

Contribution of managed honeybees (*Apis mellifera*
scutellata Lep.) to sunflower (*Helianthus annuus* L.)
seed yield and quality

by

Gebreamlak Bezabih Tesfay

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DEDICATION

I am pleased to dedicate this thesis to my brother, Niguse Bezabih Tesfay, whose stubborn determination for our family's success has led me here thank you.

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ABSTRACT

Insects are considered to be responsible for 80-85% of all pollination, with honeybees being well known for their pollination services for many crops. The effect of managed honeybee pollination on sunflower seed yield and quality (germination percentage and oil content) was investigated at the University of Pretoria experimental farm and in commercial sunflower fields at Settlers. This was done through pollinator exclusion and pollinator surveys on sunflower field plots located at different distances from managed honeybee colonies. Observations on the foraging behaviour and activity of honeybees throughout the day were also made.

The data presented in this thesis reveal that seed quantity and quality of sunflower increased significantly as a result of insect visits. Insect pollination improved the mass of 100 seeds (by 38%), as well as their germination percentage (by 38%) and oil content (by 36%). Moreover, visitation frequency, seed yield and quality were negatively correlated with distance to the honeybee hives, suggesting that the distribution of honeybee colonies is not enough to maintain an adequate pollination service throughout the large sunflower fields at Settlers. Honeybee foraging activity varied throughout the day, the highest activity being from 9h00 until 16h00, and activity was correlated with temperature.

The results of this study suggest that the use of managed honeybees in sunflower crop production can effectively increase the seed quality and quantity, but additional management measures should be considered to improve production in large monocropping farms that are currently isolated from pollinator sources. Additional provision of honeybee colonies is needed in sunflower production areas during the flowering period. Furthermore, as our results show that in the presence of pollinators other than honeybees seed yield was also improved, management measures that promote the biodiversity of sunflower visitors may also have an important contribution.

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CHAPTER 1

General Introduction

1.1 Importance of pollination for agriculture

Pollination, the transfer of pollen grains from the male organ (anther) of a plant to the female organ (stigma), is essential to produce fruit and seeds. Insect pollination is very important in determining the mating opportunities of plants and increases seed set of many fruit crops, as well as the quality of seed/fruit (e.g. oil content, pyrethrin content, rubber content) (Free, 1993). Hence pollination is a keystone process in both human managed and natural terrestrial ecosystems (Kearns and Inouye, 1993; Kevan, 1999; Donaldson, 2002; Richards and Kevan, 2002; de Jong *et al.*, 2005; Collette, 2008). Globally, up to 30% of the human food supply depends directly or indirectly on pollinators for fruit set (McGregor, 1976; Greenleaf and Kremen, 2006). The most likely reason for the unexpected yield declines detected in the past on insect pollinated crops is a decline in insect pollinators (McGregor, 1976; Richards, 2001, Ricketts, 2004). Of the six known types of pollination agents (insects, birds, wind, gravity, water and mammals) insects are by far the most important in pollination (McGregor, 1976), being responsible for 80-85% of all pollination (Johannsmeier and Mostert, 2001). The biggest groups of insect pollinators are solitary bees, bumblebees and honeybees (Free, 1993), probably due to their large number of hairs and their behavioural patterns such as feeding their young on nectar and pollen (McGregor, 1976; du Toit, 1988; Jams and Pitts-Singer, 2008).

Agricultural expansion and the associated use of agricultural chemicals (pesticides, herbicides and fertilizers) play a great role in the decline of native pollinators (managed honeybees and wild pollinators) (Radford *et al.*, 1979; Free 1993; Chandel *et al.*, 2004). This is also happening through the modification and elimination of pollinator habitats (Kearns *et al.*, 1998; Chapman and Bourke, 2001; Kremen *et al.*, 2002; Staffen-

Dewenter, *et al.*, 2005; Morandin and Winston, 2005). Practices such as destruction of hedgerows and natural verges are also responsible for the decline of many natural food sources and nesting sites of wild pollinating insects (Free, 1993; Collette, 2008; Davila and Wardle, 2008; Gallai *et al.*, 2009). Therefore, when many hectares are occupied by the same crop plant (monoculture) there may be too few insect pollinators around and it may be necessary to enhance pollinators in that area (du Toit, 1988).

Honeybees (*Apis mellifera* L.) are indigenous to Africa, the middle East and Europe (Winston, 1987) and the beekeeping industry was first started in those regions before expanding all over the world (Anderson *et al.*, 1983). Honey has long been an important component of the human diet (Adjare, 1990). Rapid development of modern beekeeping occurred in the 19th century when Langstroth discovered box-hives with suitable bee space and that revolutionized the beekeeping industry in general and honey production in particular (Anderson *et al.*, 1983). The evolution and divergence of honeybees has been closely linked to that of the angiosperm plants (Winston, 1987), as pollen is a vital food for the brood of the honeybees and many flower species depend on bees to pollinate it, this being an important mutualism. A single honeybee colony needs 34-65 kg honey and 20-50 kg pollen per year to survive (Johannsmeier and Mostert, 2001).

The contribution of managed honeybee pollination to crop production and quality has been estimated to be more than the value of honey and wax production (Shrestha, 2004). Honeybees are responsible for 70-80% of insect pollination (Johannsmeier and Mostert, 2001). The eastern honeybees (*Apis cerana*) are also maintained in India, China and Japan for commercial purposes (Free, 1993), but the western honeybee (*Apis mellifera*) is a successful, if not the most effective, pollinating agent of a large range of crops world wide (Free, 1993; Johannsmeier and Mostert, 2001), contributing to the economic growth and sustainability of human beings. As an example, in the Western Cape (South Africa) the deciduous fruit industry which is entirely dependent on honeybees as pollinators generates R1 billion per year and creates job opportunities for 80,000 people (Picker *et al.*, 2004). Pollination services in agriculture represent possibly one of the greatest means of increasing quality and quantity of crops and honeybees are being managed for this

purpose. For all the United States, the annual value of increased agricultural production in yield and quality that is attributed to honeybee pollination varied from US\$9.3 billion in 1989 to US\$14.6 billion in 2000 (Morse and Calderone, 2000). In Australia faba bean (*Vicia faba* L.) caged with honeybees produced 25% higher seed yield than without honeybees (Somerville, 1999) and Kamler (1997) reported 26% increment in Czech Republic in sunflower.

1.2 Sunflower

Sunflower importance as a crop

Sunflower (*Helianthus annuus* L.) is believed to have been domesticated from wild sunflowers around 1000 B.C. in North America and it has been distributed to South or Central America, Asia and Europe (Putman *et al.*, 1990). Sunflower is one of the most important oil crops in South Africa (Du Toit, 1988) and worldwide (Cantamutto and Poverene, 2007; Skoric *et al.*, 2007), with its cultivation for oil production expanding in both developed and developing countries (Škorić, 1992). Its oil is widely used in the human diet because of its high level of unsaturated fatty acids, lack of linolenic acid, bland flavour and high smoke points (Putman *et al.*, 1990). Moreover, sunflower has many other uses such as forage (used as silage crop for animals), food for ruminant animals plus swine and poultry, industrial applications and non-oilseed feed (use for birdfeed or in human diets as a snack) (Putman *et al.*, 1990). Sunflower oil may be also used as a raw material to extract biodiesel, which can be used as fuel in diesel engines (Antolin *et al.*, 2002)

Sunflower pollination

Self-incompatible and cross-pollinated crops need pollinating agents for efficient pollination and seed set (Thapa, 2006). Moreover, several crop species are able to self-pollinate to a certain extent (Cunningham *et al.*, 2002). Sunflower is able to self-pollinate by curling down the stigma to the pollen source but results in either poor fertilization or abortion of the seed (du Toit, 1988). Self-pollination is prevented by means of self-

incompatibility (Minckley *et al.*, 1994; Neff and Simpson, 1990), and by desynchronized flowering time of male and female florets (Free, 1993). Therefore, insect pollination is important for profitable sunflower production mainly in its quality and quantity increment (Fell, 1986; Neff and Simpson, 1990; Free, 1993; Basualdo *et al.*, 2000; Paiva *et al.*, 2002; Paiva *et al.*, 2003; Hernandez, 2008; Nderitu *et al.*, 2008; Oz *et al.*, 2009). Previous studies have showed that pollination of sunflower due to wind and insects smaller than honeybees does not exceed 9%, suggesting that the honeybee is the principal insect pollinator of sunflower crop (Low and Pistillo, 1986). The spotted maize beetle (*Astylus atromaculatus*) may contribute to pollination of sunflower, during certain times of the season or in localized areas (du Toit, 1988), for example in South Africa du Toit (1988) gave the result of pollination efficiencies of some insects in a cage experiment as follows: 72% seed set with honeybees, 76% with spotted maize beetle, 38% with flies, 44% with American bollworm larvae, 72% was found in open control plots whereas seed set with no pollinating insects was 44%. However, when many hectares are occupied by a single flowering crop the influence of these beetles would be less significant due to their clumping and irregular occurrence (du Toit, 1988). Several studies have found that managed honeybees can have an important contribution to sunflower pollination, leading to improvements in both seed yield and oil content (Langridge and Goodman, 1974; 1981; Sunmangala and Giriraj, 2003; Ndreritu, *et al.*, 2008). Pollination has also been shown to affect sunflower crop maturity positively (Noetzel, 1968; du Toit, 1988; Putnam *et al.*, 1990). Sunflower takes approximately 109 days from planting to maturity in temperate regions (Putnam *et al.*, 1990) and cultivar differences in maturity are usually associated with changes in vegetative growth. Blooming lasts 14 to 17 days (du Toit, 1988), the peak being reached approximately two weeks after initial bloom (Minckley *et al.*, 1994). However, if there is sufficient pollination this period may be shortened to 5-10 days (Noetzel, 1968).

Many investigations have consistently confirmed that yield levels can be increased to between 10 and 100% in fruits and nuts, 70 and 100% in vegetables and melons, 100% in sunflower crop and 10 and 100% in soybean and rapeseed through good management of pollinators (Morse and Calderone, 2000). Insect pollination of sunflower crop attributed

to the income of Makueni (Kenya) farmers by about US\$1.697 million in 2005 and this amount increased by 50% of the total value of sunflower in the district in that year (Ndreritu *et al.*, 2008). Morse and Calderone (2000) reported that, from the estimated value of US crop production, 100% of the sunflower crop depends on insect pollination and 90% of it was pollinated by honeybees. Furthermore, Africanized honeybees are more efficient than European bees for commercial sunflower seed production (Basualdo *et al.*, 2000).

1.3 General aim

The springbok Flats, surrounding Settlers is one of the largest commercial sunflower producing area in the province. Shortage of pollination services have been reported in commercial sunflower production areas (du Toit, 1988) which led to low yields. Even though some farmers are getting pollination services from managed honeybees, while beekeepers get sunflower honey from the farms, yet some farmers are reluctant to import hives during the flowering season and rely on natural pollination services instead. The research described in this thesis aims to improve the understanding of the use of managed honeybee colonies in sunflower pollination. To achieve this, the following objectives will be addressed:

- 1- To quantify the contribution of managed honeybee pollination to sunflower seed production and quality (Chapter 2).
- 2- To determine the influence of weather conditions and time of day on honeybee visits to sunflower head inflorescences (Chapter 3).

The findings of this project will contribute to the definition of general guidelines to maintain or improve sunflower crop pollination.

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CHAPTER 2

Importance of managed honeybees for sunflower production

Introduction

Due to the seeds' high content of vegetable oil (39-49%), sunflower (*Helianthus annuus* L.) has emerged as an economically important crop worldwide (Neff and Simpson, 1990; Putnam *et al.*, 1990; Paiva *et al.*, 2002; Paiva *et al.*, 2003; Hernandez, 2008; Nderitu *et al.*, 2008; Oz *et al.*, 2009). This annual crop is also used for animal rations and various chemical and industrial products (Putnam *et al.*, 1990). Sunflower is generally considered to be self-fertile and a self-pollinated crop, but lack of self-compatibility reduces its seed yield (McGregor, 1976; du Toit, 1988; Neff and Simpson, 1990; Free, 1993). The extent to which a plant is able to self-pollinate is influenced by the position and time of emergence of anthers in relation to the stigma (Free, 1993). In some cases the tip of the stigma of sunflower crop extends above the anthers to get pollen and to increase the chance of self-pollination, but in most cases this causes abortion (du Toit, 1988).

There are several studies on the pollination of sunflower crops in different countries: for instance Neff and Simpson (1990) in USA, Langridge and Goodman (1974) Australia, Sumangala and Giriraj (2003) India and Nderitu *et al.* (2008) Kenya. Sunflower seed yield and quality can improve after cross-pollination provided by insects (du Toit, 1988; Free, 1993; Basualdo *et al.*, 2000, Richards, 2001; Paviva *et al.*, 2003; Sumangala and Giriraj, 2003). The most important quality characteristics of a sunflower crop, increased through honeybee pollination, are percentage germination and oil content (Langridge and Goodman, 1974; El-Sarrag *et al.*, 1993; Paviva *et al.* 2003). Pollination of sunflower due to wind and insects smaller than the honeybee is negligible because of its sticky and heavy pollen, implying that honeybees are the most effective for sunflower pollination (Low and Pistillo, 1986). Furthermore, if there is a shortage of honeybees in the sunflower fields, a small seed yield is harvested (du Toit, 1988; McGregor, 1976;

Noetzel, 1968). Even though other insects and solitary bees have an important supplementary effect on sunflower pollination, honeybees are the most valuable floral visitors (Neff and Simpson, 1990; Free, 1993; Basualdo *et al.*, 2000; Hernandez, 2008; Nderitu *et al.*, 2008). Beekeepers seek out apiary sites near sunflower fields where they are utilized for honey production and honeybee colonies are commonly managed by beekeepers to improve sunflower production (Morse and Calderone, 2000). It is still not clear how many honeybee colonies per hectare are needed to maximize sunflower production (Paviva *et al.*, 2003), but generally large concentrations of honeybees are necessary in the case of hybrid sunflower seed production (Free, 1993). Several factors (e.g. surrounding crops, flowering weeds, and climate) could influence the number of honeybee colonies needed for adequate sunflower pollination (du Toit, 1988).

Many studies on the importance of managed honeybees for sunflower pollination have been conducted around the world (Free, 1993), but there is still a lack of knowledge on the pollination requirements of sunflower fields in southern Africa. In addition, the effect of distance from the honeybee colony and the response of different cultivars to insect pollination have not been considered. Therefore, we conducted this study at the experimental farm of Pretoria University and on commercial sunflower farms located in the Springbok flats, Settlers South Africa, to achieve the following objectives.

1. To compare the effect of insect pollination between bagged and un-bagged sunflower heads under field conditions and experimentally using a cage experiment (all insects, honeybees only, no insects).
2. To assess the abundance of managed honeybee foragers in relation to distance from the colonies and their impact on seed yield and quality.
3. To assess the pollination of different sunflower cultivars to insect pollination.

Materials and Methods

Effect of honeybees and other flower visitors on sunflower seed production and quality

To identify the contribution of honeybees and other flower visitors to sunflower pollination, an exclusion experiment was conducted at the experimental farm of Pretoria University (25° 45' S, 28° 16' E and 1372 m above sea level), located in a region with an average annual rainfall of 670 mm, mainly during the months of October to March (Annandale *et al.*, 1999).

Experimental design

For the set up at the experimental farm, the Monsanto DK4040 sunflower cultivar was planted in March 2009 in nine plots, each with three rows (10-15 sunflower plants in each row). All necessary agronomic practices recommended for the crop, including irrigation were carried out. The experiment was laid out in a randomized complete block design with three treatments with three replicates each. The treatments were: (1) sunflowers accessible to all flower visitors - the plots were left open for natural pollination as control; (2) sunflowers not accessible to any insects (self pollinated) – the plots were covered with a insect proof mesh cage (before the ray florets started opening) and all insects were removed from the cage; (3) sunflowers accessible only to honeybees – the plots were covered with an insect proof mesh cage and a honeybee colony with four frames (Fig. 1B) were placed inside the cage during the sunflower flowering peak (50% florets open). Insect proof mesh cages (5 m x 3 m and 2.5 m high) were made of slotted angle iron covered with 20% shade cloth. All insects were removed from all the cages before blooming, to exclude unwanted pollinators. Honeybee colonies used in this experiment received supplementary feeding (dissolved sugar) and water before and after they were placed in the cages.

Flower visitation surveys

Flower visitor surveys were done for four consecutive days (5 - 8 June 2009) in each of the open plots to assess which insect species were visiting the sunflowers. Fifteen minute surveys were done every hour from 9h00 - 18h00. Whenever identification of flower visitor species was not possible *in loco*, specimens were captured for later identification by taxonomists (see Acknowledgements). Air temperature, relative humidity, wind speed and rainfall were obtained from a weather station located at a distance of about 200 m from the plots.

Seed collection and laboratory work

When flowering had ceased and all honeybee colonies were removed from the cages, the cages were removed (five months after planting the sunflower) from the plots to insure uniformity of seed development conditions. After sunflowers had reached physiological maturity (two months after removing the cages), seven dried sunflower heads from each experimental plot were randomly harvested and the effect of insect pollination on sunflower yield was measured by comparing the yield of the three treatments based on seed yield/plot, mass of 100 seeds, (pooled across the seven sunflower heads per plot) seed germination percentage and oil content as follows.

Mass of 100 seeds, seed yield (g) per 15 m², germination percentage and oil content

The dependence of sunflower crop on honeybee pollination (i.e., the contribution of managed honeybee pollination to the crop yield) was measured. This was done by comparing the seed yield from sunflowers accessible to all flower visitors, the seed yield from sunflowers not accessible to any insects (self pollinated) and the seed yield from sunflowers accessible only to honeybees. This can be expressed formally as:

$$\text{Yield increase from all insects excluding self pollination (A\%)} = \left(\frac{Y_{all} - Y_{self}}{Y_{all}} \right) \times 100$$

$$\text{Yield increase from all insects excluding honeybee pollination (B\%)} = \left(\frac{Y_{all} - Y_{bee}}{Y_{all}} \right) \times 100$$

$$A\% - B\% = \left(\frac{Y_{all} - Y_{self}}{Y_{all}} \right) \times 100 - \left(\frac{Y_{all} - Y_{bee}}{Y_{all}} \right) \times 100$$

$$(A - B)\% = \left(\frac{Y_{bee} - Y_{self}}{Y_{all}} \right) \times 100$$

Where Y_{all} is the seed yield from sunflowers accessible to all flower visitors, Y_{self} is the seed yield from sunflowers not accessible to any insects (self pollinated) and Y_{bee} is the seed yield from sunflowers accessible only to honeybees.

A germination success study was conducted by considering the principle of maximum percentage germination using the between paper (BP) method, following the necessary steps used by the International Rules for Seed Testing (ISTA, 2009). No pre-chilling germination tests were needed to break the dormancy of the seed. Four sheets of Anchor germination paper were soaked with 100 ml of distilled water. Sunflower seeds (50 seeds per treatment) were placed horizontally on three layers of the germination paper and covered with the fourth sheet. The Anchor germination paper was then rolled up, put into a plastic bag and loosely tied to allow ventilation. The plastic bags were placed into an incubator at constant darkness at 25°C. The number of germinated seeds was counted, recorded and removed from the germination paper at 4, 7 and 10 days respectively. The seeds were considered as germinated when the visible protrusion of radical and plumules emerged from the seed coat. These germination tests were carried out for seeds from each of the nine sampling plots (3 replicates per treatment), with fifty randomly selected seeds per plot used to determine percentage germination.

Sunflower seed oil content was measured by extracting the crude fat from sampled seeds (AOAC official method of analysis, 2000). A Soxhlet extractor tube was used as fat extractor apparatus. Nine samples (one per sampling plot) of ground sunflower seeds (ground with the husk) were precisely weighed in empty thimbles. Hereafter samples were placed in filter paper (fold up wards) inside the extraction chamber, fitted with a siphon and attached between the boiler and the condenser. Pure petroleum ether (60-80 boiling points) was used as a solvent. The flask was two-thirds filled with the solvent and then fitted to the extractor. The apparatus was assembled and left to work. After 8 h (feed

samples), the apparatus was carefully dismantled and the remaining solvent was evaporated completely in an electric oven at 105°C until a constant weight was obtained. After the sample (the oil) cooled down in desiccators, the flask with the oil was reweighed and oil content was determined as a percentage using the following formula:

$$\text{Oil content (\%)} = \frac{\text{weight of extracted oil (g)} \times 100}{\text{weight of sample (g)}}$$

Statistical analyses

To determine if the mass of 100 seeds, germination percentage and oil content varied between the three pollination treatments (all insects, honeybees only, no insects), analyses of variance (ANOVA) were done using the statistical package SAS windows version 5.1.260. Significant differences between the means of treatments were further analysed using Tukey's Studentized Range (HSD) test (SAS software institute 2003).

Variation of the effect of managed honeybees at different distances to the sunflower plot and among cultivars

Study area

This section of the study was conducted in March 2009 on commercial sunflower farms located in the Springbok flats, Settlers (South Africa, 27° 57'S, 28° 32'E). Average annual rainfall in the region is 481 mm, with the highest monthly average rainfall (95 mm) occurring in January and lowest average rainfall (0 mm) in June. Average daily maximum temperatures range from 19.9°C in June to 28.8°C in January (Source: Bela-Bela climate, <http://www.saexplorer.co.za>).

Nine farms with no natural vegetation in the surrounding areas using four commercially important hybrid sunflower cultivars (sown from 2-22 January 2009) were selected (Table 1). A total of 16 plots (3 m x 3 m = 9 m² each) and three observation points per plot, located at different distances (Table 1) from managed honeybee colonies (Fig. 2),

were selected throughout the farms (one to three plots per farm depending on the size of the farm, plots separated by a minimum distance of 500 m).

Honeybee abundance surveys

In each 9 m² plot three observation points were selected. The observation points were at least one metre apart to avoid disturbance of insect foragers during observation. Sunflower heads at peak flowering (with approximately 50% of the florets open) that could be easily observed were selected (1-4 sunflower heads) at each observation point. We then recorded the number of insect visitors to the sunflower head during a 4 min period. In each plot, two survey visits (one in the morning, 09h00-13h00, and one in the afternoon, 14h00-17h00) were done with total observation time of 384 min (4 min x 16 plots x 3 observation points x 2 survey visits = 384 min). The number of colonies in proximity was recorded. Whenever identification of flower visitor species was not possible *in loco*, specimens were captured for later identification by taxonomists (see Acknowledgements). Wind speed, air temperature and relative humidity were recorded in each plot during the experimental work using an Omni-directional wind meter, thermometer and hygrometer (Skywatch Atmos, Switzerland)

Statistical analysis

To determine if the number of honeybee foragers counted at each observation point was influenced by increasing distance from honeybee hives, General Linear Mixed Models (GLMM) were used to test for differences in honeybee abundance (honeybees per head) along transects, using distances as independent variable and the different cultivars as the dependent variables. As error variance was homogeneous and data were normally distributed, we analysed data using Gaussian errors and t-tests on changes in deviance, using the R package nlme.

Exclusion experiment

In each sampling plot, 27 sunflower plants of similar physiological maturity were selected before the ray florets started opening: nine were bagged using white bag to exclude insect visitors throughout the flowering period and eighteen sunflower heads

(controls) were left un-bagged (Fig. 3). After the sunflower heads finished flowering, all pollinator exclusion bags were removed to ensure uniformity of seed development conditions. After full development was achieved (five months after planting), seed heads were harvested and placed in separate bags (the bags that was used to exclude insect visitors in the farm). Several events (*e.g.* mammal herbivores, diseases) caused a reduction of the initial number of sunflower heads, leading to a final number of 271 sunflower heads (133 bagged and 138 un-bagged). There were no differences in physical appearance between the fully developed (seed with developed embryo) and poorly developed (seed without developed embryo) sunflower seeds in some of the cultivars. Consequently, to assess the effect of honeybee visitation on pollination effectiveness and seed quality, we randomly selected 100 achenes from each sunflower head and measured yield (as the mass of 100 seeds), germination percentage and oil content.

Yield assessment

The effect of insect pollination on the mass of 100 seeds and oil content increment was checked by comparing the yield of bagged and un-bagged sunflower heads using the calculation developed by Morse and Calderone (2000).

$$\text{Yield increment (\%)} = \frac{(\text{mean yield from un-bagged heads} - \text{mean yield from bagged heads}) \times 100}{\text{mean yield from un-bagged heads}}$$

Oil content determination and germination percentage test

Oil content determination and the germination tests were done following the same procedure as in the previous experiment, but the samples in this germination percentage were different: Three flower heads were randomly selected per treatment (3 un-bagged and 3 bagged) for each plot (15); leading to a total of 90 flower heads being analyzed. Fifty seeds from each flower head were randomly selected for germination percentage assessment.

Statistical analysis

To determine the effect of distance to honeybee colonies (continuous variable), cultivar (categorical variable) and treatments (bagged and un-bagged) (categorical variable) on each of the response variables (mass of 100 seeds, germination percentage and oil content) a Generalized Linear Model (GLM) ANOVA was used. Post-hoc analyses were conducted to identify specific group differences. Analyses were done using the STATISTICA software windows version 7.0.61.0 (1984-2004).

Results

Effect of honeybees and other flower visitors on sunflower seed production and quality

Flower visitors

Average climate conditions of the experimental farm at the University of Pretoria during data collection were: air temperature 14.8⁰C, relative humidity 42.3%, rainfall 0.001 mm and wind speed 2.2 m/s. The sunflower visitor community was diverse, including insects from four orders. Hymenopteran visitors belonged to the families Sphecidea, Halictidae, Scoliidae, Megachilidae, Apidae and Ichneumonidae; while Dipteran visitors were identified as *Asarkina africana* Bezzi, 1908 (Syrphidae), *Eristalinus* sp. (Syrphidae), *Sarcophaga* sp. (Sarcophagidae), *Stomorphina rugosa* (Bigot, 1888) (Calliphoridae) and *Eristalis tenax* Linnaeus, 1758 (Syrphidae). Hymenoptera constituted the highest percentage of insects, while Coleoptera, Diptera and Lepidoptera were the least abundant orders (Table 2).

Mass of 100 seeds, seed yield (g) per 15 m², germination percentage and oil content

The only variable that varied significantly between the different pollination treatments was mass of 100 seeds. Total yield (g) per 15 m² means the seed yield (g) per 15 m² plot. The calculation of seed yield difference results in the seed production of sunflower plots visited by all types of insects being 8.3% higher than plots visited only by honeybees and 15.2% higher than plots isolated from all flower visitors. The seed yield increment of

sunflower crop was calculated using the formula according to the method and results revealed that there was a total of 11.02% seed yield increment. The post-hoc test (Tukey) revealed significant differences between the treatments in mass of 100 seeds, but did not show significant difference in yield per 15 m², germination percentage and oil content (Table 3). The overall model shows that there were no significant differences among the treatments: total yield per 15 m² ($F = 0.93$, $P > 0.47$ and $df = 2$), germination percentage ($F = 0.02$, $P > 0.98$ and $df = 2$) and oil content ($F = 0.33$, $P > 0.74$ and $df = 2$), but mass of 100 seeds shows significant difference ($F = 12.04$, $P < 0.02$ and $df = 2$).

Variation of the effect of managed honeybees at different distances to the sunflower plot and among cultivars

Flower visitor abundance

A total of 517 insects were recorded during the flower visitor surveys on commercial farms (Table 4). Average air temperature was $32 \pm 4^{\circ}\text{C}$, average wind speed was 0.9 m/s and average relative humidity was $44 \pm 9\%$. Dipteran flower visitors belonged to the Syrphidae and Sarcophagidae. Hymenopteran flower visitors included *Megachile frontalis* Smith (Megachilidae), *Tetraloniella* cf. *apicalis* (Friese) (Apidae), *Campsomeris* sp. (Scoliidae), Formicidae, *Apis mellifera* Linnaeus (Apidae) and *Lasioglossum* sp. (Halictidae).

Hymenoptera constituted the highest percentage of insects since there were honeybee colonies brought in, while Heteroptera and Lepidoptera were the least abundant orders (Table 4). Of the all insects visiting the commercial sunflower fields, honeybees were the most dominant with on average 1.54 honeybees/sunflower head recorded per 4 min observation period. A total of 416 honeybees were counted on 271 sunflower heads, accounting for 97.2% of all Hymenoptera recorded. The number of honeybee significantly declined with distance from managed honeybee colonies (Standardized within group residuals: $Q1 = -0.44$, Median = 0.05, $Q3 = 0.50$; Random effects: cultivar, StDev = 0.22; t-value (1, 27) = -2.5612, $P < 0.02$).

Sunflower seed yield

Mass of 100 sunflower seeds significantly declined with distance from honeybee colonies ($F = 32.65$, $P < 0.0001$ and $df = 1$) and it was also significantly varied between treatment (bagged vs. open sunflowers) ($F = 173.74$, $P < 0.0001$ and $df = 1$). There was no significant difference in mass of 100 sunflower seeds between different cultivars ($F = 1.82$, $P > 0.14$ and $df = 3$), but the interaction between treatment and cultivar was statistically significant ($F = 3.72$, $P < 0.03$ and $df = 3$). The result of the calculation according to the method indicated that insect visitation lead to a total mass of 100 seeds increment of 38%. The post-hoc test (Tukey) revealed significant differences between bagged and un-bagged sunflower heads and the different cultivars (Table 5). The mean mass of 100 seeds of the un-bagged sunflower cultivar was higher than the bagged sunflower cultivars in all the cultivars (Table 5).

Germination percentage

Germination percentage did not significantly decline with distance from honeybee colonies ($F = 0.47$, $P > 0.49$ and $df = 1$). Germination percentage varied significantly between treatments (bagged vs. open sunflowers) ($F = 46.8$, $P < 0.0001$ and $df = 1$) and between different cultivars ($F = 24.17$, $P < 0.0001$ and $df = 3$). Also, the interaction between cultivar and treatment was significant ($F = 8.85$, $P < 0.0001$ and $df = 3$). The calculation for comparison of treatments with respect to germination percentage revealed that seed crop in the un-bagged sunflower heads showed maximum germination percentage i.e. total of 95% seeds germinated as compared to that of bagged sunflower heads which gave total of 59% seeds germinated. The post-hoc test (Tukey) revealed significant differences between bagged and un-bagged sunflower heads and the different cultivars. The mean germination percentage of the un-bagged sunflowers was higher than the bagged sunflowers for all the cultivars (Table 6).

Oil content

Oil content declined significantly with distance from managed honeybee colonies ($F = 5.98$; $P < 0.02$ and $df = 1$). Oil content varied significantly between treatments (bagged vs. open sunflowers) ($F = 123.45$; $P < 0.0001$ and $df = 1$), and between different cultivars

($F = 51.78$; $P < 0.0001$ and $df = 3$). However, the interaction between cultivar and treatment was also significant ($F = 26.75$; $P < 0.0001$ and $df = 3$). The post-hoc test (Tukey) revealed significant differences between bagged and un-bagged sunflower heads and the different cultivars. The mean oil content of the un-bagged sunflower cultivar was higher than the bagged sunflower cultivars in all the cultivars (Table 7). The result of the calculation in oil content increment indicates that there was a total of 36% oil content increment due to insect pollination.

Discussion

Effect of honeybees and other flower visitors on sunflower seed production and quality

Sunflower seed yield and quality parameters (seed yield (g) per 15 m², germination percentage and oil content) did not show significant differences between the treatments (all insects, honeybees only, no insects) in the experimental farm at the University of Pretoria. However, higher mean yield and quality were recorded in open pollination (pollinated by all insects) compared to the other treatments. Contrary to the previous studies (Langridge and Goodman, 1974) germination percentage and oil content were not statistically significantly affected. Mass of 100 seeds was, however, significantly affected by insect pollination (Table 3). This agrees with previous studies (El-Sarrag *et al.*, 1993; Oz *et al.* 2009; DeGrandi-Hoffman and Chambers, 2006) which reported a significant difference in mass of 100 seeds due to insect pollination. The possible reason why the slight increment in oil content and germination percentage was not significantly affected by insect pollination is possibly due to the cultivar that is not very dependent on cross pollination and/or might be because of a weather effect. When the sunflower cultivar started setting seed a heavy winter frost and lower air temperature (14.8°C) occurred, harming the plant (the sunflower head was wilted) and this may have confounded the effect of treatments on all parameters evaluated.

Variation of the effect of managed honeybees at different distances to the sunflower plot and among cultivars

Large scale field results indicated that pollinators are very important for sunflower crop seed yield and quality. This is in agreement with previous studies (Langridge and Goodman, 1981; Neff and Simpson, 1990; Joksimovic *et al.*, 2005; Greenleaf and Kremen, 2006; Cantamutto and Poverene, 2007; Ndreritu, *et al.*, 2008), but contradicts the results obtained in the experimental farm with the cultivar Monsanto K4040, suggesting that this cultivar is less dependent on cross pollination than the most common cultivars used in commercial sunflower farms. This explanation is supported by Krause and Wilson (1981) who showed that cultivar differences can cause yield differences. Mass of 100 seeds and oil content declined significantly with increasing distances from the managed honeybee colonies, revealing that the highest honeybee population and highest production of sunflower was found near honeybee colonies, this indicated that honeybee foragers prefer near distance to far distance if the same resource is available.

Honeybees were by far the most frequently recorded insects on sunflower. The high proportion of honeybees compared to other insects visiting the flowers indicated that honeybees were the major pollinators of the sunflower crop at our field site, with both honeybee abundance and seed yield decreasing with increasing distance from the colonies. In a previous study conducted in the same region of South Africa, honeybees were also ranked first in terms of abundance (du Toit, 1988). Du Toit (1988) concluded that the low presence of other insects can be due to the intensive cultivation of crops and application of chemicals. Other findings indicated that honeybees comprised 99% of flower visitors in sunflower crops in Australia (Langridge and Goodman, 1974, 1981). Ndreritu *et al.* (2008) in Kenya also reported that on average honeybees represented 72% of the individuals of bee species observed. Johannsmeier and Mostert (2001) observed that honeybee foragers were more efficient up to 100 m from the hive and started decreasing in number after 250 m. Steffan-Dewenter and Tschardt (1999) reported that, habitat isolation affects both bee diversity and seed set of self-incompatible crop plants. Generally, we can conclude that pollination services increased with increasing proximity

to managed honeybee colonies and honeybees were by far the main floral insect observed on sunflower cultivars.

In this experiment we noted that there was significant interaction between different sunflower cultivars and treatment with respect to the mass of 100 seeds. This would seem to indicate that some of the cultivars (e.g. Pannar 7033) had large individual seeds compared with the other cultivars (Table 5). Other researchers have reported that there was significant difference in mass of 100 seeds between different cultivars (e.g. cougar and hybrid 894) collected from bagged sunflower heads (Krause and Wilson, 1981).

Un-bagged sunflower heads had significantly more developed seeds and higher total mass of 100 seeds than bagged heads. These results are in agreement with those of Low and Pistillo (1986) who observed an increase in seed yield in open pollinated compared to that of bagged and different cultivars differ in their seed weight. Kamler (1997) reported different sunflower cultivars react differently to pollination. Putman *et al.* (1990) reported that all sunflower varieties produce some sterile seeds in the absence of pollination, but varieties differ in their degree of dependence on insect pollination. In another study Krause and Wilson (1981) reported that, un-bagged (open) sunflower heads had significantly more developed seeds and higher total seed weight than bagged heads. A wide range in terms of sunflower seed yield increment was reported in different countries due to insect visitation (Table 8). The differences in the seed yield increment can be attributed to the fact that researchers used different sunflower cultivars than the cultivars we used here or may also attributed to other factors such as weather conditions.

Germination percentage significantly differed and is in agreement with previous studies (Langridge and Goodman, 1974; Paiva *et al.* 2003) where germination percentage was higher in the un-bagged sunflower heads. Furthermore, cultivar differences contributed significantly to variation in germination percentage in all the treatments. In this experiment we found that germination percentage of the sunflower crop was improved and this improvement might be as a result of insect pollination mainly honeybees.

Seeds obtained from un-bagged sunflower heads had significantly higher oil content compared to from bagged heads. Oil content decreased significantly as the distance from honeybee colonies increase. A wide range of oil content increment has been reported in different countries due to insect pollination (Table 9). Krause and Wilson (1981) reported that, even though there was no statistically significant difference in percent oil content between un-bagged and bagged sunflower cultivars, an increment in the mean oil content of un-bagged (36.9%) and bagged (35.2%) sunflower cultivars was observed. In our study, there were also marked differences in oil content increment among the different sunflower cultivars due to insect pollination. For instance, Cultivar Panner 7355 and Pannar 7033 had higher mean oil content in the open pollination than the bagged once, whereas for Syngenta and Monsanto 6822 the mean oil content increment was less (Table 7). The high self-fertility of Syngenta and Monsanto 6822 may explain the low yield increment (bagged versus un-bagged sunflower heads) compared to other cultivars.

Implications of results to sunflower farming

While a wide range of insects visited the commercial sunflower crop in Settlers as well as the University of Pretoria experimental farm, the most important insects contributing to pollination of sunflower crop were Hymenoptera (mainly honeybees), Coleoptera, and Diptera. Counting insects at increasing distances from honeybee colonies confirmed that; as the distance increases, honeybee forager number decreases. Seed quantity and quality also followed this pattern. Other natural pollinators did not fill the gap of the decreasing number of honeybee foragers because in this study we used fields far from natural habitat; it is also likely due to the low number of other natural pollinators. If we assume that wild honeybee populations and other natural pollinators are invariably not adequate for sunflower pollination, bringing in honeybee colonies to sunflower farms may be an easy and simple way of producing high yield and good quality seeds.

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Table 1. Commercial sunflower farms details

Farm no	Cultivar name	Number of colonies	Distance from colonies (m)	Total number of plots
1	Pannar 7033	8	90, 500	2
2	Pannar 7033	59	1100	1
3	Pannar 7033	43	230, 520, 1070	3
4	Pannar 7033	2	985	1
5	Pannar 7355	10	1900	1
6	Pannar 7355	15	1200	1
7	Pannar 7355	15	100, 540, 1000	3
8	Syngenta	19	70, 490, 970	3
9	Monsanto 6822	10	990	1

Table 2. Insect visitors on sunflower crop at University of Pretoria experimental farm

Insect order	Total number	Percentage of total (%)
Hymenoptera	479	45%
Coleoptera	272	25%
Diptera	209	19%
Lepidoptera	115	11%
Total	975	100%

Table 3. Effect of pollination on seed quantity and quality (mean $\pm$ SD) of sunflower crop at University of Pretoria experimental farm, the results of the General Linear Model (GLM) with post-hoc test (Tukey) are shown.

Parameters	all insects	only honeybees	no insects
Mass of 100 seeds (g)	5.22 ^a $\pm$ 0.89	4.78 ^{ab} $\pm$ 0.45	4.29 ^b $\pm$ 0.38
Total yield (g) per 15 m ²	2046.60 ^a $\pm$ 1050.74	1734.30 ^a $\pm$ 344.98	1508.7 ^a $\pm$ 232.38
Germination (%)	28 ^a $\pm$ 8.54	24 ^a $\pm$ 7.81	22 ^a $\pm$ 7.23
Oil content (g/100g DM)	35.14 ^a $\pm$ 0.39	36.08 ^a $\pm$ 3.94	33.89 ^a $\pm$ 2.76

Means in the same row having same letters in common are not significantly different at 5% level of probability.

Table 4. Total number of insect orders recorded at 16 sampling plots at Settlers commercial sunflower farms during four minutes observation per sampling point (3 points per plot)

Insect orders	Total number of insects	Percentage total (%)
Hymenoptera	428	82.79%
Coleoptera	59	11.41%
Diptera	14	2.71%
Heteroptera	4	0.77%
Lepidoptera	3	0.58%
Not identified	9	1.74%
Total	517	100%

Table 5. Comparing the effects of treatments (bagged v. un-bagged) and cultivars on the mass of 100 seeds (mean $\pm$ SD) of sunflowers at Settlers commercial sunflower farms. Overall statistics: $R^2 = 0.51$, $n = 271$ and $P < 0.01$. The results of the Generalized Linear Model (GLM) with post-hoc test (Tukey) are shown.

Cultivar	Treatments	
	Bagged	Un-bagged
Pannar 7033	3.57 ^{cd} $\pm$ 0.71	5.79 ^a $\pm$ 1.10
Pannar 7355	3.41 ^d $\pm$ 1.20	4.98 ^b $\pm$ 1.36
Syngenta	3.03 ^d $\pm$ 0.19	5.70 ^a $\pm$ 1.42
Monsanto 6822	2.6 ^d $\pm$ 0.96	4.82 ^{bc} $\pm$ 1.10

Means within column and row sharing the same letters are not significantly different at 5% level of probability and.

Table 6. Comparing the effects of treatments (bagged v. un-bagged) and cultivars on germination percentage (mean $\pm$ SD) of sunflowers at Settlers commercial sunflower farms. Overall statistics: $R^2 = 0.70$, $n = 90$ and $P < 0.01$. The results of the Generalized Linear Model (GLM) with post-hoc test (Tukey) are shown.

Cultivar	Treatments	
	Bagged	Un-bagged
Pannar 7033	66 ^b $\pm$ 21	98 ^a $\pm$ 4.8
Pannar 7355	30 ^c $\pm$ 13.7	86 ^{ab} $\pm$ 24
Syngenta	83 ^{ab} $\pm$ 8.8	100 ^a $\pm$ 1
Monsanto 6822	93 ^{ab} $\pm$ 12.7	100 ^a $\pm$ 0

Means within column and row sharing the same letters are not significantly different at 5% level of probability.

Table 7. Comparing the effects of treatments (bagged v. un-bagged) and cultivars on oil content (mean $\pm$ SD) of sunflowers at Settlers commercial sunflower farms. Overall statistics: $R^2 = 0.86$, $n = 90$ and $P < 0.01$. The results of the Generalized Linear Model (GLM) with post-hoc test (Tukey) are shown.

Cultivar	Treatments	
	Bagged	Un-bagged
Pannar 7033	28.6 ^d $\pm$ 7.26	37.8 ^{bc} $\pm$ 3.20
Pannar 7355	12.2 ^e $\pm$ 2.88	38.0 ^{bc} $\pm$ 3.91
Syngenta	35.3 ^c $\pm$ 4.09	43.2 ^{ab} $\pm$ 2.52
Monsanto 6822	39.9 ^{abc} $\pm$ 1.84	47.6 ^a $\pm$ 1.17

Means within column and row sharing the same letters are not significantly different at 5% level of probability.

Table 8. Amount of sunflower yield increased (%) in different countries

Number	Authors	Country	Amount of yield increased (%)
1	Langridge and Goodman (1974)	Australia	60
2	Langridge and Goodman (1981)	Australia	11
3	du Toit (1988)	South Africa	38
4	Kamler (1997)	Czech Republic	26
6	Paiva <i>et al.</i> (2003)	Brazil	93.6
7	Nderitu <i>et al.</i> (2008)	Kenya	53
8	Oz <i>et al.</i> (2009)	Turkey	93-94
9	This study	South Africa	38

Table 9. Amount of sunflower oil content increased (%) in different countries

Number	Name of people	country	Amount of oil increased (%)
1	Langridge and Goodman (1974)	Australia	3
2	El-Sarrag <i>et al.</i> (1993)	Sudan	30
3	Kamler (1997)	Czech Republic	2
4	Nderitu et al. (2009)	Kenya	17.9
5	This study	South Africa	36



Fig. 1. Insect exclusion treatments were applied: (A) open pollination and plots caged without insects; (B) honeybee colony with four frames inside a cage.



Fig. 2. Managed honeybees distributed for sunflower crop pollination at Settlers commercial sunflower farms.



Fig. 3. Insect exclusion treatments in sunflower crop pollination at Settlers commercial sunflower farms, including bagged and un-bagged sunflower heads in three parallel rows at different distances.

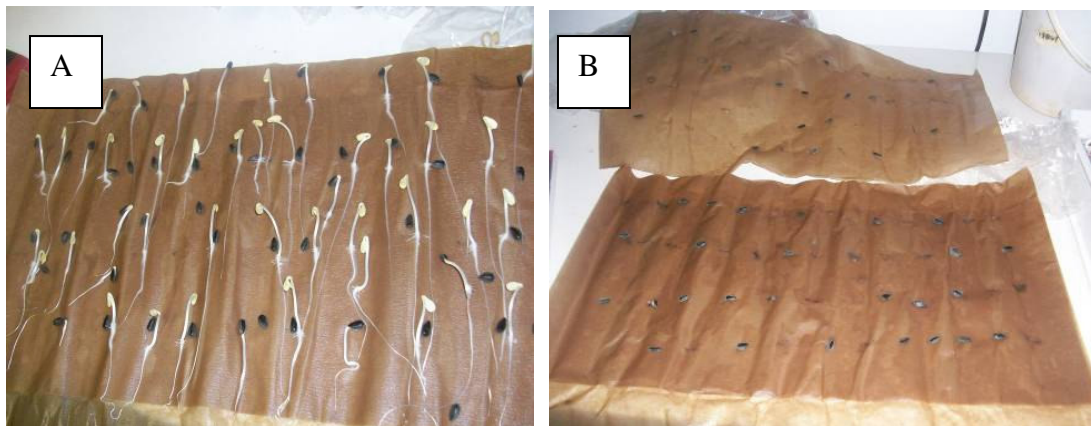


Fig. 4. Germination percentage of sunflower seeds: (A) Sample from un-bagged sunflower head and; (B) sample taken from bagged sunflower head attached to Anchor germination paper during the fourth day of germination time (first count).

CHAPTER 3

Preliminary observations on foraging behaviour of honeybees

Introduction

Pollination is an “accidental” action performed by honeybee foragers when collecting nectar and/or pollen from flowering plants for their own energetic needs and to provide the colony with food. Intensive pollination is essential in modern agricultural practices in order to obtain greater yield and quality of fruits, nuts, vegetables, fruit and seeds of crops (McGregor, 1976; Morse and Calderone, 2000). Honeybees play an effective role in the pollination of sunflower but their function in pollination depends on environmental conditions, such as temperature, field size, availability of water and competing nectar and pollen sources (du Toit, 1988). Transporting hives into agricultural fields for intensive pollination has increased sunflower seed yield and quality (Langridge and Goodman, 1974; McGregor, 1976; Langridge and Goodman, 1981; Low and Pistillo, 1986; Free, 1993; Paiva *et al.*, 2003; Morse and Calderone, 2000, Aizen and Harder, 2009, Oz *et al.*, 2009).

Various biotic (e.g. size of nectary, position of flower on plant, diameter of shoot, cultivar, and age of flower) and abiotic (e.g. weather, soil characteristics) factors can influence nectar production (du Toit, 1988; Johannsmeier and Mostert, 2001), indirectly affecting honeybee foraging activity. A scarcity of rain and/or moisture stress during flowering negatively affects nectar production, while hot weather and high atmospheric humidity is conducive to maximum nectar production (Johannsmeier and Mostert 2001). Du Toit (1988) showed that periods of continued high or low temperature and rainy weather have a direct influence on sunflower nectar production and may result in low honeybee foraging activity. Moreover, environmental factors (wind, temperature, availability of water) can also affect honeybee foraging activity directly (du Toit, 1988; Ajdare, 1990; Martins, 2004). Honeybee foraging activity is good at relatively high

temperature (up to 35°C) and decreases when the temperature is below 20°C or remains above 37°C for a period of time (Adjare, 1990). Martins (2004) reported that honeybee foraging activity patterns varied with temperature throughout the day. Honeybee pollen collecting activity is also known to vary through the day (Zaitoun and Vorwohl, 2003), being higher early in the morning and in the afternoon for sunflower crops (Free, 1993). This peak foraging time in the morning is probably related to anther dehiscence. However, for other crops the peak of honeybee foraging activity can occur at a different time of the day (Yücel and Duman, 2005). It is possible that honeybee foragers precisely synchronize their foraging activity with the time of availability of the food source (Moore *et al.*, 1989); however further studies are needed to clarify the influence of environmental factors and food availability on the daily pattern of foraging activity of honeybee foragers. The objective of this study was to record observations on the initiation, cessation and peak hours of honeybee foraging activities, to compare the amount of bee collected pollen and to determine the influence of weather conditions on honeybee visits to sunflower head inflorescences.

Materials and methods

Honeybee foraging activity

To investigate the daily pattern of honeybee foraging activity, observations on the foraging behaviour of managed honeybees were done in nine hives placed at the border of commercial sunflower farms in South Africa (Settlers, Limpopo, 27° 57'S, 28° 32'E) in April 2009. The number of honeybees entering the hive for a period of 5 minutes was recorded each hour from early morning (7h00) until late evening (18h00) (Fig. 5A) for one day (04/ April 2009), this comparison was done for one day because we were comparing the foraging activity of honeybees between the times of the day in a bright sky, and this research was also conducted as a preliminary study for further investigation. Honeybees entering the hive with pollen in their corbiculae were classified as pollen collectors, and those without as nectar collectors. In addition, the weight of collected pollen was determined by placing pollen traps in front of the entrance of three hives early in the morning (before observations) and removing the pollen collected at 10h00, 14h00

and 18h00 (Fig. 5B). To test the impact of weather conditions of the day on honeybee activity, air temperature and relative humidity were recorded during the experimental work using a thermometer and hygrometer (Skywatch Atmos, Switzerland) in each observation time from early morning (07h00) until late evening (18h00).

Statistical analyses

The effect of time of the day on honeybee abundance data (total honeybees, nectar collectors and pollen collectors), was tested using the statistical package SAS (Software Institute 2003, Windows version 5.1.260.), and the means were compared using General Linear Model (GLM) by one-way ANOVA. Factorial ANOVA was used to determine the effects of temperature and relative humidity on honeybee abundance. Pollen weight of the three honeybee colonies against the time of the day was also analysed using General Linear Model (GLM) by one-way ANOVA.

Results

Honeybee foraging activity

A total of 31,617 honeybees were counted during the whole day on the nine colonies; of which 84.5% (26,708) were nectar collectors and 15.5% (4,909) were pollen collectors. The number of nectar and pollen collectors varied significantly with the time of the day (nectar collectors: $F = 24.42$, $P < 0.0001$ and $df = 11$; pollen collectors: $F = 9.51$, $P < 0.0001$ and $df = 11$), as did the total number of honeybees ($F = 26.10$, $P < 0.0001$ and $df = 11$). The lowest number of honeybees (35 pollen collectors and 239 nectar collectors) was recorded at 18h00, whereas the highest number of honeybees (3,188 nectar collector and 902 pollen collector) was recorded at 11h00 (Fig. 6). Total number of foragers typically increased in the morning at 9h00 and peaked at 11h00 and then gradually declined towards the end of the day (Fig. 6).

Temperature and relative humidity vary inversely throughout the day, consequently honeybee foraging activity (number of honeybees per 5 min) varied with air temperature and relative humidity (Fig. 7 and 8). The results of the analyses indicated that number of

honeybees per 5 min varied significantly with temperature and humidity (Temperature: $F = 19.4$, $P < 0.0001$ and $df = 18$) relative humidity ($F = 3.44$, $P < 0.001$ and $df = 12$) and interaction temperature-relative humidity ($F = 1.33$, $P < 0.253$ and $df = 1$). Early in the morning (07h00) when average temperature was $18.7 \pm 0.7^\circ\text{C}$ and average relative humidity was $74.3 \pm 1.3\%$ honeybee activity commenced, although abundance was very low. Honeybees attained their maximum foraging activity at 11h00 when the average hourly temperature was $31 \pm 0.5^\circ\text{C}$ and average relative humidity was $42 \pm 0.7\%$. Later at 18h00, a sharp decline in honeybee foragers was observed with decrease in average air temperature to $25 \pm 0.4^\circ\text{C}$ and increase in average relative humidity to $45 \pm 0.9\%$.

Although the mean weight of pollen decreased with the time of day in the three colonies, this effect was not significant ($F = 0.15$, $P > 0.86$ and $df = 2$). The largest sunflower pollen weight was obtained at 10h00 (16.11 ± 7.38 g) and 14h00 (16.08 ± 5.24 g), but a relatively low mean weight was recorded at 18h00 (11.66 ± 4.27 g).

Discussion

This study confirms that foraging behavior of honeybees on a sunflower crop varies during the day. Similarly, observations by du Toit (1988) in the area showed a steep increase of honeybee activity early in the morning (6h30) and the peak was reached between 9h00 and 10h00. The results also agree with those of Nderitu *et al.* (2008) who reported that the number of both *Apis* and non-*Apis* bee foragers on sunflowers peaked between 10h00 and 14h00. Yücel and Duman (2005) showed, for onion crops in Turkey higher activities of *Apis mellifera* workers between 11h00 and 12h00. Fell (1986) in Virginia reported that total number of honeybee foragers typically increased in the morning and peaked between 13h00 and 14h00 and then gradually decreased towards the end of the day. Paiva *et al.* (2002) observed a higher foraging activity of honeybees at 14h00. Ajare (1990) concluded that honeybee foraging activity varies with time of the day, but no honeybee forager was found by 18h30.

In agreement with the results obtained for foraging activity, there were great hourly fluctuations in the average weight of the pollen loads carried by honeybee workers, carrying more pollen loads during the morning (du Toit, 1988). However, honeybee activity changes may also be related to the change in the resource foraged (nectar vs. pollen) and due to the colony fulfilling its food requirements, with previous studies showing that the peak of activity occurs sooner for pollen foragers than for nectar collectors (du Toit and Swart, 1994). Similarly to our findings a study conducted in Brazil by Paiva *et al.* (2002) showed that nectar gatherer honeybee foragers in sunflower crop were more abundant than pollen collectors. Nectar collectors are very important to sunflower crop pollination in Settlers because of their numerical dominance. In addition, nectar collectors settled among the florets forcing their head to reach the nectary, this resulted in bees being copiously dusted with pollen leading to better pollination.

In accordance with previous studies (Adjare, 1990; Puškadija *et al.*, 2007), the results show that temperature and humidity affect honeybee foraging activity. It is therefore likely that pollination could be more effective if flowering occurs during warm and clear weather (Adjare, 1990). The peak foraging activity of honeybees recorded at 11h00 could be because of the favourable conditions such as temperature. Such factors are shown to have great contribution to honeybee flight (Adjare, 1990). The change in honeybee number may be related to light intensity and wind speed. Reports show that honeybees are fully active at temperatures higher than 14°C and solar radiation higher than 300w/m² (Vicens and Bosch, 2000). Honeybees do not leave the hive at temperatures lower than 14°C or at wind speeds higher than 30km/hr (Adjar, 1990). Furthermore, in agreement with Puškadija *et al.* (2007), our results also showed that the foraging activity of honeybees increased as temperatures increased up to 31±0.5°C, but when the average temperature exceeded 31±0.5°C, foraging intensity tended to plateau and then decline (Fig. 7). Other factors like colony stores, amount of available pollen and honey in the hive, the cyclic nectar and pollen production at the commercial sunflower farm, light intensity, total brood in the colony and fanning behavior of honeybees for ventilation of the colony during high temperatures may also have effects on the number and behavior of

honeybee foraging activities. Future studies on honeybee pollination should thus be restricted to times of the day where resource availability is likely to be the same.

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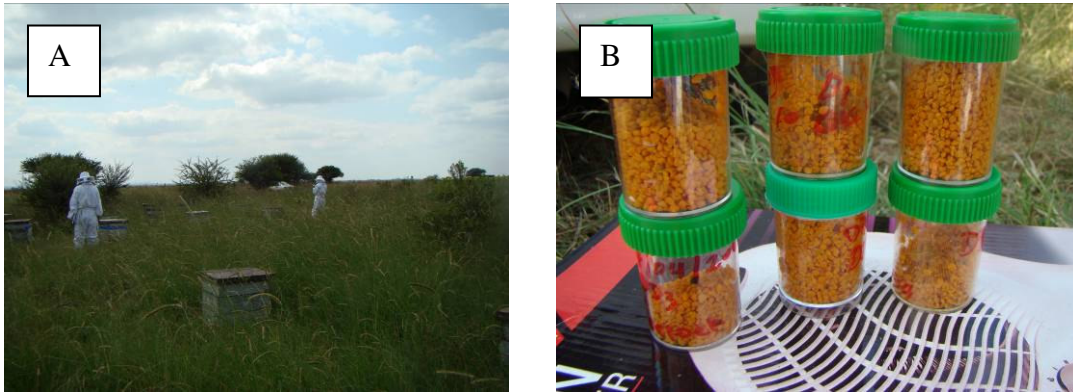


Fig. 5. Foraging activity of managed honeybees: (A) different honeybee colonies distributed at the border of sunflower crop with observations taking place on the number of pollen collectors and nectar collectors at the hive entrance; (B) sunflower pollen collected using a pollen trap at 10h00, 14h00 and 18h00 from three different colonies.

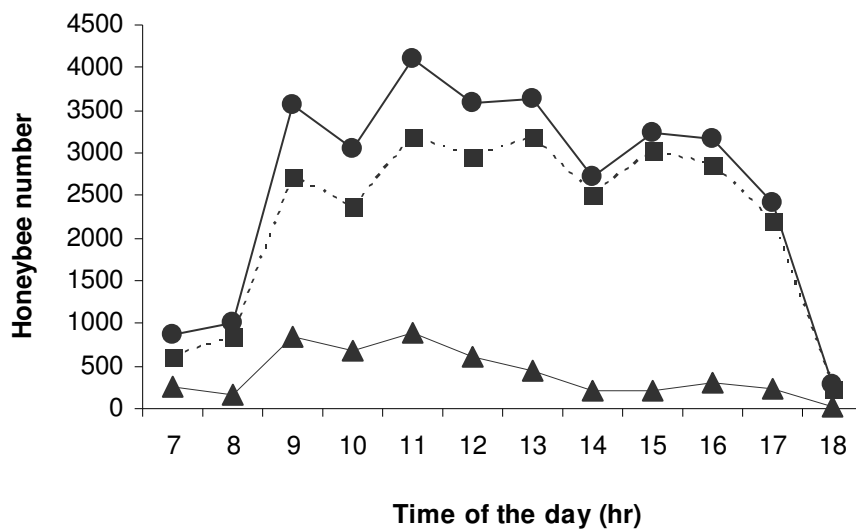


Fig. 6. Total number of honeybees (—●—), nectar collectors (---■---) and pollen collectors (—▲—) observed at different time intervals at Settlers commercial sunflower farms during 07/ April 2009.

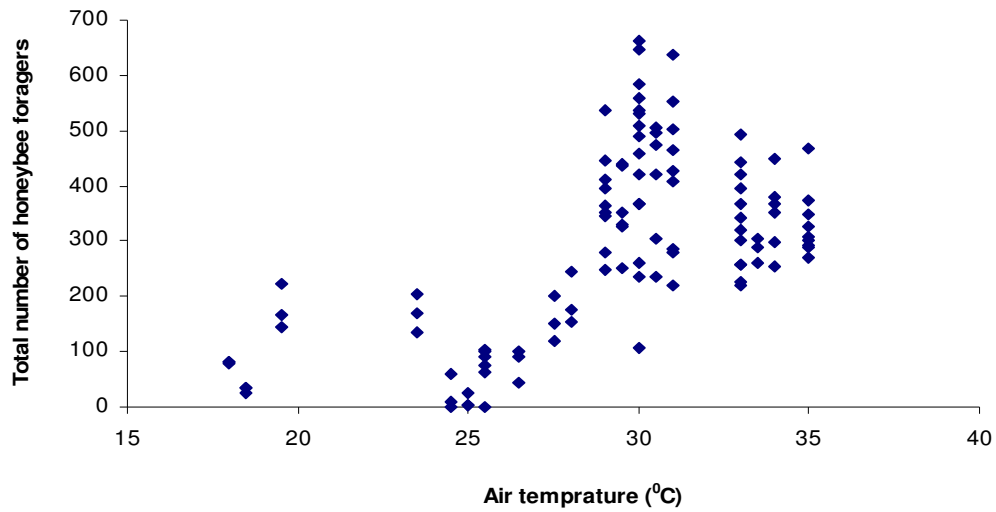


Fig. 7. Influence of air temperature (°C) on number of honeybees visiting sunflower inflorescences during peak flowering at Settlers commercial sunflower farms during 07/ April 2009.

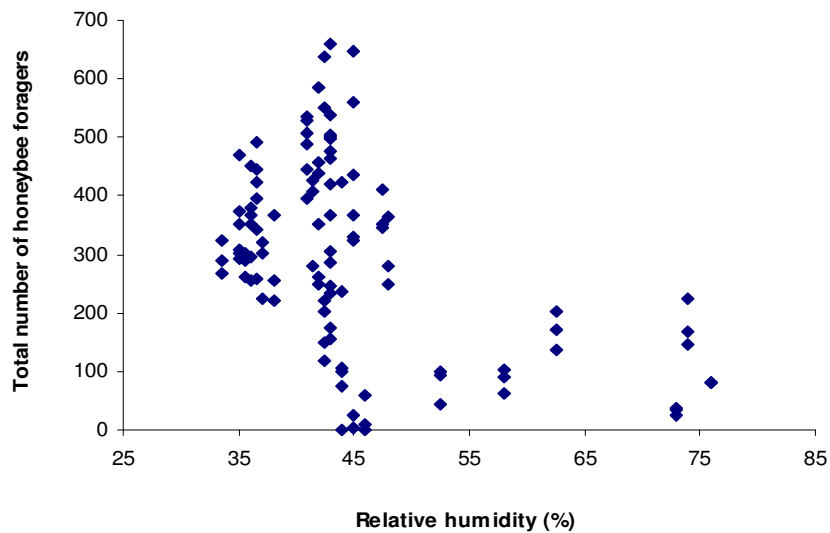


Fig. 8. Influence of relative humidity (%) on number of honeybees visiting sunflower inflorescences during peak flowering at Settlers commercial sunflower farms during 07/ April 2009.

CHAPTER 4

General discussion

Most agricultural and horticultural crops are adapted for insect pollination (Free, 1993), sunflower being one of these (Sunmangala and Giriraj, 2003; Ndreritu, *et al.*, 2008). The results presented in this thesis reveal that honeybees and other insect visitors are very important for sunflower pollination and the most dominant insects in the experimental areas were honeybees. As a result, the mass, germination percentage and oil content of sunflower seeds are significantly affected by insect pollination.

Sunflower pollination in South Africa

The research results from the University of Pretoria Experimental Farm indicated that yield and quality of sunflower seeds were slightly increased as a result of insect pollination, but most of the parameters except seed mass were not statistically significantly different between treatments (Chapter 2). Other studies elsewhere have reported that, even though different cultivars exhibit different degrees of self-fertility, the absence of insect visitors, particularly honeybees, has an adverse effect on seed yield and quality of commercial sunflower (Kamler, 1997). The absence of significant differences in this study might be because of the cultivar we used and/or because of adverse weather effects, i.e. at the time when the sunflower starts setting seed heavy winter frost occurred. Frost damage could have harmed the plant and possibly confounded the effect of treatments on all parameters evaluated. In another study, Holman *et al.* (2004) reported that compared with spring wheat and canola, sunflower crop has a low frost tolerance.

Among the diverse sunflower visitors at Settlers commercial farms, the most abundant and important insects believed to contribute to sunflower pollination were hymenopterans and of those, the most influential (97%) were honeybees. This high number could be because of honeybee colonies physically brought to the commercial farms for effective sunflower pollination. Therefore, honeybees are likely very important

for sunflower pollination because of their large number of hairs, their behavioural patterns and their numerical dominance.

In this experiment isolation from honeybee colonies leads to significant declines in the number of honeybee foragers and in the yield and quality of sunflower seeds. Previous studies elsewhere in the world reported that, the abundance of honeybee depends primarily on distance from the nearest apiary (Steffan-Dewenter, 1999). This also fits well with the expected negative relationship between foraging distance of honeybees and seed yield of sunflower (Free, 1993). The decreasing abundance of honeybee foragers may be a result of their preference for collecting nectar and pollen near to their hives (Paranhos *et al.*, 1997; Blanche *et al.*, 2006). Therefore, we can conclude that the quality and quantity of sunflower seed increased with increasing proximity to managed honeybee colonies and honeybees are the most important pollinating insects for commercial sunflower crops.

Insect pollination increased the total mass of 100 seeds by about 38% (see results of exclusion experiment, Chapter 2). Studies elsewhere in the world have also reported increases in sunflower seed yield through insect pollination of 38% (du Toit 1988, in South Africa) and 53% (Ndreritu *et al.* 2008, in Kenya). Our results are also in agreement with previous studies (Langridge and Goodman 1974; Krause and Wilson 1981; du Toit, 1988; Kamler, 1997; Paiva *et al.*, 2003; Nderitu *et al.* 2008; Oz *et al.* 2009) in which it was reported that, despite the difference in amount of increment, sunflower seed yield increased on exposure to insect pollination. In conclusion, this study revealed that sunflower is largely dependent on honeybee pollination for increasing seed yield, and moving honeybee colonies to commercial sunflower farms during the flowering period is the most essential input for sunflower seed production.

Seeds obtained from un-bagged sunflower heads had significantly higher oil content compared to those obtained from bagged heads, and significant differences were also observed between the different cultivars. In agreement with previous studies (Langridge and Goodman, 1974; Krause and Wilson, 1981; El-Sarrag *et al.*, 1993; Kamler, 1997;

Nderitu *et al.*, 2009), the absence of insect pollinators had an adverse influence on increment of oil content in commercial sunflower crops. Furthermore, as also illustrated before (Langridge and Goodman 1974; Paiva *et al.*, 2003), this study showed that insect pollination of sunflowers led to significant increases in germination rates (Chapter 2). Although overall insect pollination led to differences in germination percentage, paired comparisons between cultivars revealed that only the most common cultivars in the study year (Pannar 7355 and Pannar 7033) show a statistically significant difference in germination percentage, whereas germination of less common cultivars (Syngenta and Monsanto 6822) did not vary significantly with insect pollination. Syngenta and Monsanto 6822 might have higher self-fertility ability than Pannar 7355 and Pannar 7033. It is concluded that the reason for the reduction in germination percentage in the bagged sunflower heads in all the cultivars could be the absence of insect pollinators, which are mainly honeybees. All the cultivars used in this study were self-pollinated and self-fertile, but there was an overall increase in seed mass, germination percentage and oil content when the sunflower head was exposed to insect pollination.

The single head bag method is the most important method to exclude insects from visiting the sunflower head (du Toit, 1988 and Free, 1993), but the uncertain mechanical pollination occurring as a result of the bag touching the florets in windy conditions has to be considered. From this study we observed that, as distance increases from honeybee colonies, honeybee per head was significantly decreased resulting in decreasing seed set, germination percentage and oil content. Therefore, not only placing honeybee colonies in the commercial sunflower farm, but also the distribution of colonies to give thorough coverage of the entire crop is also very important. It may also be vital to consider the strength of the colony (the number of honeybee foragers in the hive).

The daily pattern of honeybee foraging activity fluctuated during the course of a day. A steep increase was observed in the morning after light intensity made flight possible, this trend increased and moderate foraging activity was reached at 9h00. After this, activity was more or less constant throughout the day till late afternoon, when a sharp decline in number of honeybee forgers was observed until foraging ceased after 18h00. The

decrease in foraging activity and number of honeybee workers in the afternoon might be due to the colony fulfilling its food requirements or it might be related to temperature, relative humidity and nectar and pollen flow change over the day. These results are partially in accordance with those of Sabir *et al.* (1999) who observed that honeybee foragers foraging from 7h00-15h00 and the most intense visitation was between 11h00-12h00. This variation in honeybee numbers might be affected by the amount of available pollen and nectar. For instance there were hourly fluctuations in the average weight of pollen loads carried by honeybee workers, more pollen load was collected from the pollen trap at (10h00) flowed by 14h00 whereas relatively little pollen was collected at 18h00. Honeybee foraging activity also depends on weather and temperature: as temperatures increased, so did the foraging activity of honeybees, but once average temperature reached approximately $31\pm0.5^{\circ}\text{C}$, foraging intensity tended to plateau and then slightly decreased. A total of 84.5% nectar collector and 15.5% pollen collector honeybee foragers were observed from this experiment in 07/April 2009. The high number of nectar collecting compared to pollen collecting honeybee foragers may be related to the fact that pollen is important for the diet of the honeybee colony (Crailsheim *et al.*, 1992; Human and Nicolson, 2006), whereas nectar is used for making the large amount of honey after the extra moisture content is removed and elaborated by the honeybees (Crailsheim *et al.*, 1992; Abu-Tarboush *et al.*, 1993). This means that more nectar than pollen collector honeybees visit sunflowers throughout the day, with first mentioned being very important to sunflower crop pollination in Settlers.

Conclusion

The following main findings can be reported from this study:

1. Honeybees (*Apis mellifera* L.) were the main pollinator of sunflower crop.
2. Honeybee abundance was influenced by the distance of the field from the colony.
3. Sunflower seed yield and quality were also influenced by the distance of the field from the honeybee colony, most probably because of decreasing honeybee abundance.
4. Total exclusion of pollinators in Settlers area may result in a loss of about 38% mass of 100 seeds, 38% germination percentage and 36% oil content.

5. Seed production of sunflower plots visited by all types of insects in University of Pretoria experimental farm was 8.3% higher than plots visited only by honeybees and 15.2% higher than plots isolated from all flower visitors.
6. The difference in seed yield and quality from bagged sunflower heads among the cultivars could be indicative of cultivar difference for the response of pollination.
7. Honeybee foraging activity depends on daily weather and temperature and the daily pattern of honeybee foraging activity fluctuated during the course of a day.
8. Nectar collector honeybees were more abundant than pollen collectors throughout the day and those foragers are very important to sunflower crop pollination in Settlers.

Recommendation and implications

Pollination is an essential input in the production of the sunflower crop and this crop requires pollination for improving quality as well as enhancing yield. As modern agricultural production has come to rely on large monocropping farms, dependence on wild insects living on the surrounding area for pollination has become less feasible. This is because of a decline in the availability of wild pollinators, likely due to disturbance of nesting habitats and food sources with the introduction of modern agricultural practices. Our results demonstrate the great importance of insect pollinators, essentially honeybees, on seed yield, germination percentage and oil content, since caged and bagged sunflower heads produce lower quality seeds than the open heads. It is recommended that moving honeybee colonies to sunflower production areas during the flowering period is essential for maximum seed production and quality increment. Combining all parameters measured with pollination, cultivars Pannar 7033 and Pannar 7355 were the most responsive cultivars to pollination. Therefore, using the advantage of the information provided by the results from this study, planting these cultivars in areas of no insect pollinators present may lead to a reduction in sunflower seed yield and quality.

This study did not investigate the exact distance and direction of honeybee colonies for good pollination of sunflower crop. In addition the exact number of honeybee colonies needed for maximum production of sunflower crop was not investigated. The potential of

sunflower crop for honey production that may be important for beekeepers was also not investigated further. Therefore, future research about these would complement this study.

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APPENDIX 1

Differential attractiveness to honeybee foragers of sunflower cultivars and flowering stages

Introduction

The frequency of interactions between plants and pollinators depend on the plant floral rewards and the energy needed for the pollinators (Free, 1993). Plant floral rewards can differ greatly from one plant species to another, as well as among different cultivars of the same species (Abrol, 2007), and even flowers on a single plant (Goulson *et al.*, 2007). Honeybee foragers may respond differently to genetically modified cultivars of the same crop (Srivastava and Singh, 2006). Previous studies revealed that honeybee foraging preference has a positive relationship with nectar production and nectar availability of sunflower crop (Fell, 1986; Kamler, 1997; Zaj  cz, *et al.* 2008). Honeybee foragers may be differently attracted to different sunflower cultivars because of pollen/nectar availability (Fell, 1986) and it can also be affected by pollen quality (Cook *et al.*, 2003).

Materials and Methods

To evaluate whether honeybee foragers discriminate between sunflower cultivars and different floral stages, a three day survey was conducted on 17 sunflower cultivars at Toowoomba Agricultural Research Station (Limpopo, South Africa, 27⁰ 57'S, 28⁰ 32'E) in March 2008. All cultivars were flowering but the flowering stage differed between cultivars. For each cultivar, the visitation frequency of honeybees was recorded by walking slowly along two transects (70 m long), one in the morning (10h00-11h30) and another in the afternoon (15h00-16h30). Whenever a honeybee was detected, the sunflower floral stage (25%, 50%, 75% and 100% developed florets) was recorded.

Statistical analyses

Factorial ANOVA was used to determine the effects of cultivar and flowering stage on honeybee abundance. Data was analyzed using the statistical package SAS (software institute 2003, windows version 5.1.260).

Results

During the study period, a total of 2581 honeybees was recorded on the 17 sunflower cultivars. The lowest number of honeybees per sunflower head was observed on cultivar PAN 7050 (0.63 ± 0.83) compared to the other cultivars. Conversely, the highest number of honeybees was observed in cultivar PAN 7033 (2.58 ± 0.78) (Table 10). Honeybee number was significantly different between sunflower cultivars and floral stage. There were significant differences in the number of honeybees per head between cultivars ($F = 5.24$, $P < 0.0001$ and $df = 16$) and between flowering stage ($F = 22.09$, $P < 0.0001$ and $df = 3$). However, differences between cultivars were only detected in certain flower stages (interaction cultivar-Flowering stage: $F = 3.26$, $P < 0.0001$ and $df = 48$). The highest number of honeybees per head was recorded in the 75% and 50% flowering stages respectively, whereas the lowest number of honeybees per head were recorded in the 100% flowering stage (Table 11).

Discussion

The reason why we found large numbers of wild honeybees during this pilot study might be that the field was 200-300 m from natural habitat. Steffan-Dewenter and Tscharntke (1999) found that isolation from natural habitats diminishes abundance and species richness of bees, which are the most important flower-visiting insects. Our results show that flowering stage has an important effect on honeybee visitation. The considerable, lower number of honeybees observed on cultivar PAN 7050 when compared to the other cultivars (Table 10) is likely related to its floral stage (it was less than 25% flowering stage) during the period of the study. However, cultivars with the same stage of flowering

were visited by a significantly different number of honeybee foragers, suggesting that honeybee foragers can discriminate among cultivars. Earlier studies indicated that honeybee foragers discriminate between cultivars of the same crop and flowering stages if a choice is offered (DeGrandi-Hoffman and Chambers, 2006). Plant genetic composition affects the chemical composition of flower odors with an indirect effect on insect pollinator behaviour (Sapir, 2009). Wright *et al.* (2002) worked with canola and snapdragon and suggested that, even though other factors may also influence floral discrimination, both intensity and odor quality affect the ability of honeybees to differentiate among floral perfumes.

In agreement with the results here presented, a previous study done in the same region found that sunflower insect visitation rates were affected by flowering stage, strongly decreasing after 80% flowering stage (du Toit, 1988). This result might be also related to the availability of resources. For instance, honeybee foragers were observed moving quickly over the florets in 100% flowering stage without gathering nectar or pollen and sometimes hovering in front of a flower, sometimes briefly touching the sunflower head and then departing without snooping into the florets. Conversely, pollen and nectar collectors were clearly observed gathering resources at the 75%, 50% and 25% stages. The nectar collectors settled among the florets, forcing their head to reach the nectary and pollen collectors went quickly over the florets and accumulated the pollen in their pollen basket. We can conclude that future studies on crop pollination need to consider differences in cultivar and flowering stage and researchers need to be careful with generalizations when their studies are only based on one cultivar. In addition to that, honeybee foragers might have the capability of identifying and discriminating among cultivars and flowering stages. Therefore, introducing colonies to the commercial sunflower crop at exact time of flowering is important to produce high and good quality seeds.

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Table 10. Number of honeybees per sunflower head (means $\pm$ SD) observed in 17 sunflower cultivars and in four different flowering stages at Towoomba agricultural research centre in the morning and afternoon combined.

<i>Plot no.</i>	<i>Cultivar name</i>	<i>Mean number of honeybees/ cultivar</i>	<i>Mean number of honeybees/ sunflower head at 25%</i>	<i>Mean number of honeybees/ sunflower head at 50%</i>	<i>Mean number of honeybees/ sunflower head at 75%</i>	<i>Mean number of honeybees/ sunflower head at 100%</i>
1	Agsun 8251	1.9 $\pm$ 0.83	1.73 $\pm$ 0.64	2.30 $\pm$ 0.30	2.57 $\pm$ 0.29	1.0 $\pm$ 1.0
2	AFG 271	2.18 $\pm$ 0.86	2.43 $\pm$ 0.20	2.80 $\pm$ 0.21	2.47 $\pm$ 1.0	1.0 $\pm$ 0.25
3	PAN 7033	2.58 $\pm$ 0.78	2.63 $\pm$ 0.25	3.43 $\pm$ 0.50	2.70 $\pm$ 0.36	1.57 $\pm$ 0.51
4	Agsun 5671	2.07 $\pm$ 1.15	2.40 $\pm$ 0.36	2.70 $\pm$ 0.62	2.83 $\pm$ 0.49	0.33 $\pm$ 0.58
5	Sirena	1.78 $\pm$ 1.02	1.00 $\pm$ 1.00	1.50 $\pm$ 1.32	2.60 $\pm$ 0.30	2.03 $\pm$ 0.86
6	DK 4040	2.20 $\pm$ 1.12	1.17 $\pm$ 2.02	2.37 $\pm$ 0.32	2.87 $\pm$ 0.50	2.40 $\pm$ 0.26
7	Agsun 5672	2.21 $\pm$ 0.89	1.77 $\pm$ 1.66	2.47 $\pm$ 0.25	2.43 $\pm$ 0.06	2.17 $\pm$ 1.04
8	NK Ferti	1.74 $\pm$ 1.07	0.67 $\pm$ 1.15	1.60 $\pm$ 1.39	2.43 $\pm$ 0.31	2.27 $\pm$ 0.12
9	PAN 7050	0.63 $\pm$ 0.83	1.67 $\pm$ 0.58	0.83 $\pm$ 0.76	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
10	AFG 272	1.61 $\pm$ 1.07	0.00 $\pm$ 0.00	2.00 $\pm$ 1.00	2.23 $\pm$ 0.21	2.20 $\pm$ 0.20
11	PAN 7726 HO	1.78 $\pm$ 1.29	0.67 $\pm$ 0.58	1.60 $\pm$ 1.04	3.20 $\pm$ 1.57	1.67 $\pm$ 0.58
12	PAN 7049	1.13 $\pm$ 1.13	2.07 $\pm$ 0.12	2.10 $\pm$ 1.01	0.33 $\pm$ 0.58	0.00 $\pm$ 00
13	Agsun 5383	2.23 $\pm$ 0.67	2.57 $\pm$ 0.51	2.37 $\pm$ 0.40	2.53 $\pm$ 0.46	1.43 $\pm$ 0.75
14	DKF 68-22	2.17 $\pm$ 0.60	2.43 $\pm$ 0.32	2.37 $\pm$ 0.21	2.53 $\pm$ 0.31	1.33 $\pm$ 0.58
15	NK Armoni	2.15 $\pm$ 0.60	2.47 $\pm$ 0.42	2.23 $\pm$ 0.23	2.57 $\pm$ 0.15	1.33 $\pm$ 0.58
16	Agusun 5551	2.03 $\pm$ 0.92	1.33 $\pm$ 1.53	2.50 $\pm$ 0.44	2.60 $\pm$ 0.17	1.67 $\pm$ 0.58
17	PAN 7048	1.93 $\pm$ 1.23	2.83 $\pm$ 0.35	2.47 $\pm$ 0.42	2.40 $\pm$ 0.69	0.00 $\pm$ 0.00

Table 11. Number of honeybees observed per head (means $\pm$ SD) of sunflower in the four different floral stages all cultivars combined.

Flowering stage	Mean number of bees/head
75%	2.31 $\pm$ 1.10
50%	2.21 $\pm$ 0.83
25%	1.75 $\pm$ 0.93
100%	1.32 $\pm$ 0.95