that the number of bees leaving the hive had a direct effect on the number of bees counted on the onion umbels. Thus, if the number of bees leaving the hive decreased, the number of bees counted on the onion umbels also decreased. This shows that even though there were alternative food sources available, the honeybees preferred the onion flowers.

- With regard to the overall pollinators on the onion umbels, including honeybees and other insects, the number of honeybees on the onion flowers was far greater than the other insects. This shows that honeybees are of great importance for onion pollination and that the number of hives brought to the field should be of good pollination standards.

7.2 Recommendations

- Additional research is needed on irrigation management on onion cultivars in the earlier stages of development, as it has been found that water stress on onions in the early growth phases is the most important.
- More research must be done to determine the attractiveness of different onion cultivars, pertaining to the nectar and pollen compositions. When a baseline of cultivar attractiveness has been established, it could be possible to breed cultivars with more attractive pollen and nectar to honeybees.
- In conjunction with the research on cultivar attractiveness, it is extremely important to establish pollen compatibility among hybrid cultivars. An emphasis must be put on the investigation of non-viable pollen in Hybrid onion cultivars.
- Lastly, it is crucial to assess the foraging behaviour of honeybees with regard to the natural factors, namely temperature, humidity, rainfall, hive activity, and insect activity. This data will enable the industry to use it as an indication of possible complications regarding honeybee pollination on onion cultivars.
- Monitor soil moisture, evaluate nectar composition and nutritional balance of nectar and pollen, of onion flowers.

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Appendix 4. 1: Amount of water applied to each treatment during the 36 days from the start of differentiated irrigation (20th of October) until the harvesting of the onion seeds (16th of December)

Treatment	Date	Julian Day	Day	Total Bee Counts	Total insect Count	Water application in hours
A	30/10/2015	303	0	240	0	0
A	31/10/2015	304	1	19	0	2
A	01/11/2015	305	2	.	0	0
A	02/11/2015	306	3	22	0	0
A	03/11/2015	307	4	50	41	0
A	04/11/2015	308	5	511	108	0
A	05/11/2015	309	6	35	71	0
A	06/11/2015	310	7	376	71	2
A	07/11/2015	311	8	657	82	0
A	08/11/2015	312	9	272	83	0
A	09/11/2015	313	10	613	68	2
A	10/11/2015	314	11	392	14	0
A	11/11/2015	315	12	145	129	0
A	12/11/2015	316	13	.	.	0
A	13/11/2015	317	14	1082	34	2
A	14/11/2015	318	15	13	19	0
A	15/11/2015	319	16	.	.	0
A	16/11/2015	320	17	88	36	0
A	17/11/2015	321	18	732	41	2
A	18/11/2015	322	19	690	45	0
A	19/11/2015	323	20	781	43	2
A	20/11/2015	324	21	12	18	0
A	21/11/2015	325	22	.	.	0
A	22/11/2015	326	23	792	36	0
A	23/11/2015	327	24	826	51	2
A	24/11/2015	328	25	.	.	0
A	25/11/2015	329	26	230	21	0
A	26/11/2015	330	27	.	.	2
A	27/11/2015	331	28	.	.	0
A	28/11/2015	332	29	304	71	0
A	29/11/2015	333	30	121	23	0
A	30/11/2015	334	31	149	70	2
A	01/12/2015	335	32	305	44	0
A	02/12/2015	336	33	67	8	0
A	03/12/2015	337	34	142	64	0
A	04/12/2015	338	35	49	21	2
B	30/10/2015	303	0	241	.	4
B	31/10/2015	304	1	15	.	4

B	01/11/2015	305	2	.	.	0
B	02/11/2015	306	3	32	.	0
B	03/11/2015	307	4	45	37	4
B	04/11/2015	308	5	490	117	8
B	05/11/2015	309	6	51	65	4
B	06/11/2015	310	7	352	46	8
B	07/11/2015	311	8	755	69	8
B	08/11/2015	312	9	346	74	8
B	09/11/2015	313	10	670	57	8
B	10/11/2015	314	11	402	16	4
B	11/11/2015	315	12	161	115	4
B	12/11/2015	316	13	.	.	8
B	13/11/2015	317	14	1205	48	4
B	14/11/2015	318	15	19	27	4
B	15/11/2015	319	16	.	.	8
B	16/11/2015	320	17	108	27	4
B	17/11/2015	321	18	857	57	8
B	18/11/2015	322	19	794	48	8
B	19/11/2015	323	20	1008	43	8
B	20/11/2015	324	21	28	15	4
B	21/11/2015	325	22	.	.	4
B	22/11/2015	326	23	897	49	8
B	23/11/2015	327	24	815	51	4
B	24/11/2015	328	25	.	.	8
B	25/11/2015	329	26	245	25	8
B	26/11/2015	330	27	.	.	4
B	27/11/2015	331	28	.	.	8
B	28/11/2015	332	29	373	54	8
B	29/11/2015	333	30	101	14	4
B	30/11/2015	334	31	205	53	12
B	01/12/2015	335	32	428	43	12
B	02/12/2015	336	33	75	8	8
B	03/12/2015	337	34	164	68	12
B	04/12/2015	338	35	71	15	12
C	30/10/2015	303	0	211	.	4
C	31/10/2015	304	1	17	.	0
C	01/11/2015	305	2	.	.	0
C	02/11/2015	306	3	23	.	0
C	03/11/2015	307	4	52	42	4
C	04/11/2015	308	5	450	102	4
C	05/11/2015	309	6	58	50	4
C	06/11/2015	310	7	405	51	4
C	07/11/2015	311	8	632	84	4
C	08/11/2015	312	9	501	75	0
C	09/11/2015	313	10	609	70	4
C	10/11/2015	314	11	440	11	0

C	11/11/2015	315	12	229	120	4
C	12/11/2015	316	13	.	.	4
C	13/11/2015	317	14	1157	35	0
C	14/11/2015	318	15	19	16	4
C	15/11/2015	319	16	.	.	4
C	16/11/2015	320	17	133	38	0
C	17/11/2015	321	18	922	64	8
C	18/11/2015	322	19	762	48	4
C	19/11/2015	323	20	906	40	4
C	20/11/2015	324	21	14	24	0
C	21/11/2015	325	22	.	.	0
C	22/11/2015	326	23	810	33	4
C	23/11/2015	327	24	835	50	0
C	24/11/2015	328	25	.	.	4
C	25/11/2015	329	26	225	21	4
C	26/11/2015	330	27	.	.	4
C	27/11/2015	331	28	.	.	4
C	28/11/2015	332	29	346	47	4
C	29/11/2015	333	30	121	17	0
C	30/11/2015	334	31	149	78	4
C	01/12/2015	335	32	315	51	0
C	02/12/2015	336	33	70	9	4
C	03/12/2015	337	34	118	78	4
C	04/12/2015	338	35	48	12	4
D	30/10/2015	303	0	184	.	0
D	31/10/2015	304	1	16	.	0
D	01/11/2015	305	2	.	.	0
D	02/11/2015	306	3	18	.	0
D	03/11/2015	307	4	36	31	0
D	04/11/2015	308	5	414	95	0
D	05/11/2015	309	6	48	56	0
D	06/11/2015	310	7	352	49	0
D	07/11/2015	311	8	576	78	4
D	08/11/2015	312	9	454	51	0
D	09/11/2015	313	10	536	71	0
D	10/11/2015	314	11	429	19	0
D	11/11/2015	315	12	246	122	0
D	12/11/2015	316	13	.	.	4
D	13/11/2015	317	14	1100	32	0
D	14/11/2015	318	15	21	22	0
D	15/11/2015	319	16	.	.	0
D	16/11/2015	320	17	80	21	0
D	17/11/2015	321	18	821	48	4
D	18/11/2015	322	19	705	53	0
D	19/11/2015	323	20	787	26	0
D	20/11/2015	324	21	16	19	0

D	21/11/2015	325	22	.	.	0
D	22/11/2015	326	23	812	38	0
D	23/11/2015	327	24	749	51	0
D	24/11/2015	328	25	.	.	0
D	25/11/2015	329	26	238	27	0
D	26/11/2015	330	27	.	.	0
D	27/11/2015	331	28	.	.	0
D	28/11/2015	332	29	364	53	4
D	29/11/2015	333	30	125	18	0
D	30/11/2015	334	31	156	78	0
D	01/12/2015	335	32	296	50	0
D	02/12/2015	336	33	53	6	0
D	03/12/2015	337	34	122	51	4
D	04/12/2015	338	35	34	21	0
E	30/10/2015	303	0	173	.	0
E	31/10/2015	304	1	17	.	2
E	01/11/2015	305	2	.	.	0
E	02/11/2015	306	3	22	.	0
E	03/11/2015	307	4	31	33	0
E	04/11/2015	308	5	378	114	0
E	05/11/2015	309	6	52	53	0
E	06/11/2015	310	7	389	54	0
E	07/11/2015	311	8	624	65	0
E	08/11/2015	312	9	461	56	0
E	09/11/2015	313	10	547	73	4
E	10/11/2015	314	11	429	21	0
E	11/11/2015	315	12	202	94	0
E	12/11/2015	316	13	.	.	0
E	13/11/2015	317	14	1100	47	0
E	14/11/2015	318	15	18	24	0
E	15/11/2015	319	16	.	.	0
E	16/11/2015	320	17	99	48	0
E	17/11/2015	321	18	786	64	2
E	18/11/2015	322	19	692	56	0
E	19/11/2015	323	20	789	29	2
E	20/11/2015	324	21	22	18	0
E	21/11/2015	325	22	.	.	0
E	22/11/2015	326	23	819	41	0
E	23/11/2015	327	24	791	51	0
E	24/11/2015	328	25	.	.	2
E	25/11/2015	329	26	253	20	0
E	26/11/2015	330	27	.	.	0
E	27/11/2015	331	28	.	.	2
E	28/11/2015	332	29	361	53	0
E	29/11/2015	333	30	137	21	0
E	30/11/2015	334	31	147	73	0

E	01/12/2015	335	32	294	45	4
E	02/12/2015	336	33	53	6	0
E	03/12/2015	337	34	127	68	0
E	04/12/2015	338	35	36	19	0
F	30/10/2015	303	0	264	.	0
F	31/10/2015	304	1	22	.	2
F	01/11/2015	305	2	.	.	0
F	02/11/2015	306	3	19	.	0
F	03/11/2015	307	4	32	29	0
F	04/11/2015	308	5	400	119	0
F	05/11/2015	309	6	60	73	0
F	06/11/2015	310	7	392	41	0
F	07/11/2015	311	8	654	74	0
F	08/11/2015	312	9	482	65	0
F	09/11/2015	313	10	586	66	4
F	10/11/2015	314	11	394	15	0
F	11/11/2015	315	12	167	85	0
F	12/11/2015	316	13	.	.	0
F	13/11/2015	317	14	1048	31	0
F	14/11/2015	318	15	21	18	0
F	15/11/2015	319	16	.	.	0
F	16/11/2015	320	17	117	24	2
F	17/11/2015	321	18	821	56	2
F	18/11/2015	322	19	652	53	0
F	19/11/2015	323	20	835	42	0
F	20/11/2015	324	21	22	18	0
F	21/11/2015	325	22	.	.	0
F	22/11/2015	326	23	768	43	4
F	23/11/2015	327	24	770	52	0
F	24/11/2015	328	25	.	.	0
F	25/11/2015	329	26	222	27	0
F	26/11/2015	330	27	.	.	0
F	27/11/2015	331	28	.	.	0
F	28/11/2015	332	29	332	55	0
F	29/11/2015	333	30	117	15	2
F	30/11/2015	334	31	134	70	0
F	01/12/2015	335	32	282	39	0
F	02/12/2015	336	33	48	5	0
F	03/12/2015	337	34	115	57	0
F	04/12/2015	338	35	31	15	2
G	30/10/2015	303	0	239	.	0
G	31/10/2015	304	1	16	.	0
G	01/11/2015	305	2	.	.	0
G	02/11/2015	306	3	21	.	0
G	03/11/2015	307	4	58	48	0
G	04/11/2015	308	5	473	115	0

G	05/11/2015	309	6	30	69	0
G	06/11/2015	310	7	329	71	0
G	07/11/2015	311	8	683	64	0
G	08/11/2015	312	9	273	81	0
G	09/11/2015	313	10	574	62	0
G	10/11/2015	314	11	387	17	0
G	11/11/2015	315	12	148	128	0
G	12/11/2015	316	13	.	.	0
G	13/11/2015	317	14	1015	34	0
G	14/11/2015	318	15	15	12	0
G	15/11/2015	319	16	.	.	0
G	16/11/2015	320	17	80	38	0
G	17/11/2015	321	18	662	58	0
G	18/11/2015	322	19	608	44	0
G	19/11/2015	323	20	602	36	0
G	20/11/2015	324	21	15	18	0
G	21/11/2015	325	22	.	.	0
G	22/11/2015	326	23	756	28	0
G	23/11/2015	327	24	670	41	0
G	24/11/2015	328	25	.	.	0
G	25/11/2015	329	26	195	21	0
G	26/11/2015	330	27	.	.	0
G	27/11/2015	331	28	.	.	0
G	28/11/2015	332	29	348	48	0
G	29/11/2015	333	30	176	23	0
G	30/11/2015	334	31	147	52	0
G	01/12/2015	335	32	322	54	0
G	02/12/2015	336	33	65	5	0
G	03/12/2015	337	34	125	50	0
G	04/12/2015	338	35	41	14	0

Appendix 4. 2: The complete honey foraging data for the study

Treatment	Plot No.	Replicas	Bee Count AM	Bee Count PM	Insect AM	Insect PM	Seed yield (kg)	Germination (%)	Abnormal %	Dead %	Average tensiometer readings kPa
A	6	1	443	907	75	54	1,22	93	3	4	13
A	7	2	467	1008	116	50	1,34	89	6	5	14
A	14	3	330	841	121	74	1,39	91	4	5	11
A	16	4	281	893	123	71	1,09	93	3	4	7
A	21	5	204	892	68	71	1,08	94	3	3	11
A	22	6	272	950	75	67	1,41	97	3	.	13
A	25	7	217	858	73	62	1,22	93	3	4	9
A	34	8	241	911	108	104	1,8	91	7	2	15
B	1	1	457	1025	86	41	1,23	86	7	7	0
B	10	2	544	1083	120	87	1,5	89	5	6	1
B	20	3	261	981	80	64	1,32	95	4	1	2
B	23	4	222	879	69	57	1,22	92	5	3	1
B	24	5	223	801	71	41	1,11	94	3	3	0
B	32	6	277	1000	106	89	1,28	91	7	2	3
B	33	7	372	1242	109	106	1,99	90	4	6	2
B	56	8	446	1140	67	48	1,56	94	3	3	1
C	2	1	550	1170	75	63	1,8	93	4	3	6
C	5	2	524	1028	89	62	1,52	92	5	3	3
C	26	3	213	879	85	69	1,16	91	5	4	5
C	29	4	245	910	111	85	1,1	92	4	4	6
C	31	5	315	952	86	99	1,31	88	5	7	5
C	40	6	316	1000	79	67	1,42	96	3	1	1
C	46	7	313	899	70	64	1,16	90	6	4	10
C	48	8	390	873	86	76	1,22	87	8	5	7
D	11	1	489	1022	115	72	1,4	88	9	3	9
D	17	2	282	885	104	52	1,22	93	4	3	7
D	28	3	214	813	96	57	1,12	92	5	3	7
D	41	4	282	916	60	59	1,21	92	5	3	1
D	42	5	280	895	60	56	1,25	96	2	2	18
D	45	6	304	872	68	51	1,09	95	3	2	30
D	47	7	367	906	74	81	1,34	93	4	3	.
D	50	8	355	907	93	88	1,25	93	7	.	9
E	9	1	517	1004	146	77	1,45	90	4	6	21
E	12	2	371	877	113	65	1,15	88	4	8	14
E	37	3	273	880	91	62	1,16	90	5	5	10
E	39	4	290	883	73	56	1,13	87	8	5	35
E	44	5	223	717	39	52	0,9	95	4	1	47
E	49	6	366	888	97	76	1	96	3	1	18

Appendix 5. 1: Details of bee and insect counts, temperature, relative humidity and rainfall

Time	Day	Bee Count	Insect Count	Temperature	Humidity	Rainfall mm
8:00 cloudy	30/10/2015	228	.	20,67	73,9	.
15:00	30/10/2015	1324	.	27,67	46,28	.
7:30 cloudy	31/10/2015	122	.	22,17	56,07	.
rain	01/11/2015	.	.	16,16	90,7	18
8:00 Flowers to vat after the rain	2/11/2015	.	.	15,16	66,04	4
15:00 cloudy	02/11/2015	157	.	20,17	43,76	.
08:00	03/11/2015	304	261	18,17	54,26	.
08:00 bee stop working 8:30	04/11/2015	387	489	21,67	49,37	.
15:00	04/11/2015	2729	281	33,66	17,89	.
08:00	05/11/2015	334	437	25,67	41,21	.
08:00 start to rain	06/11/2015	83	89	21,67	49,99	2
15:00	06/11/2015	2512	294	30,67	32,12	.
08:00	07/011/2015	139	325	22,17	59,05	.
15:00	07/011/2015	4442	191	31,17	26,78	.
08:00	8/11/2015	1604	202	24,17	54,26	.
15:00	8/11/2015	1183	283	33,66	22,71	.
08:00	9/11/2015	109	270	23,17	49,37	.
15:00	9/11/2015	4026	197	30,67	32,78	.
15:00	10/11/2015	2873	113	23,67	48,14	.
08:00	11/11/2015	456	326	19,17	60,82	.
15:00	11/11/2015	842	467	23,67	40,57	.
08:00	13/11/2015	2383	150	23,17	45,64	.
15:00	13/11/2015	5324	111	29,67	20,66	.
08:00 could wind	14/11/2015	126	138	19,67	48,14	.
08:00	16/11/2015	705	232	21,67	49,37	.
08:00	17/11/2015	2006	238	25,67	34,75	.
15:00	17/11/2015	3595	150	32,17	22,71	.
08:00 cloudy	18/11/2015	94	212	19,67	53,05	.
15:00	18/11/2015	4809	135	32,17	22,03	.
08:00	19/11/2015	2258	195	24,67	41,21	.
15:00 strong wind	19/11/2015	3449	64	39,65	8,73	.
08:00 cloudy	20/11/2015	129	130	20,17	57,86	.
08:00	22/11/2015	755	148	19,67	46,89	.
15:00	22/11/2015	4899	120	30,67	13,7	.
08:00	23/11/2015	787	183	23,67	47,51	.
15:00	23/11/2015	4672	164	35,66	19,97	.
08:00	25/11/2015	1608	162	26,67	43,75	.
08:00 rain	26/11/2015	.	.	16,16	90,7	23
15:00 rain	26/11/2015	.	.	24,17	46,89	.
8:00 could wind	27/11/2015	.	.	18,67	51,83	.

bees do not work						
15:00 could wind bees do not work	27/11/2015	.	.	28,17	22,71	.
08:00	28/11/2015	416	184	22,17	48,14	.
15:00	28/11/2015	2012	197	35,16	19,28	.
08:00	29/11/2015	898	131	25,67	43,75	.
15:00	30/11/2015	1087	474	42,14	13,7	.
08:00	01/12/2015	1202	140	32,17	30,13	.
15:00	01/12/2015	1040	186	36,66	24,08	.
08:00	02/12/2015	431	47	24,17	63,15	.
08:00	03/12/2015	640	137	27,67	51,22	.
15:00	03/12/2015	273	299	39,65	18,59	.
08:00	04/12/2015	310	117	28,67	48,76	.

Appendix 5. 2: Number of bees leaving the beehive

Date collected	30 Oct (08:00)	30 Oct (15:00)	31 Oct (9:00)	2 Nov (9:00)	2 Nov (15:00)	3 Nov (9:00)	4 Nov (9:00)	4 Nov (15:00)	11 Nov (9:00)	11 Nov (15:00)	23 Nov (9:00)	23 Nov (15:00)
Hive number	Bees working per minute per hive											
A (143)	0	48	6	No activity due to wind and rain	0	0	8	43	No activity	More than 100 bees per minute. Bees start to sting.		More than 100 bees per minute. Bees start to sting.
B (161)	18	78	26		21	21	24	More than 100 bees per minute. Bees start to sting.	37		51	
C (313)	40	106	65		34	54	48		69		83	
D (457)	30	99	43		14	45	21		48		66	
E (19)	21	80	16		13	14	27		39		61	