Effect of Insect Pollinator Species Deployment and Interactions with Parental Inbred Lines in Hybrid Carrot Seed Production

By

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PREFACE

The research contained in this dissertation was completed by the candidate while based in the Discipline of Plant Breeding, School of Agricultural, Earth and Environmental Science of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by Sakata Seed Southern Africa (Pty) Ltd.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Signed: Prof Julia Sibiya

Date: 10/06/2021

DECLARATION: PLAGIARISM

I, **Tebogo Lucky Skosana**, declare that:

- The dissertation has not been presented for any degree or examination at any other University. Also, the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work.
- This dissertation does not contain other persons 'data, pictures, graphs, or additional information unless expressly acknowledged as being sourced from other persons.
- This dissertation does not contain other persons' writing unless expressly cited from other researchers' information. The information from other sources that initially written are stated, then:
    - Their words have been re-written and acknowledged from the general information attributed to them. The wording or statement used is exact words used from the source, are written or structure in italic, and reference inside quoted marks.
- The figures, tables, pictures, or text contained in this dissertation are not pasted or copied from the internet either from books or journals unless expressly acknowledged. In theory, sources are being detailed and referenced.

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Co-Supervisor: Dr. Cousin Musvosvi Signature:

ABSTRACT

Insect pollinator species are highly valued for their contribution towards cross-pollination in many vegetable crops for food and seed production. Honeybees (*Apis mellifera*) are a significant main pollinator not only in entomophilous crop, but for many other plants in their natural habitats. Moreover, attempts to increase seed production through the introduction of an alternative pollinator species (such as Calliphorides flies) throughout the world have encouraged growers and breeders to think more precisely about the management of these pollinators for the future. However, several constraints, including climate, have resulted in low success of pollinators, thereby failing to meet pollination demand for hybrid carrot seed production, both nationally and internationally. The goal of this study was to identify alternative non-bee insect species that can be used as agents of pollination in commercial hybrid carrot seed production. The research experiment was conducted in Matjiesrivier farm (33º23'31.86" S and 22º05'14.91" E) that is situated under the Oudtshoorn district municipality, which is a Cango valley of Western Cape Province. Carrot parents were three cytoplasmic male sterile (CMS) lines, which were pollinated by two pollen donor-male inbred lines. Two insect species, honeybees (*Apis mellifera*) and Calliphorid flies (*Chrysomya chloropyga*), were used as agents of pollination. The experiment was arranged in a 2x2x3 factorial with two replications. The weight of umbels, seed weight and germination percentage data were collected to achieve research study objectives. Statistical analysis for all data was done using SAS (SAS Institute Inc, 2018) and R (R Core Team, 2019) statistical computation software. The data were subjected to analysis of variance (ANOVA) for individual umbel level (order) harvests. The TUKEY post hoc test was done at a 5% level of probability to compare the treatments. From the results, flies were comparably effective as honeybees during pollination, while analysis of variance for quantitative traits (germination percentage, seed weight, and umbels weight) was highly significant implying that the traits differed among the advanced lines and the deployment of the two species during pollination. The trait variability was influenced by the umbel stages of different CMS lines and their interaction with pollinator by male fertile and

male sterile lines. This information will be useful in a breeding program that focuses on hybrid seed production in carrots and a combination of the two insect pollinators' deployments to improve cross breeding for future management would be essential.

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DEDICATION

I dedicate this dissertation to my family heroes:

- Mother (R.I Skosana), sister (T.L Skosana), you are my pillars of strength and you believed in me that I can, and I shall.

- My fiancée and my son who have been supportive throughout this journey even though it was difficult for them since I had to devote my time on this research study.

- My late father, grandmothers (both paternal and maternal side) may their souls rest in peace!!

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ABBREVIATIONS

SVC	Sakata Vegetable Carrot
ANOVA	Analysis of variance
TRT	treatment
CV	Coefficient of variation
DF	Degree of freedom
F	Flies (*Chrosomya chloropyga*)
B	Bees (*Apis mellifera*)
SYP	Seed yield potential
SS	Seed set
WU	Weight of umbels
SW	Seed weight
M1	Male inbred line 1 (SVC 111)
M2	Male inbred line 2 (SVC 131)
CMS	Cytoplasmic male sterile
F1	Female inbred line 1 (SVC 211)
F2	Female inbred line 2 (SVC 212)
F3	Female inbred line 3 (SVC 213)

CHAPTER 1: INTRODUCTION

1.1 Background

Carrot (*Daucus carota L.*) is the most popular seed-propagated vegetable crop species in the world, with numerous uses. The edible storage roots are usually orange, white or red, or white blend in colour with a crisp texture when fresh (Department of Agriculture Forestry and Fisheries, 2018). These roots are rich in vitamin C, B1 and B2 and are particularly rich in carotene, the pro-vitamin A (Stolarczyk and Janick, 2018), Besides being one of the most economically important crops in the vegetable seed production business, carrot can be processed either alone or with other vegetables, for example, in the production of carrot juice, carrot cake, and in fodder production (Department of Agriculture Forestry and Fisheries, 2010), among other uses.

According to Muneer *et al*. (2019), carrot flowers are protandrous, hence they require cross-pollination and that is why insects are the main pollinating agents of carrot. Whereas pollination is the most critical aspect of vegetable seed production, it is often the most poorly understood and least optimized process. Bees are still the most effective and efficient pollinators, even though sometimes they are compromised due to unfavourable weather conditions. However, there is an increasing concern of honeybees decline, thus impacting food crops, due to their role as pollinators in different crops such as carrots. It is, therefore, essential to identify alternative non-bee insect pollinators that can be efficiently utilized and managed as crucial resources in carrot seed production (Cunningham *et al.* 2002; Klein *et al*. 2007; Winfree, 2008; Aizen *et al.* 2009; Gallai *et al*. 2009).

Consequently, understanding other pollinator insects that can influence pollination is essential to enhance growth in the agricultural sector (Ahmad *et al*. 2002). For example, an association between Calliphorid flies (*Calliphoridae*) and carrot flowers has been established, resulting in Perez-Banon *et al*. (2007) suggesting their possible utilization to supplement pollination in carrots.

However, these flies have been implicated in the pollination of wild-growing carrots. Nevertheless, even in experiments with other crops such as onions (*Allium cepa* L.) in cage enclosures, the species have been shown to be effective pollinators (Clement *et al*., 2007). Thus, there is a need for researchers to observe and compare the effectiveness of bees versus flies as pollination agents in carrots. There are instances when the conditions are not favourable for the bees to be effective pollinators; in such scenarios, effective alternative insect pollinator species would be required. Thus, understanding the behaviour and effectiveness of Calliphorid flies and bees and their interaction with carrot varietal strains in seed production is crucial.

1.2 Problem statement

Due to the absence or insufficient knowledge of potential non-bee insect species pollinators, hybrid seed production in carrots relies mostly on managed honeybees to successfully provide good pollination service. However, problems begin when bees cannot feed and sometimes they are constrained by weather conditions simply because they cannot adapt, survive or do well under certain conditions, thus resulting in decreased effectiveness as pollination agents. Weather conditions occurring in the Matjiesrivier area, Western Cape, South Africa have been observed to constrain bee pollinations during carrot hybrid seed production. In some instances, other than in the open field, bees are not active in the net-house or cages and tend to sit at the net corners or edges due to confusion/disorientation at specific periods under these conditions. In addition, the effectiveness of crop pollination depends on the biological timing of both the crop and its pollinators.

1.3 Motivation of the study

Results from this study will provide seed industries and farmers/growers with broad understanding and information about the importance of visitation by pollinators in carrots seed production.

Identification of non-bee insect pollinator species for hybrid carrot seed production can have a huge impact on food security and diversity, human nutrition, and carrot market prices, which all rely strongly on pollinators. Therefore, ensuring reliable pollination may be one of the best ways of improving the economical production of many crops including carrot. The knowledge about how insect pollinators interact with certain carrot varieties can help growers in optimization of seed production. Securing effective pollinators could increase pollination effectiveness, and consequently the yield and quality of the produce could also be increased.

1.4 Research objectives

1.4.1. Research goal

The study aimed to identify alternative non-bee insect species that can be used as agents of pollination in commercial hybrid carrot seed production.

1.4.2. Hypotheses

- Calliphorid flies are comparably effective as honeybees in pollination in carrot seed production.
- There are significant differences between female and male inbred lines for seed production and quality traits in carrots.
- Insect pollinators have significant interaction effects with parental inbred lines in carrot hybrid seed production and seed quality.

1.4.3. Specific objectives

- To compare the effectiveness of pollinator species (bees and Calliphorid flies) deployed on carrot inbred line parents during the pollination period in hybrid seed production.
- To determine if there are any differences between female and male inbred lines for seed production and quality traits.

- To determine if there are significant insect pollinator species by inbred line parent interaction effects on seed production and quality traits.

1.5. Dissertation outline

This dissertation consists of six logically linked chapters and follows the traditional dissertation format. The referencing system used in the chapters of this dissertation is based on the Crop Science journal. This is an example of the recommended formats by the University of KwaZulu-Natal. The structure of the dissertation is given below.

Chapter 1: Introduction

This chapter presents a brief background to the study undertaken, outlining the problem to be addressed by the study, the objectives to be met and the hypothesis behind each objective. Through this chapter, the gaps in research on the topic at hand are identified.

Chapter 2: Literature review

This chapter reviews several topics including the origin, spread, centre of diversity, taxonomy and domestication and genetics of the crop and general implications. Furthermore, it addresses the implication of pollinators and pollinations, carrots flower biology, controlled pollination and roles of insects (Bees and alternative insect pollination). A need for male sterility and carrots seed production constraints is also discussed and the effect of climate and weather on pollination and pollinators. Finally, it highlights the effect of supplementary pollination on carrot seed production and quality, and the important traits and traits association in seed production.

Chapter 3: Materials and Methods

This chapter outlines the different materials that were employed to meet the set objectives in chapter one as well as the methods used in the analysis of the collected data.

Chapter 4: Results

Results of the field trials and their analysis are outlined in this chapter.

Chapter 5: Discussion of results

A critical discussion and interpretation of the results obtained from the study has been conducted with reference to comparative studies.

Chapter 6: Conclusion and recommendation

This chapter relates the findings of the study to the objectives set in chapter one as well as making some recommendation for future breeding programmes.

CHAPTER: 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, several topics are covered that relate to the objectives of the study. The chapter covers the origin, spread, centre of diversity and taxonomy. Furthermore, domestication of the crop and general implications of pollinators and pollination are discussed. Carrots flower biology, controlled pollination, and roles of insects (Bees and alternative insect pollination) are given. The need for male sterility and carrots seed production constraints, with climate and weather effect on pollination and pollinators are also discussed. Finally, the effect of supplementary pollination on carrot seed production, seed quality, and important traits and traits association in seed production are highlighted.

2.2 Origin, spread, centre of diversity and taxonomy

Carrot (*Daucus carota subsp. Sativus*) is a biennial plant belonging to the Apiaceae/Umbelliferae family (Muneer *et al.* 2019). It is the domesticated form of the wild carrot, *Daucus carota*, native to Europe and Southwestern Asia (Meyer *et al*. 2012). According to Stolarczyk and Janick (2018), carrot was originally wild in different parts of Europe and Asia, but was domesticated first in Afghanistan, which is now recognized as the primary centre of diversity. It then spread over to other regions and countries such Mediterranean, Asian and Turkey being recognized as secondary centre of diversity. The greatest development and improvement of the original wild carrot that had thin, long roots took place in France (Department of Agriculture Forestry and Fisheries, n.d., accessed on 20 January 2021). Carrot has been an important vegetable crop in South Africa since the early settlement at the Cape. It is currently grown all over South Africa, particularly near urban areas including in Stellenbosch, Johannesburg, Greytown and Pretoria (Department of Agriculture Forestry and Fisheries, 2018).

2.3 Domestication and genetics of the crop

Selection criteria or process of domestication of carrots often has directed more attention to quality traits such as colour, shape, flavour, and physiological traits that contribute to uniformity (Doebley *et al*. 2006). Cultivated carrot are mainly classified into the anthocyanin or eastern-type carrot (e.g., yellow or purple) or western-type carrot (yellow, orange, or red) and the carotene based on the pigmentation in the roots. Analysis of the genetic structure of wild and cultivated crops in combination with archaeological and historical evidence, has provided insight into the geographic and temporal details of domestication to reveal, where, and how many times a crop was domesticated (Meyer *et al*. 2012).

2.4 General implication of pollinators during pollination

Poor pollination is a problem for the carrot hybrid seed growers worldwide. Over time, the crop may experience fluctuations in pollinator visitation since the carrot flowering duration in a single crop is long, approximately six weeks (Erickson *et al*. 1979). However, numerous factors influence pollinators' effectiveness and efficiency, depending on geographic location and the environment. Although bees are the common pollinator insects, researchers in some countries have proved that calliphorid flies could do effective pollination without bees in cages. Thus, in this study, pollination using the calliphorid flies will be examined for their potential in hybrid carrot seed production for future management. Most vegetable crop species require insect pollination, depending on whether the formation of the propagation organ and or harvestable product relies on successful cross-pollination.

According to Vicens and Bosch (2000), weather conditions impact insect pollinator flower visitation. For instance, bee species (including *Apis mellifera*) are restricted by cloudy, humid, windy, and cold weather (Kevan and Baker, 1983). In comparison, some other potential pollinator species, including Diptera, have excellent tolerance towards these weather conditions (Vicens and Bosch, 2000). The problem and issues that have arisen with pollination have pushed research toward identifying active, effective, and efficient pollinators on crops. Particularly noteworthy are the periods when it is critical to obtain successful pollination during less favourable conditions. However, information on pollinator species' effectiveness and abundance during critical periods is limited (Howlett *et al*. 2009). A wide range of insect species may act as significant pollinators of open-pollinated carrot seed crops (Gaffney *et al*. 2011). However, the effectiveness of the species for controlled pollinations needs to be well ascertained. As supported by literature, the reliability of honeybees as pollinators of hybrid carrot seed crops in the future can be problematic (Howlett, 2012).

2.5 Carrot flower biology

Carrot is a biennial cool season plant which belongs to the family Apiaceae (Umbelliferae) of the order Apiales (Alessandro and Galmarini, 2007; Muneer *et al*. 2019). Carrot flower consists of a primary umbel, which is essential for seed production, and secondary and tertiary umbels, of individual florets. The flower is the most critical biological structure for ensuring angiosperm reproduction. The flowering of the crops often provides an essential resource for many pollinators. Hence, the flowers entice pollinators by variation in morphology, colour, and scent. When visiting the flower, pollinators provide pollination service by delivering of pollen at an appropriate time and place for ovule fertilization in every entomophilous crop species worldwide (Klein *et al.* 2007). Still, the short duration of floral availability, low diversity of floral and nesting resources, pesticide application compromises their capacity to support diverse and abundant pollinator communities (Pott *et al*. 2010; Williams *et al*. 2010).

According to Brittain *et al*. (2013), due to complementary resource use arising from variation in morphology, pollinator species may visit different parts within a flower, inflorescence, or other flowers within the same plant (high versus low flower), thus increasing pollination. Although carrot nectar is not abundant, it is exposed to the petals and readily accessible to all insects. The florets are tiny and quickly worked by minute insects (Bohart and Nye, 1968) except those with long, slender tongues. Successful hybrid seed production is the results of perfect synchronization of stigma receptivity and male parent pollen viability(Vishal *et al.* 2018). Furthermore, the quantity of pollen deposited on the stigma can be manipulated by adjusting females' cross-ratio to male flowers (Vishal *et al*. 2018).

2.6 Controlled pollination and roles of insects

2.6.1. Bees

The manageable bees are well known as effective pollinators of economic importance. However, hybrid carrot crops are known mostly to require supplementary cross-pollination, which is accomplished through pollinators such as bees (pollinators of hybrid). The reliability of managed bees for hybrid carrot crop pollination seems to become less because of increased variability between lines (Rodet *et al.* 1960). This variability (male and female inbred lines) in managed bee-mediated pollination has led to the idea or perception to focus on alternative pollinators such as Calliphorid flies for future purposes (Howlett *et al*. 2015).

2.6.2. Alternative insect pollinators

Non-bee pollinators include flies, beetles, butterflies, and others, even though some are not good or suitable for use in seed production. Alternative pollinators provide potential insurance against bee population decline and a valuable service (Brittain *et al*. 2013). Research studies indicate that non-bee insects are equally if not more important for seed production of some crops (Larson *et al*. 2001). They can provide pollination service at different times of the day especially when weather conditions are not favourable and bees cannot forage (Rader *et al.* 2013). Besides, non-bee insects may be efficient in transferring pollen for some crops under certain conditions (Howlett, 2012) and carry pollen further distance than some bees (Rader et al., 2011).

According to Hawthorn *et al*. (1960), insects such as Calliphorides flies are among the unmanaged pollinators visiting the carrot flower. The strong association between these flies and the carrot suggests high potential for them to be utilized (Howlett, 2012). These flies have been confirmed to successfully pollinate wild-growing carrot within an isolated area/cages used in the absence of bees (Perez-Banonet *et al.* 2007). If successfully developed as managed pollinators, their potential to replace bees for crops suitable to their pollination might provide supplementary crop pollination alongside bees (Howlet, 2012). Due to their difference in foraging a combination of both pollinators could improve the rate of crop pollination, especially in areas in which climate is challenging to predict.

2.7 Need for male sterility in carrot seed production

Cytoplasmic male sterility has enabled commercial production of hybrid seed for many crops to be possible and more cost-effective. Cytoplasmic male sterile (CMS) lines Should have a stable sterility with the absence of pollen, provide good flowering and contribute to hybrid vigour (Xuli *et al*. 2017; Shu *et al*. 2016). Plants with the CMS trait have been used for many years as female parents in F1 hybrid breeding, including carrots (Nothnagel *at al.* 2000), the absence of pollen production prevents self-pollination . There are two different types of male sterility in carrots; one is where anthers are transformed into petals (petaloid sterility), which is maternally inherited and often used by breeders and the other one is called the brown-anther sterility (Barbara *et al*. 2010). According to Bach *et al.* (2002), the cytoplasmic male sterility of petaloid type is more stable than the brown-anther type. Meaning the mitochondrial dysfunction of the flower male organs can be either kept or suppressed by specific nuclear gene functions, leading in the latter case, to restored male fertility (Linke *et al*. 2003).

Therefore, CMS plants are significant in seed production, and have been extensively used by plant breeders to achieve cross pollination in the development of hybrid cultivars of multiple crops (Saxena and Hingane, 2015). The male sterile line is phenotypically and genotypically different from male fertile plants that are used in hybrid production. However, successful production of seed requires pollinators to transfer pollen from fertile to sterile plants (Howlett, 2012). Frequent movement should be ideal for pollinators between the inbred lines (Male fertile and sterile) to maximize seed set in carrot hybrid crop and the pollen flow for seed yield. Therefore, CMS lines in cross-pollination are the seed parents, and they are interspaced with beds of a fertile male line with the ratio of 2:1 or 3:1 depending on the variety. It is important to consider the distance between the inbred lines as this might have an effect on the pollination.

2.8 Carrots seed production constraints

For several reasons scientist have looked at alternative pollinators to improve the hybrid carrot seed production. This includes observations made in after several research studies that noticed a decline in bees' activities in either open field or net cages. It is thus difficult or not safe to assume that bees will provide all future pollination needs (Mader *et al*. 2010). An important reason behind the observed decline is thought to be the loss of habitat that supports host plants (Scheper *et al*. 2014) and nesting plants since different pollinators respond differently to disturbances (Cariveau *et al.* 2013; Rader *et al*. 2014). It is essential to have a correct transplanting date for both male and female inbred lines to have a good synchrony during flowering stage in order for pollinators to forage successfully for cross-pollination.

The isolation distance from natural area affects the optimal foraging and in turn affects the mean levels of pollinator's richness, visitation rate, and ultimately pollination of the crop flowers

(Cresswell *et al*. 2000). Temperature is one of the most important factors because it influences insect behaviour and affects pollinators' foraging patterns (Abrol, 2006). For example, bees such as honeybees are sensitive to temperature below 12.8ºC, rain and winds stronger than 32-40 kph, and will not forage (Eric *et al.* 2010). Furthermore, due to the expected climate change , the elevated temperature may negatively impact some pollinator species, thus affecting pollinator foraging in the future (Gaffney *et al.* 2018). Therefore, maximizing pollinator species that are efficient in all vegetable crops, especially when the period of extreme weather overlaps with the pollination window, is essential (Gaffney *et al*. 2018).

2.9 Climate and weather effect on pollination and pollinators

Responses of the plant to climate change (global warming) and other environmental factors that impact or alter flowering, nectar, and pollen production, could modify floral resource availability, distribution, and visitation quality (Rathee and Dalal, 2017). Thus, climate change is one of the essential drivers affecting pollinators and plant-pollinator interaction (Bartomeus *et al*. 2013; Thomson, 2016). Furthermore, increasing temperature, drought, and more frequent extreme events all suggest a significant impact on pollinator species distribution (Kerr *et al.* 2015). The different pollinator species respond to different environmental conditions depending on the type of insects a farmer/grower uses for pollination. Although honeybees (*Apis mellifera*) are considered the most important pollinator for many crops, bumblebees have been found to be more effective than the honeybees under certain climatic conditions such as early spring, where they can work long hours, carry more pollen, and are more active (Stubbs and Drummond, 2001).

According to Shrestha *et al*. (2018), climate and weather can enhance or disrupt biological systems, but little is known about how organism plasticity may facilitate adaptation to localized climate variation. Gradual changes to these weather patterns and climate have been predicted to increase following factors such as summer drought and floods, and all this could affect pollinators (for example, loss of synchronicity with their forage plants) and flowering time during pollination. The effects of elevated temperature on the number of flowers is ambiguous, with both increase and decrease in the number of flowers in different species (Scaven and Rafferty, 2013) having been noted. These changes in flowering phenology and potential changes in climate conditions may hold important implication for plants traits, including leaf emergence, flowering time, and germination (Hegland *et al*. 2009).

2.10 Effect of supplementary pollination on carrot seed production and quality

Limited information is available about insect pollination's possible effects on seed quality parameters that affect the market value (Bommarco *et al.* 2012). According to Garibaldi *et al*. (2013), the increase in wild insect visitation is higher, significant, and twice as much as honeybees' visitation in 41 crop systems worldwide. However, several studies have showed that supplementary pollinators are practical and efficient enough to increase seed quantity and quality with their greater visitation to vegetable crops. Thus, their foraging activity and seed set are far greater than in the managed pollinators such as honeybees (Howlett, 2012). Furthermore, it was observed that, with these supplementary pollinators, the quantity and quality of seed production was more than that obtained with manged pollinators under constrained climatic conditions (Vicens and Bosch, 2000; Kevan and Baker, 1983). Therefore, for successful pollinations in future, these supplementary pollinators should be considered. Their potential as a supplement highlights the possibilities and development of strategies to improve the seed quality in hybrid carrot pollination (Gaffney *et al.* 2011).

2.11 Important traits and traits association in carrot seed production

Most of the inbred lines in carrot seed production rely on biotic pollination for a successful cross-pollination to produce a hybrid (Ahmad *et al*. 2002). Carrot germplasm consist of several inbred lines (male fertile and male sterile plants), which are phenotypically and genotypically different. Genetically, the male sterility of carrots demonstrated a nuclear-cytoplasmic interaction for both cytoplasmic male sterility types (Petaloid and brown-anther). Female inbred lines (petaloid) have dominant alleles of each of the three duplicate nuclear genes necessary to maintain sterility for both cytoplasm, and dominant alleles at one or more epistatic loci that could restore fertility (Bach *et al*. 2002). Genetic and molecular mechanisms of restoration vary among the different cytoplasmic male sterility systems (Linke *et al*. 2003).

Phenotypically plants with good traits are essential in seed production and are needed for successful pollination, such as good seed set, high yield in male sterile plants, and abundant pollen production in male fertile plants with quality foraging. However, plants that lack intense attractiveness may cause the pollinators to neglect the crop (Scheper *et al*. 2014). In addition, inbred lines that have traits which enable them to withstand adverse environmental conditions are important because they will be able to support plant reproductivity - flowering time and plant interaction with pollinators - duration of pollination period (Mader *et al*. 2010). Therefore, selection of crops must always consider the morphological, adaptation, and reproductive traits as they impact seed production.

Summary

From this literature review it can be acknowledged that it is imperative to consider future management of supplementary pollinators for good successful cross-pollination. As a result of several constraints, in future it will be difficult or challenging to rely on the manageable honeybees' pollinators. There is evidence that alternative pollinators are comparably effective and efficient as honeybees for pollination. However, more analysis and detail in terms of research focusing on climate, habitat, crop traits, and environmental factors that contribute to pollination failure by other pollinators is needed. The review also noted that the Calliphorides flies are far greater than the bees in terms of visitation for foraging, seed set, and quality. The review showed that the flies were more effective in transferring pollen, travelled longer distances than bees, and were better adapted under unfavourable conditions that affected bee activity. Therefore, it is vital to consider the alternative pollinators for future use by farmers and seed industries to maximize pollinations in seed production. Considering the combination of the two pollinators (bees and flies) could result in better pollination success, particularly for carrot hybrid seed production, thus justifying the focus of this study.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site description

The study was conducted at Nefdt Farm in Matjiesrivier (33º23'31.86'' S and 22º05'14.91'' E) with an altitude of about 749 meters above sea level as shown in Figure 1. The farm is situated under the Oudtshoorn district municipality, in the Cango valley of Western Cape Province, South Africa. The summers are hot, the winters are cold, and it is dry and mostly clear year-round. The mean annual rainfall the area receives is about 170 mm, which occurs throughout the year with the lowest rain (10 mm) in January and the highest (22 mm) in March. On average, the warmest month is January and the coldest month is July, and the average annual temperatures are minimum (10.0°) and maximum (25.0°), respectively.

Figure 1: Image of the experimental research area. Picture taken by Google Earth on 28 August 2019.

3.2 Preparation of plots

The land was ploughed using a tractor-drawn plough. After that, the field was disced twice to break up the clods and provide good soil tilth. Weeding was done through application of the herbicide - Lanigan® SC (Active ingredient: linuron (urea) 500g/l) at 37.5 ml per 16 L of knapsack to control broad leaves weed species, and additionally with the assistance of hoes and spades to clean in between the rows. Drip irrigation was installed (at a spacing of 30 cm between and within the dripper lines) before transplanting to water the soil to field capacity. The planting material (carrot roots) was collected from a different location seedbed through root selection and transported to the study site area for transplanting. Tape measure, T-markers, hoes, rakes, ropes were used during planting time to open the rows and close them well for good roots stand.

3.3 Plant materials, experimental design, and layout

The experiment was a randomized complete block design in a 2x2x3 factorial arrangement with two replications. The three factors and their levels were: three cytoplasmic male sterile (CMS) carrot lines (SVC 211, SVC 212, SVC 213) which were essentially females; two pollen donors (two male inbred lines – SVC 111, SVC 131) and two insect pollinator species *viz*: honeybees (*Apis mellifera*) and Calliphorid flies (*Chrysomya choropyga*) used to transfer pollen from pollen donors for pollinating the CMS lines. Four net cages, two for pollinating with bees and the other two for pollinating with flies (Figure 2), were constructed at bolting stage for pollinator isolation and control to attain the study's objectives. Inside each cage, one of the male inbred lines was grown in two 9 m rows; in the same cage the three CMS lines were grown in 2.5 m rows replicated twice, bordering the male rows as shown in Figure 2. A distance of 0.4 m separated the female inbred lines. Thus, the three CMS lines were found in all the four cages and were pollinated by either SVC111 or SVC131 in separate cages, with bees or flies (in separate cages) as agents of pollination.

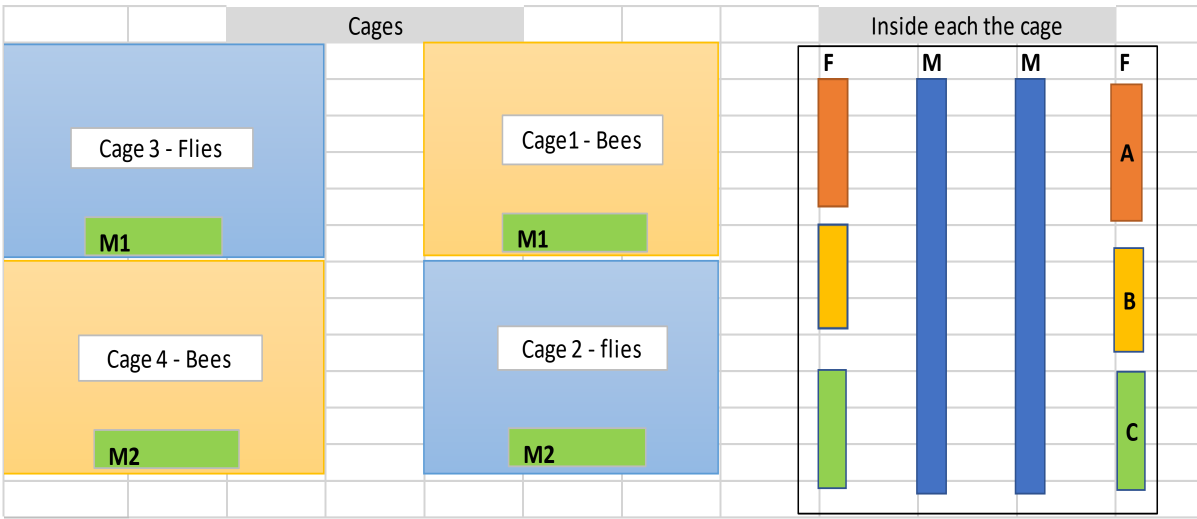

Figure 2: Experimental layout. M1 = SVC 111, M2 = SVC 131, A = SVC 211, B = SVC 212, C = 213

3.4 Caging of plots and deployment of insect pollinators

Four cages (Figure 3) were constructed immediately when the plant materials (male and female inbred lines) started to bolt. The net material used to construct the cages was a Skadunet® white 20% P/M knittex, and steel poles supported the net. Beehives were collected from the beekeeper and placed in net cages during pollination, and this was done two days after pupa flies had been placed in the other cages, so that the deployment activity of both pollinators would begin on the same date. Bees (*Apis mellifera*) deployment actual dates for both cage 1 (male SVC 111) and cage 4 (male – SVC 131) were different due to the male inbred lines blooming phase not being on the same date. The flies (*Chrosomya chloropyga*) were collected from flies' breeder and given as pupa to be placed in cages (2 & 3). Similarly, as for the bee cages, the fly cages (cage 2 - male SVC 131 and cage 3 - male SVC 111) had different deployment dates of flies. Their deployment was every two weeks from the initial date of deployment (cage 3 - (13 November 2019 until 25 December 2020 and cage 2 - 19 November 2019 until 27 December 2020) to increase the number of visitations per umbel e.g., 4 – 7 flies per umbels (e.g., as shown in Figure 5). For the bees, deployment was only one beehive in a cage from the initial date (cage 1 - 13 November 2019 until 25 December 2020 and cage 4 - 19 November 2019 until 27 December 2020). The experimental cages are shown in Figure 3. Figure 6 (A) shows 100% blooming of the male inbred line (SVC111) – on the right in cage 3 of flies' deployment and similarly Figure 6 (B) indicates second male inbred line (SVC131) – on the right 100% blooming in cage 4 for bees' deployment.

Figure 3: Experimental cages used to ensure isolation and control of pollinators

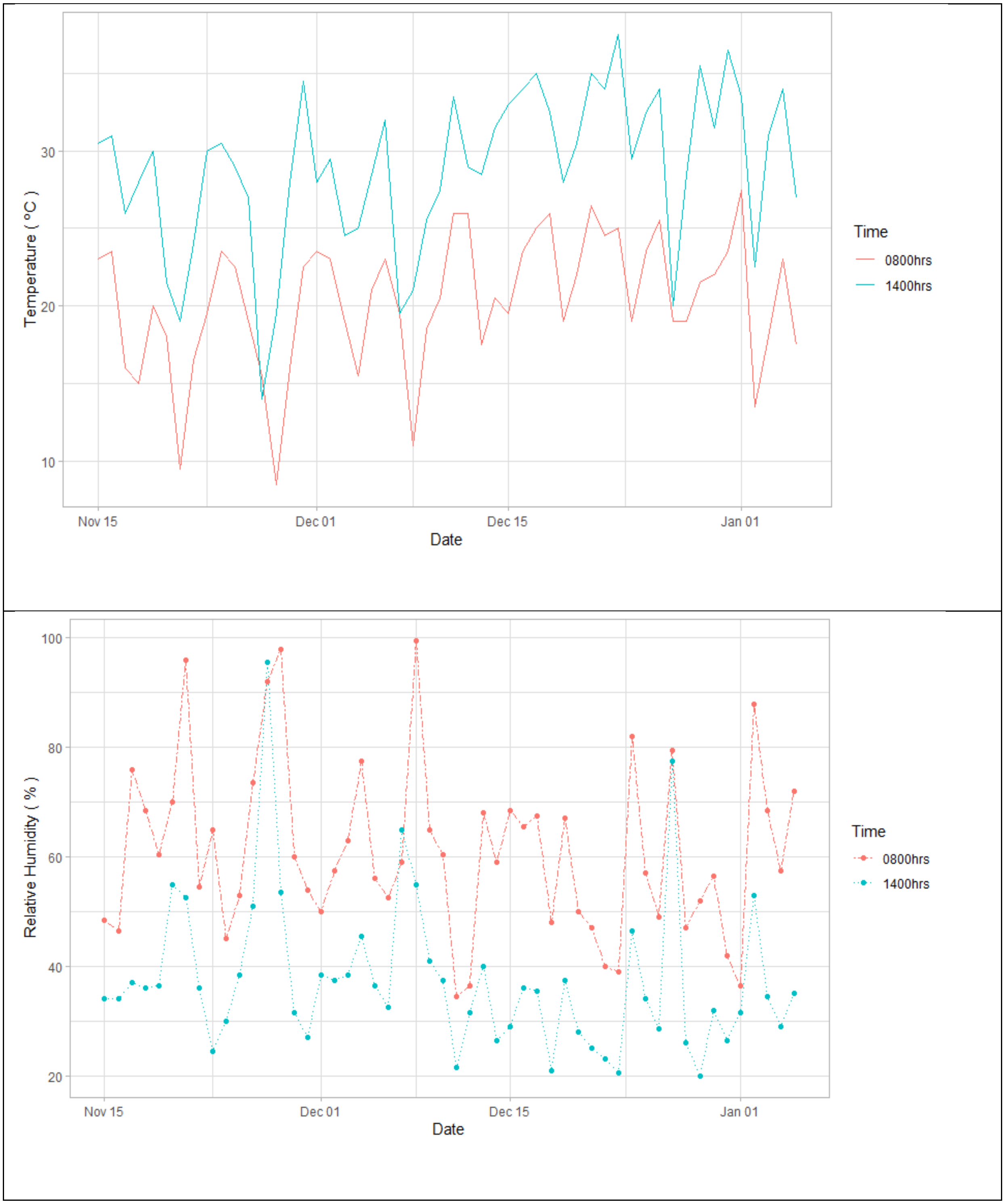

Figure 4. Weather conditions during the period of insect pollinator species deployment.

The graphs in Figure 4 show the prevailing weather conditions (temperature and relative humidity) under which deployment of pollinators was done during pollination. The two time slots were recorded in the morning and afternoon to identify high/low peak of weather condition from 15 November 2019 to 5 January 2020.

3.5 Data collection

The following parameters were recorded to achieve research study objectives: bolt start date, number of days to 50% flowering, date of 50% blooming, seed set rate, seed yield potential, the weight of umbels, seed weight, germination %, plant vigour, temperature, and relative humidity. The measurements were done as indicated below:

i). Bolt start date

The bolting date was recorded when 50% of the plants in a plot had bolted to determine which inbred-line, male or female was quicker in the production of a flowering stem.

ii). Number of days to 50% flowering

Recorded as the number of days when 50% of the plants in a plot have flowered and it helps in determining whether the variety flowers early or late.

iii) Date to 50% blooming

Recorded as the date when 50% of the plants in a plot have fully bloomed and is essential to know the right or accurate stage of introducing the pollinators.

iv). Weight of umbels

Obtained by weighing the different orders of umbels (1st, 2nd, and 3rd) per inbred line in grams per plot.

v). Seed weight

Obtained by weighing the seed harvested from different umbel orders per inbred line in grams per plot.

vi) Germination test

The results for germination test was conducted at the lab. The standardized germination methods as prescribed by ISTA (International Seed Testing Association) were used. Each species is tested using specific germination substrates, temperatures (20^{o}C – 30^{o}C) and evaluation is done on specific counting days (first count at seven days and final count on fourteen days). Each test is done on 4 x 100 seeds. The 4 replicates of 400 seeds are tested and evaluated individually. After testing each of the 4 replicates results are added for normal, abnormal, and dead seeds. A tolerance table is subsequently used to make sure that the 4 replicates do not differ significantly as per the average results. If the results are within tolerance of each other the test results are accepted.

vii). Temperature and relative humidity

Data loggers were used to measure temperature and relative humidity (%) of the area

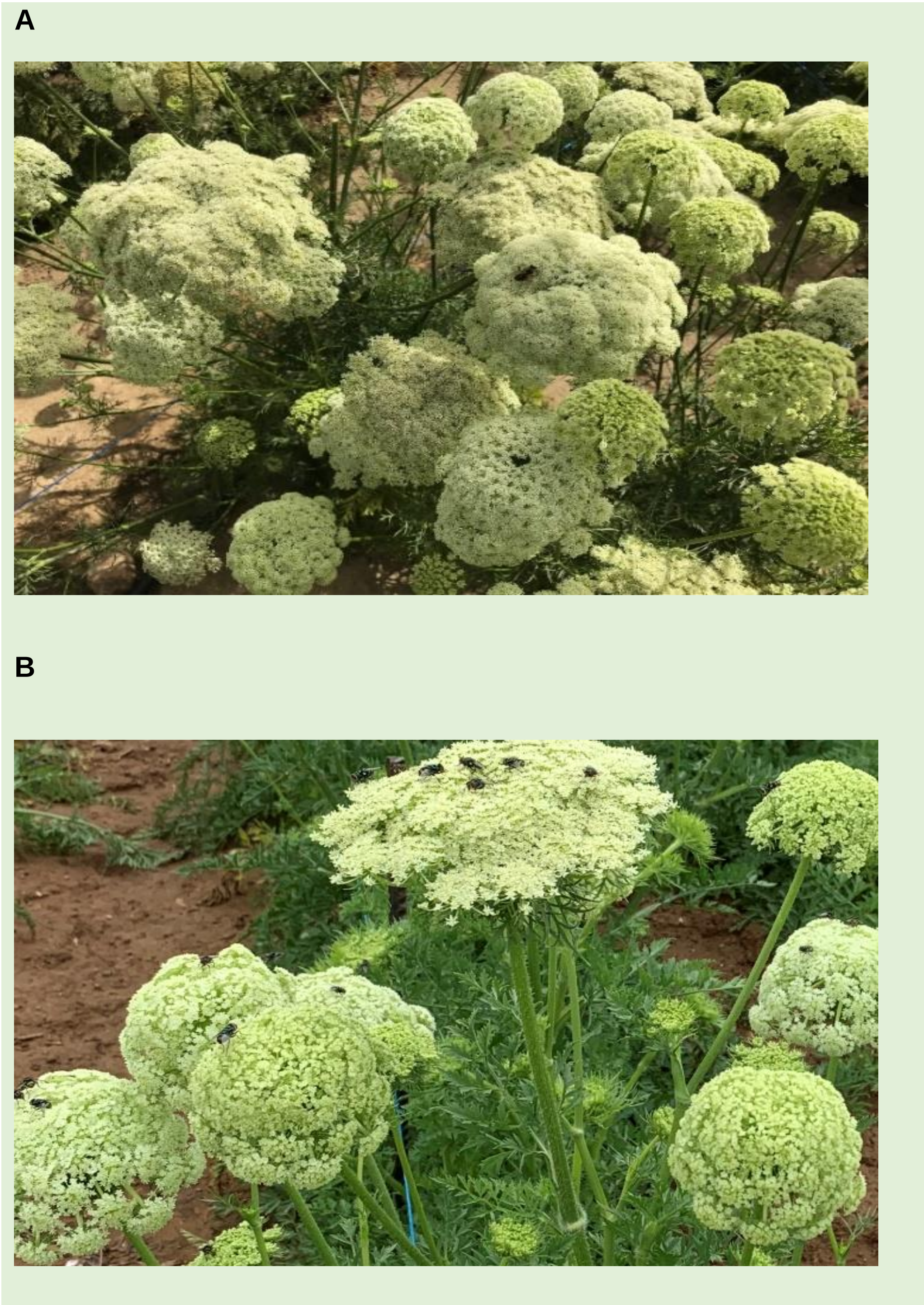

Figure 5: Insect pollinators deployed during pollination: A. -Bees (*Apis mellifera*) and B. Flies (*Chrosomya chloropyga*)

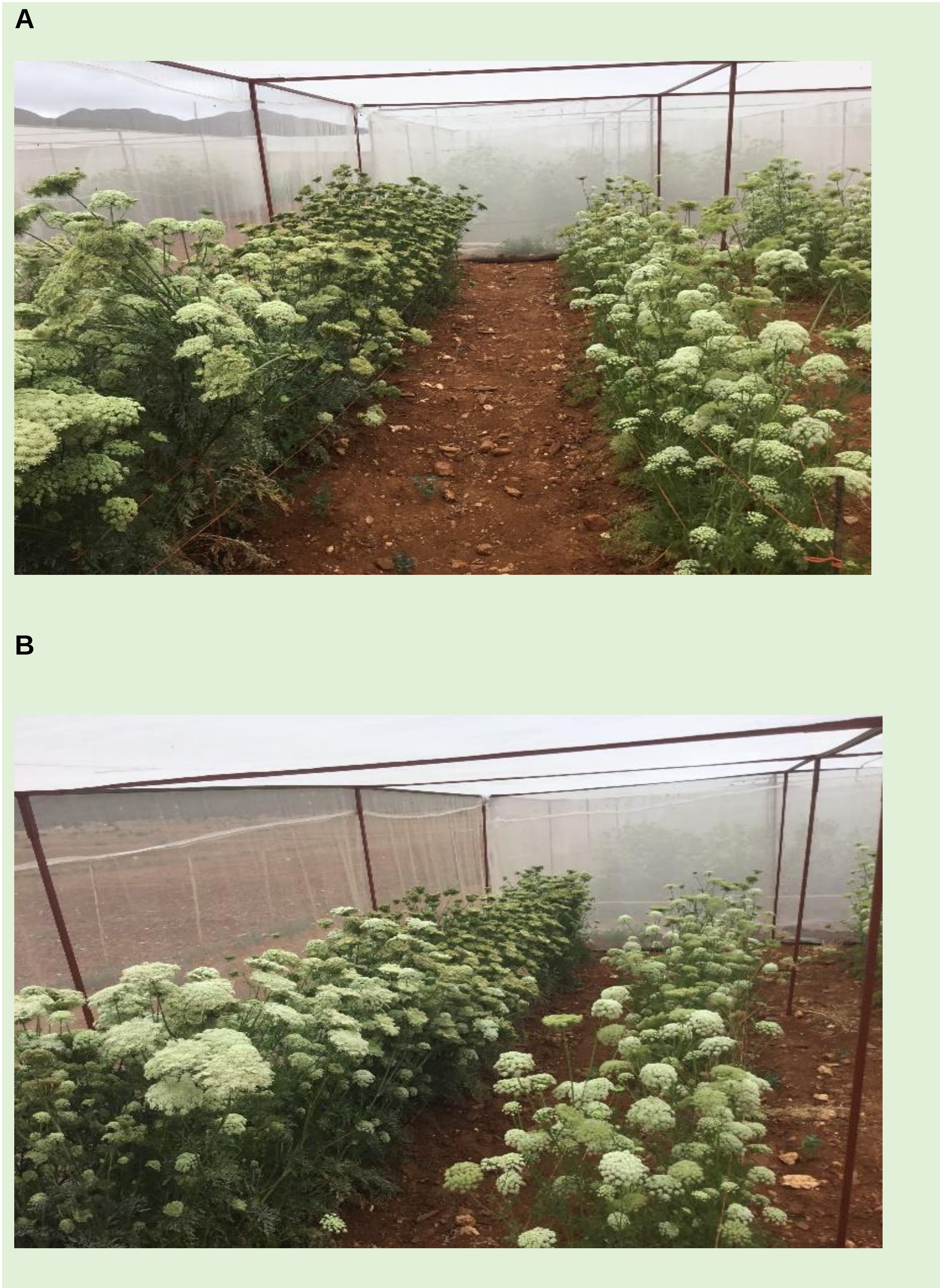

Figure 6: A view of the male inbred lines used at 100% blooming A. – Early blooming male inbred line (SVC111) – on the right; B -– Late blooming male inbred line (SVC131) – on the right

3.6 Data analysis

Analysis of all quantitative data was done using SAS (SAS Institute Inc, 2018) and R (R Core Team, 2019) statistical computation software. The data generated from the experimental research was subjected to analysis of variance (ANOVA) for individual umbel level (order) harvests, following the general linear model (GLM) presented in Equation 1 and Table 1. Analysis of variance was also performed using combined data from all umbel orders with ‘umbel order’ included as an additional factor in the ANOVA model. If significant differences were detected by ANOVA, a TUKEY post hoc test was done at 5% level of probability. The bar graphs with error bars were used for graphical representation of differences among main treatment effects and their interactions.

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \delta_l + \varepsilon_{ijkl} \qquad \text{Equation 1}$$

Where, i = 2; j = 3; k = 2; l = 2

Y_{ijkl} = response in the l^{th} replicate due to i^{th} pollinator and j^{th} female inbred line and k^{th} male inbred line

μ = general effect

α_i = effect due to pollinator

β_j = effect due to female inbred line

γ_k = effect due to male inbred line

$(\alpha\beta)_{ij}$ = interaction effect of the i^{th} pollinator and j^{th} female inbred line

$(\alpha\gamma)_{ik}$ = interaction effect of the i^{th} pollinator and k^{th} male inbred line

$(\beta\gamma)_{jk}$ = interaction effect of j^{th} female inbred line and k^{th} male inbred line

$(\alpha\beta\gamma)_{ijk}$ = interaction effect of i^{th} pollinator j^{th} female inbred line and kth male inbred line

δ_l = effect due to l^{th} replication

ε_{ijkl} = random error

Table 1: Skeleton analysis of variance (ANOVA) table for seed production and quality traits.

Source of variation	Degrees of Freedom
Replication	r – 1 = 1
Treatment	pmf – 1 = 11
Pollinator	p – 1 = 1
Male	m – 1 = 1
Female	f – 1 = 2
Pollinator x Male	(p – 1)(m – 1) = 1
Pollinator x Female	(p – 1)(f – 1) = 2
Male x Female	(m – 1)(f – 1) = 2
Pollinator x Male x Female	(p – 1)(m – 1)(f – 1) = 2
Error	(r – 1)(pmf – 1) = 11
Total	rpmf – 1 = 23

CHAPTER 4

RESULTS

This study was carried out to understand the main and interaction effects of insect pollinators, female carrot inbred lines and male carrot inbred lines in seed production. Observations were made for weight of umbels, seed weight and germination %, and this was done separately on primary, secondary, and tertiary umbel harvests from the female inbred lines. It is important to note that the male inbred lines only acted as pollen donors and the insect pollinators were the agents of pollination. Analysis of variance and means for main and interaction effects are reported.

4.1 Analysis of variance

Primary umbels

The primary order umbel harvests showed significant treatment effects for all the recorded variables (Figure 2). The pollinator effect was not significant for all the variables, and female effect was significant for all variables, whereas the male effect was significant for all variables except weight of umbels. The pollinator x female effect was significant for all variables except germination %, whilst the pollinator x male effect was significant only for germination %. Interaction effect of females x males was significant for all variables. The interaction effect of pollinator x female x male was highly significant for weight of umbels and seed weight but not significant for germination %.

Table 2: Analysis of variance for seed production and seed quality parameters in the Primary Umbels

Source of variation	Degrees of freedom	Weight of Umbels (g plot^{-1})	Seed weight (g plot^{-1})	Germination (%)
Replication	1	12.04	126.04	10.67
Treatment	11	552.56***	2207.13***	285.55***
Pollinator	1	2.04	77.04	6.00
Female	2	637.88***	1563.54***	65.38*
Male	1	7.04	13490.04***	2281.50***
Pollinator*Female	2	1682.79***	1523.29***	11.38
Pollinator*Male	1	51.04	145.04	368.17***
Female*Male	2	301.29***	1383.79***	146.38***
Pollinator*Female*Male	2	387.04***	812.54***	19.54
Error	11	20.77	35.86	9.67

*** = significant at $P<0.001$, ** = significant at $P<0.01$ and * = significant at $P<0.05$

Secondary umbels

In this category of umbels, treatment effect was highly significant for all variables (Table 3). The pollinator and male effect were significant only for germination %, whereas the female effect was significant for all variables. A similar trend as for the primary umbels was observed wherein the pollinator x female effect was significant for all variables except germination % whilst the pollinator x male effect was significant only for germination %. the interaction effect of females and males, and the three-factor interaction of pollinators, females and males were significant for all recorded variables.

Table 3: Analysis of variance for seed production and seed quality parameters in the Secondary Umbels

Source of variation	Degrees of freedom	Weight of Umbels (g $plot^{-1}$)	Seed weight (g $plot^{-1}$)	Germination (%)
Replication	1	7385.04	2109.38	1.50
Treatment	11	26031.77***	14656.83***	309.26***
Pollinator	1	3151.04	1305.38	112.67**
Female	2	90890.67***	30179.63***	96.29**
Male	1	8932.04	6305.04	2053.50***
Pollinator*Female	2	16342.17*	26190.38***	26.54
Pollinator*Male	1	1820.04	1365.04	450.67***
Female*Male	2	13645.17*	5729.54*	219.13***
Pollinator*Female*Male	2	15345.17*	14025.29***	50.54*
Error	11	2912.68	837.73	8.41

*** = significant at P<0.001, ** = significant at P<0.01 and * = significant at P<0.05

Tertiary umbels

As regards to this category of umbels, the treatment effect was significant only for weight of umbels (Table 4). The pollinator effect was not significant for all variables. The female effect was significant only for weight of umbels whereas the male effect was significant for all variables recorded. The interaction effect of females and males was significant for weight of umbels and germination % but not significant for seed weight. All other interaction effects were not significant for all the variables.

Table 4: Analysis of variance for seed production and seed quality parameters in the Tertiary Umbels

Source of variation	Degrees of freedom	Weight of Umbels (g plot^{-1})	Seed weight (g plot^{-1})	Germination (%)
Replication	1	165.38	590.04	54.00
Treatment	11	18909.01**	2798.28	55.71
Pollinator	1	1488.38	3337.04	32.67
Female	2	32291.38**	1453.88	35.29
Male	1	84372.04***	16380.38**	253.50**
Pollinator*Female	2	23.63	289.54	23.04
Pollinator*Male	1	35.04	392.04	2.67
Female*Male	2	28682.79**	3552.88	101.63*
Pollinator*Female*Male	2	54.04	39.54	2.04
Error	11	2855.74	1031.50	21.91

*** = significant at P<0.001, ** = significant at P<0.01 and * = significant at P<0.05

Across umbel orders

Overall analysis of variance across umbel orders (Table 5) revealed highly significant effect of umbel order, treatment, umbel order x treatment, and the male parent, for all recorded variables. The pollinator, umbel order x pollinator, pollinator x female, umbel order x pollinator x male, and umbel order x female x male effects were not significant for all variables. The umbel order x male, pollinator x male, and female x male interaction effects were significant only for germination %. On the other hand, the umbel order x pollinator x male, and umbel order x pollinator x female x male interaction effects were significant only for seed weight.

4.1.1 Analysis of variance across umbel orders

Table 5: Analysis of variance for weight of umbels, seed weight and germination percentage across umbel orders.

Source of variation	Degrees of freedom	Weight of Umbels (g $plot^{-1}$)	Seed weight (g $plot^{-1}$)	Germination (%)
Umbel order	2	508210.89***	154422.06***	1061.17***
Rep * Umbel order	3	2520.82	941.82	22.06
Treatment	11	18609.26***	6543.50***	537.15***
Pollinator	1	120.13	308.33	2.00
Female	2	48460.93***	6496.68	187.79***
Male	1	48724.01***	34892.01***	3960.50***
Pollinator * Female	2	3063.29	5618.44	3.04
Pollinator * Male	1	572.35	1577.33	589.39***
Female * Male	2	21903.01	2124.60	440.38***
Pollinator * Female * Male	2	4215.43	3360.68	47.18
Umbel order * Treatment	22	13442.04***	6559.37***	56.68***
Umbel order * Pollinator	2	2260.67	2205.56	74.67
Umbel order * Female	4	37679.49***	13350.18***	4.58
Umbel order * Male	2	22293.56	641.72	314.00***
Umbel order * Pollinator * Female	4	7492.65	11192.39***	28.96
Umbel order * Pollinator * Male	2	666.89	162.39	116.06
Umbel order * Female * Male	4	10363.12	4270.81	13.38
Umbel order * Pollinator * Female * Male	4	5785.41	5758.34***	12.47
Error	33	1929.73	635.03	13.33

*** = significant at P<0.001, ** = significant at P<0.01 and * = significant at P<0.05,

4.2 Means of the main and interaction effects

4.2.1 Main effects

Considering each umbel order, the mean values for the levels of pollinator were statistically not different for all the variables, for all umbel orders except for germination % (Table 6). The flies recorded higher germination rate (72.58%) than the bees (68.25%) when the secondary umbel seed was tested. Male inbred line SVC111 recorded higher mean values than the other male (SVC131) for all variables except weight of primary umbels of which there was no statistical difference. The mean values for the females were significantly different for all the variables except seed weight and germination rate of the tertiary umbels.

Means for levels of umbel order were significant for all variables (Table 6). Secondary umbels registered highest mean value for weight of umbels and seed weight. Primary umbels had highest germination rate though not significantly different from secondary umbels. There was no difference in mean values of all the variables for the pollinators across all levels of umbel order. Male inbred line SVC111 performed better than the other male (SVC131) for all variables across all levels of umbel order. Means for the levels of female inbred lines were different, over all levels of umbel orders. Regarding weight of umbels, female SVC212 recorded the highest mean value though it was not statistically different from female SVC213, and this trend was repeated for seed weight. Female lines SVC211 and SVC213 were not different in respect of germination rate which was lower than that of female SVC212.

Table 6: Main effect of levels of pollinator and parental inbred lines on seed production and seed quality assessed from each umbel order.

Factor		Primary umbels			Secondary umbels			Tertiary umbels		
		Weight of Umbels (g $plot^{-1}$)	Seed weight (g $plot^{-1}$)	Germination (%)	Weight of Umbels (g $plot^{-1}$)	Seed weight (g $plot^{-1}$)	Germination (%)	Weight of Umbels (g $plot^{-1}$)	Seed weight (g $plot^{-1}$)	Germination (%)
Pollinators	Bees	133.83a	148.50a	73.00a	387.33a	259.25a	68.25b	169.50a	126.17a	61.25a
	Flies	134.42a	144.92a	72.00a	410.25a	274.00a	72.58a	153.75a	102.58a	58.92a
Male inbred lines	SVC 111	133.58a	170.42a	82.25a	418.08a	282.83a	79.67a	220.92a	140.50a	63.33a
	SVC131	134.67a	123.00b	62.75b	379.50a	250.42b	61.17b	102.33b	88.25b	56.83b
Female inbred lines	SVC 211	144.25a	162.75a	70.38b	278.13b	198.25b	67.25b	121.50b	110.00a	58.25a
	SVC 212	130.75b	137.13b	75.75a	438.13a	284.50a	74.13a	234.88a	129.50a	62.38a
	SVC 213	127.38b	140.25b	71.38b	480.13a	317.13a	69.83b	128.50b	103.63a	59.625a

Means followed by the same letter(s) for each factor and variable, are not significantly different; different letter(s) indicate significant differences

Considering the main effect of levels umbel order, male inbred lines, and female inbred lines the mean values were statistically different for all the quantitative traits (weight of umbels, seed weight and germination percentage) (Table 7). There was no significant difference in mean values of pollinators across all levels of traits. Male inbred line SVC111 recorded higher mean values in germination percentage 75.08% than the other male SVC131 by 60.25% which there was significant difference statistical. The mean values for the females were significantly different for all the variables except weight of umbels (SVC 212 = 267.92 g and SVC 213 = 245.33 g) and seed weight (SVC 212 = 183.71 g and SVC 213 = 187.00 g).

Table 7: Main effect of levels of pollinator, parental inbred lines and umbel order on seed production and seed quality.

Factor		Weight of Umbels (g plot^{-1})	Seed weight (g plot^{-1})	Germination (%)
Umbels order	Primary	134.13b	146.71b	72.50a
	Secondary	398.79a	266.63a	70.42a
	Tertiary	161.63b	114.38c	60.08b
Pollinators	Bees	230.22a	177.97a	67.50a
	Flies	232.81a	173.83a	67.83a
Male inbred lines	SVC 111	257.53a	197.92a	75.08a
	SVC131	205.50b	153.89b	60.25b
Female inbred lines	SVC 211	181.29b	157.00b	65.29b
	SVC 212	267.92a	183.71a	70.75a
	SVC 213	245.33a	187.00a	66.96b

Means followed by the same letter(s) for each factor and variable, are not significantly different; different letter(s) indicate significant differences.

4.2.2 Treatment and interaction effects

Table 8 shows that treatment means were different for all the recorded variables in the three umbel categories except for seed weight and germination rate of the tertiary umbel harvests.

For the primary umbel category, with respect to weight of umbels, Treatment 2 (Bees x SVC111 X SVC212) recorded lowest mean of 103.5 g and Treatment 7 (Flies x SVC111 x SVC211) recorded the highest mean of 153.0 g, and the same treatments had minimum (137.5 g) and maximum (206.0 g) seed weight, respectively. However, Treatment 1 (Bees x SVC111 x SVC211) was not statistically different from Treatment 7 in respect of seed weight. The highest germination rate (89.5%) was observed on Treatment 8 (Flies x SVC111 x SVC212) and the lowest (53.0%) on Treatment 10 (Flies x SVC131 x SVC211).

In the secondary umbel category, the highest values for weight of umbels and seed weight were 595 g and 430 g respectively, both for Treatment 2 (Bees x SVC111 x SVC212), and the lowest values were 205 g and 177 g, respectively, both for Treatment 1 (Bees x SVC111 x SVC211). Mean seed weight for Treatment 9 (Flies x SVC111 x SVC213) which was 426 g was not significantly different from that for Treatment 2. The highest germination rate (92%) was observed on Treatment 8 (Flies x SVC111 x SVC212) and the lowest (50%) was observed on Treatment 10 (Flies x SVC131 x SVC211).

As regards the tertiary umbel treatment means, there was no significant difference for seed weight and germination %. However, Treatment 2 (Bees x SVC111 x SVC212) recorded the highest value (375 g) for weight of umbels and Treatment 10 (Flies x SVC131 x SVC211) recorded the lowest value (79.5 g).

Factor interaction effects that were detected as significant by analysis of variance are graphically represented (Figures 7 to 10).

Table 8: Means for recorded variables of different treatments for the first, second and third umbel harvests

Treatment	*Factor combinations*	Primary umbels			Secondary umbels			Tertiary umbels		
		Weight of Umbels (g $plot^{-1}$)	Seed weight (g $plot^{-1}$)	Germination (%)	Weight of Umbels (g $plot^{-1}$)	Seed weight (g $plot^{-1}$)	Germination (%)	Weight of Umbels (g $plot^{-1}$)	Seed weight (g $plot^{-1}$)	Germination (%)
1	Bees x SVC111 x SVC211	148.5ab	197.0a	83.5abc	205d	177c	80bcd	160abc	173a	65a
2	Bees x SVC111 x SVC212	103.5d	137.5c	78.0abc	595a	430a	70cde	375a	182a	68a
3	Bees x SVC111 x SVC213	143.5ab	189.5ab	75.0cbd	446abc	242cb	69.5ed	155cb	114a	59.5a
4	Bees x SVC131 x SVC211	134.0cb	121.0c	58.5ef	300cd	209c	54fg	95c	84a	52a
5	Bees x SVC131 x SVC212	131.5cb	120.0c	72.0cd	359.5bcd	236cb	70.5cde	113.5c	97a	63a
6	Bees x SVC131 x SVC213	142.0ab	126.0c	71.0cde	418.5abcd	261cb	65.5ef	118.5c	107a	60a
7	Flies x SVC111 x SVC211	153.0a	206.0a	86.5ab	305cd	197.5c	85ab	151.5cb	127a	65a
8	Flies x SVC111 x SVC212	148.5ab	165.5b	89.5a	400abcd	224cb	92a	350ab	150a	63.5a
9	Flies x SVC111 x SVC213	104.5d	127.0c	81.0abc	557.5ab	426.5a	81.5abc	134c	97a	59a
10	Flies x SVC131 x SVC211	141.5ab	127.0c	53.0f	302.5cd	209.5c	50g	79.5c	56a	51a
11	Flies x SVC131 x SVC212	139.5ab	125.5c	63.5defde	398abcd	247.5cb	64ef	101c	89a	55a
12	Flies x SVC131 x SVC213	119.5dc	118.5c	58.5ef	498.5abc	339ab	63ef	106.5c	96.5a	60a

Means followed by the same letter(s) in a column are not significantly different; different letter(s) indicate significant differences.

Across all umbel harvests, Treatment 2 (Bees x SVC111 x SVC212) recorded the highest weight of umbels and seed weight (Table 9). whereas the lowest values for the same variables were recorded for Treatment 1 (Bees x SVC111 x SVC211) Treatment 10 (Flies x SVC131 x SVC211), respectively. Treatment 8 (Flies x SVC111 x SVC212) was the best in respect of germination %, and Treatment 10 was the worst-performer for the same.

Table 9: Means for recorded variables of different treatments across all umbel order harvests

Treatment	*Factor combinations*	Weight of Umbels (g $plot^{-1}$)	Seed weight (g $plot^{-1}$)	Germination (%)
1	Bees x SVC111 x SVC211	171.2d	182.3bc	76.2ab
2	Bees x SVC111 x SVC212	357.8a	249.8a	72.0bcd
3	Bees x SVC111 x SVC213	248.2bcd	181.8bcd	68.0cde
4	Bees x SVC131 x SVC211	176.3cd	138.0cd	54.8gh
5	Bees x SVC131 x SVC212	201.5cd	151.2cd	68.5cd
6	Bees x SVC131 x SVC213	226.3bcd	164.7cd	65.5def
7	Flies x SVC111 x SVC211	203.2cd	176.8bcd	78.8ab
8	Flies x SVC111 x SVC212	299.5ab	179.8bcd	81.7a
9	Flies x SVC111 x SVC213	265.3bc	216.8ab	73.8bc
10	Flies x SVC131 x SVC211	174.5d	130.8d	51.3h
11	Flies x SVC131 x SVC212	212.8bcd	154.0cd	60.8efg
12	Flies x SVC131 x SVC213	241.5bcd	184.7bc	60.5fg

Means followed by the same letter(s) in a column are not significantly different; different letter(s) indicate significant differences.

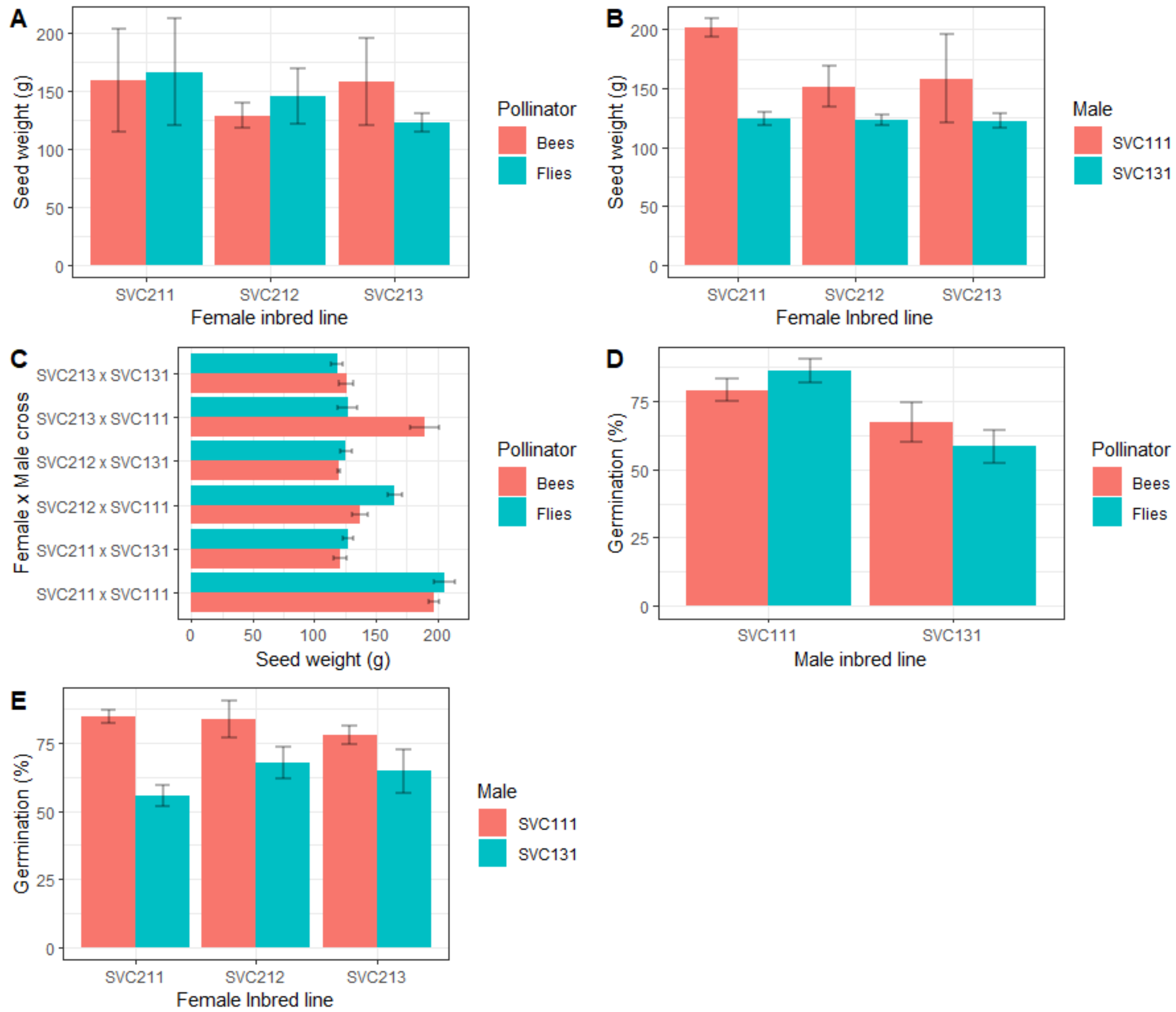

Figure 7: Significant factor interaction effects on seed weight in grams per plot (A to C) and germination percentage (D to E) of primary umbel harvests.

There was significant female inbred line by pollinator interaction effect on seed weight of primary umbel harvests (Figure 7.A). The seed weight from SVC211 x Flies was the highest; this was followed by SVC213 x Bees. Combinations SCV212 x Bees and SCV213 x Flies recorded relatively low seed weights.

The female x male interaction effect was significant for seed weight of the primary order umbel harvests (Figure 7.B). Combination SVC211 x SVC111 recorded the highest seed weight, followed by SVC213 x SVC111 and SVC212 x SVC111, in that order. It is also evident that Male SVC111 was superior to SVC131.

The three-factor interaction effect of pollinator x female x male was significant for seed weight of the primary umbel harvest (Figure 7.C). Highest amount of seed by weight was harvested from female SVC211 x SVC111 cross that was pollinated by flies, followed by the same combination of female and male pollinated by bees. However, these combinations were not significantly different from SVC211 x SVC111 pollinated by bees.

There was a significant interaction effect of males and pollinators for germination % of primary umbel harvests, as shown in Figure 7.D. The effect of SVC111 x Flies recorded the highest germination rate and it differed significantly from the next best combination (SVC111 x Bees). The lowest germination % was realised for SVC131 in combination Flies, followed by SVC131 in combination with Bees.

The female x male interaction effect was significant for germination % of primary umbel harvests (Figure 7.E). Higher germination rates were observed when the pollen source was SVC111 than when it was SVC131. The female by male combination SVC211 x SVC111 recorded the highest germination % followed by SVC212 x SVC111; and SVC211 x SVC131 recorded the lowest germination %.

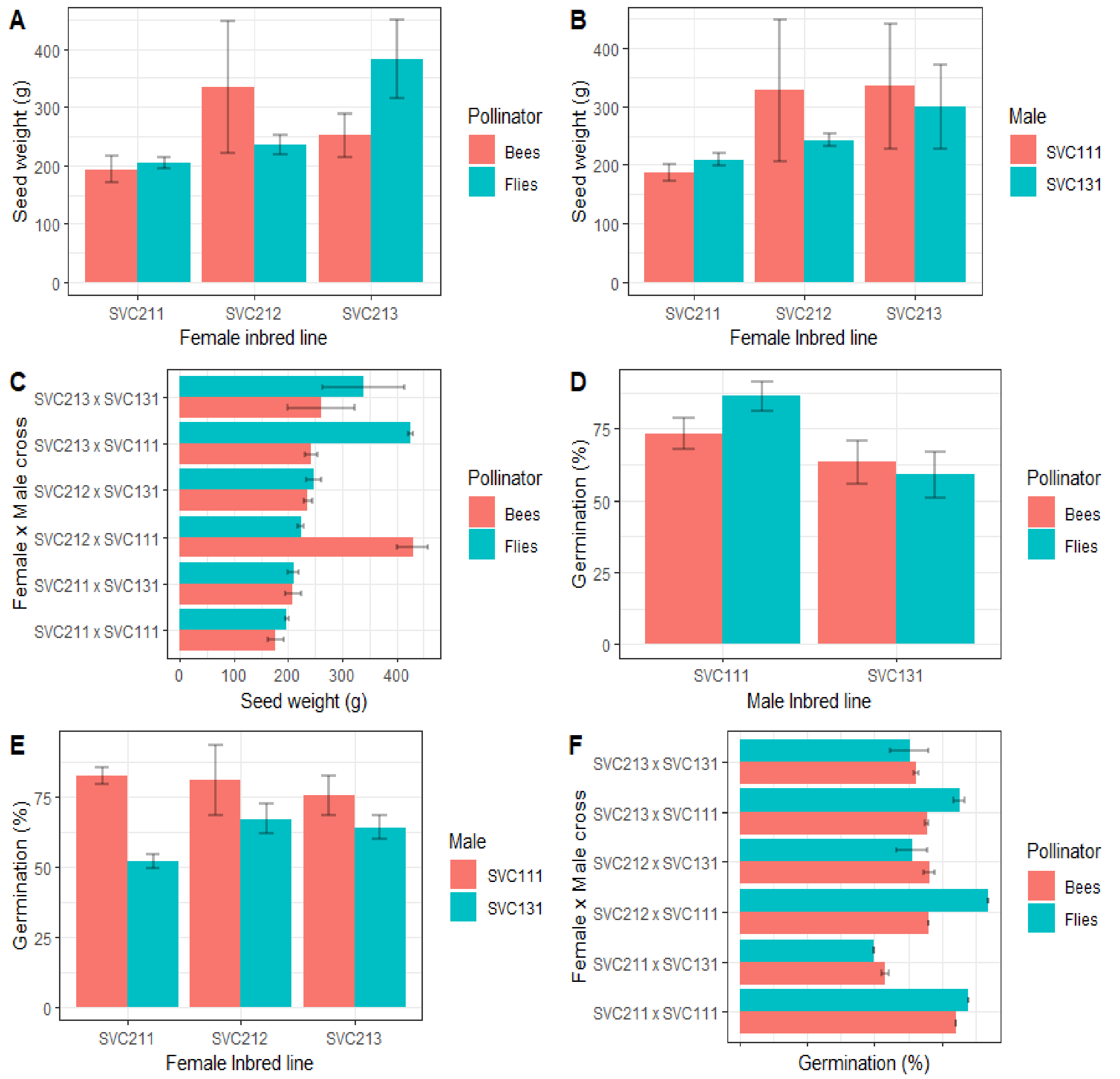

Figure 8: Significant factor interaction effects on seed weight in grams per plot (A to C) and germination percentage (D to F) of the secondary umbel harvests.

Interaction of female parents by pollinators was significant for seed weight (Figure 8.A). Female SVC213 x Flies recorded the highest seed weight followed by SVC212 x Bees. the combination SVC211 x Bees recorded the lowest seed weight.

The female x male interaction effect on seed weight is illustrated in Figure 8.B. Female SVC213 by male SVC111 gave the highest seed quantity but was not statistically different from female SVC212 by male SVC111. Lowest seed quantity was recorded for female SVC211 x male SVC111.

A higher order interaction effect, that is, pollinator x female x male, is illustrated for seed weight of secondary umbel harvests (Figure 8.C). Bees x SVC212 x SVC111, and Flies x SVC213 x SVC111 were not significantly different, and they recorded highest seed weight. The minimum seed weight was recorded on Bees x SVC211 x SVC111 followed by Flies x SVC211 x SVC111.

Pollinator x male parent graphs for germination % of secondary umbel harvests shows that Flies x SVC111 was the most desirable combination. Next best was Bees x SVC111, and the least desirable combination was Flies x SVC131.

Female by male interaction effect was significant for germination % of the secondary umbel harvests. Combination SVC211 x SVC111 had highest germination % followed by SVC212 x SVC111, and SVC213 x SVC111, in that order. The lowest germination rate was recorded on the SVC211 x SVC131 combination.

Three factor interaction effect on germination % of secondary umbel harvests is represented in Figure 8.F. Top three combinations were: Flies x SVC212 x SVC111 which recorded the highest value, followed by Flies x SVC211 x SVC111, and then Flies x SVC213 x SVC111. The lowest germination rate was recorded for Flies x SVC211 x SVC131.

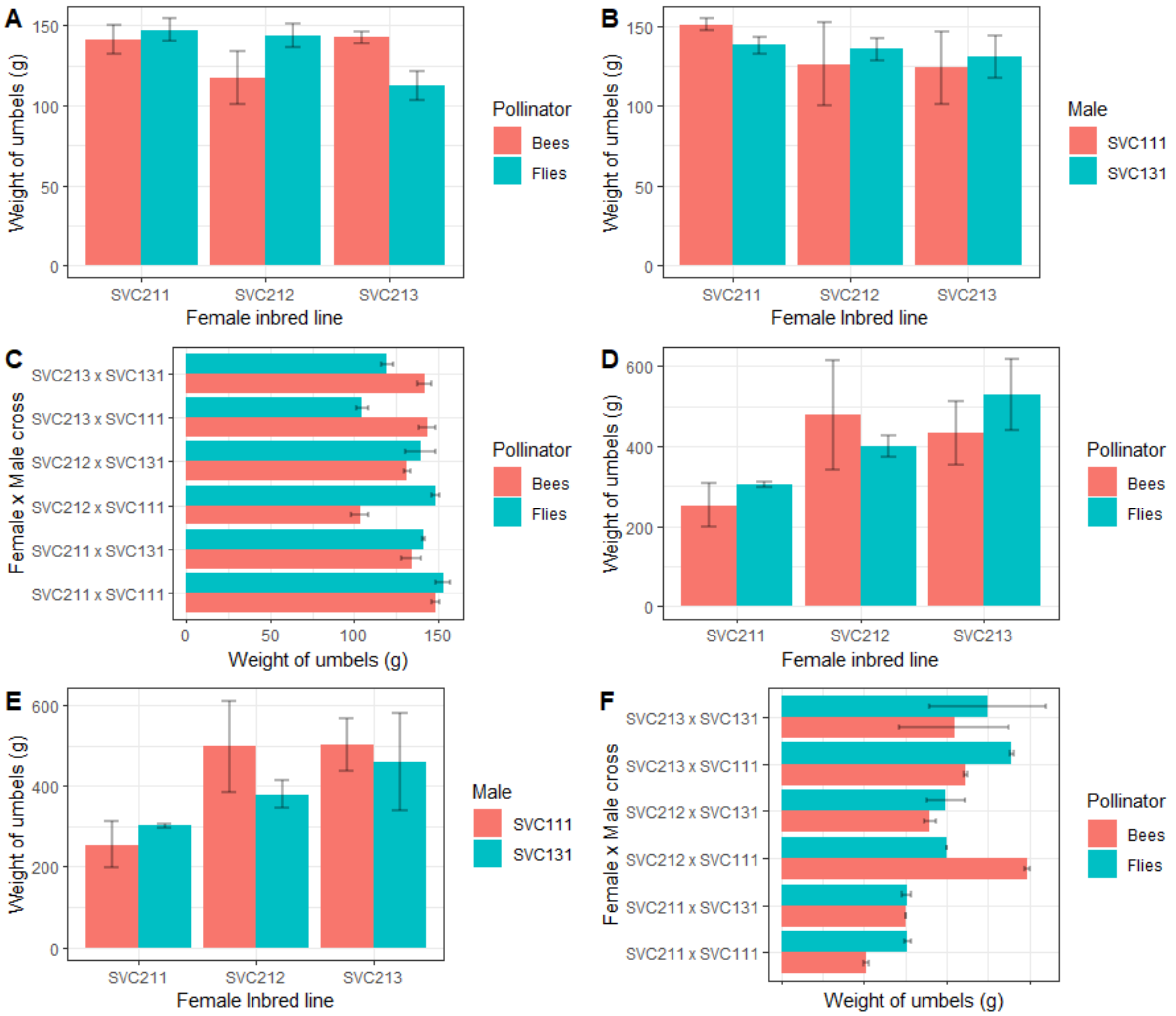

Figure 9: Significant factor interaction effects on weight of umbels of primary umbel harvests (A to C) and secondary umbel harvests (D to E) in grams per plot.

Figure 9 clearly shows the significant factor interaction effects on weight of umbels of the primary and secondary umbel order harvests. In the primary umbel order category, female x pollinator (Figure 9.A), female x male (Figure 9.B), and female x male x pollinator (Figure 9.C) were significant. In Figure 9A, SVC211 x Flies, SVC212 x Flies, SVC213 x Bees, and SVC211 x Bees recorded high umbel weights not significant from each other. The combinations SVC212 x Bees and SVC213 x Flies recorded relatively low umbel weights.

In Figure 9B, there are slight differences between the combinations of females and males. However, SVC211 x SVC111 recorded the highest umbel weight.

There were slight mean differences in weight of primary order umbels when three factor combinations were compared (Figure 9C). However, combinations SVC213 x SVC111 x Flies, and SVC212 x SVC111 x Bees, had relatively low umbel weights.

The female x male interaction effect on weight of secondary umbels presented in Figure 9D shows that SVC213 x Flies was the best combination followed by SVC212 x Bees. The least desirable combinations in this umbel category were SVC211 x Bees and SVC211 x Flies.

In Figure 9E, females SVC212 and SVC213 in combination with male SVC111 recorded highest weight of umbels, followed by SVC213 x SVC131. The female SVC211, pollinated by either male, gave relatively low umbel weights.

In Figure 9F, the best combination for weight of umbels was SVC212 x SVC111 x Bees, followed by SVC213 x SVC111 x Flies. The lowest umbel weight was recorded for SVC211 x SVC111 x Bees.

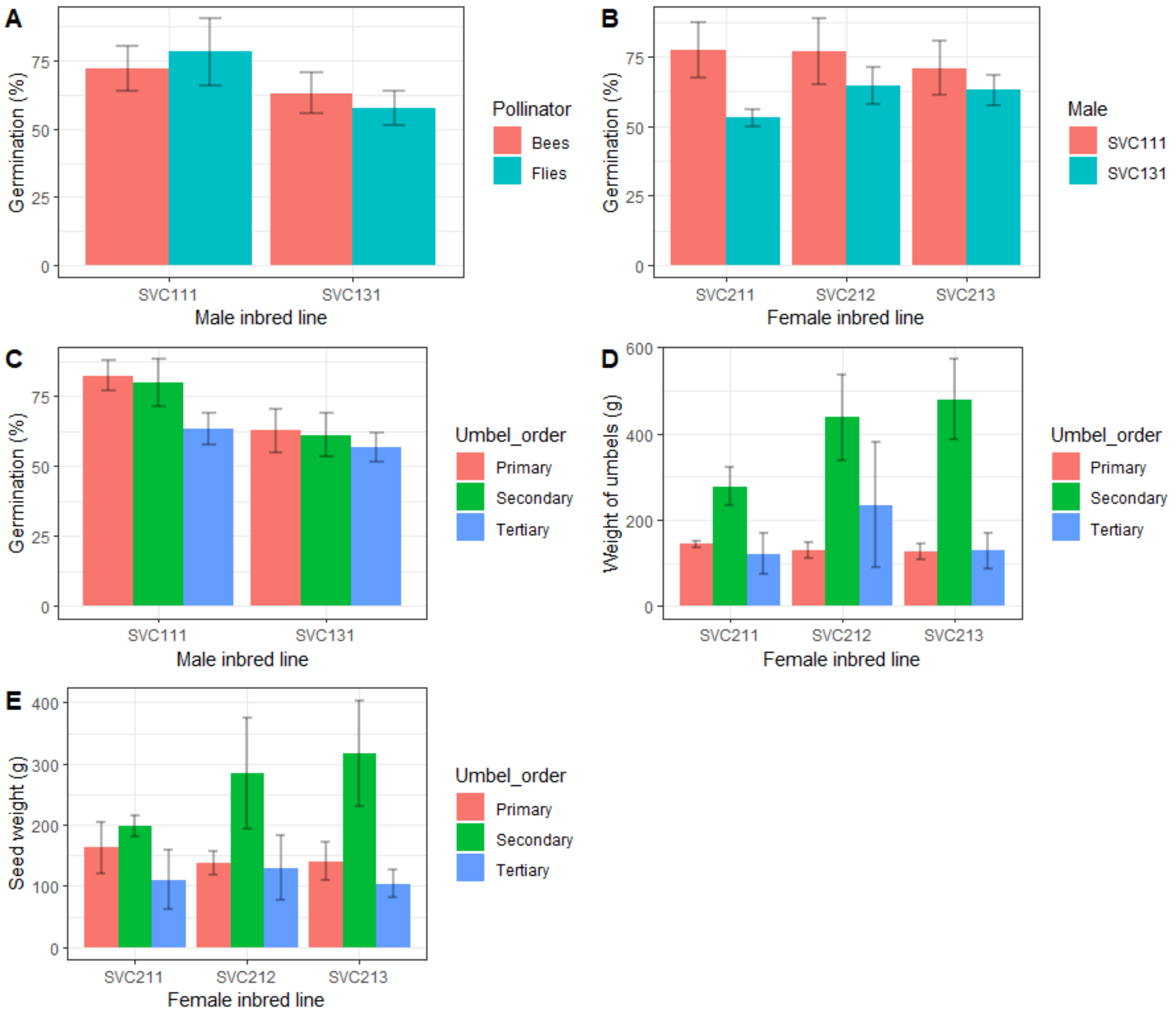

Figure 10: Significant two factor interaction effects on germination percentage (A to C), weight of umbels in grams per plot (D), and seed weight in grams per plot (E) across all umbel order harvests.

Significant interaction effects across all umbel order harvests are presented in Figures 10. Figure 10 shows significant two factor interaction effects on weight of umbels, seed weight and germination %.

Figure 10A shows male x pollinator effect of germination %. The highest germination rate was observed on combination SVC111 x Flies, followed by SVC111 x Bees. The combinations involving male SVC131 had relatively lower germination %.

Female x male interaction effect on germination % is illustrated in Figure 10B. Highest germination % was recorded by SVC211 x SVC111 and SVC212 x SVC111, followed by

SVC213 x SVC111. Combinations involving male SVC131 exhibited relatively lower germination %.

The performance of males regarding germination % in each umbel order is shown in Figure 10C. Male SVC111 was superior to SVC131 in all umbel orders. Highest percentage value of germination was recorded for SVC111 in the primary umbel category, followed by the same male in the secondary umbel category, and then again, the same male in the tertiary umbel category. Germination % decreased when advancing from primary umbel order to secondary umbel order, and from the secondary order to the tertiary umbel category.

Another significant two factor interaction effect was that of the female parent and the umbel order. This showed the performance of each female in each umbel order for weight of umbels and seed weight, and in this regard, almost the same trend was observed for the two variables. Highest weight of umbels value was observed on SVC213 in the secondary umbel category, followed by SVC212 and SVC211 in the same umbel category. The same ranking of top three observations was exhibited for seed weight.

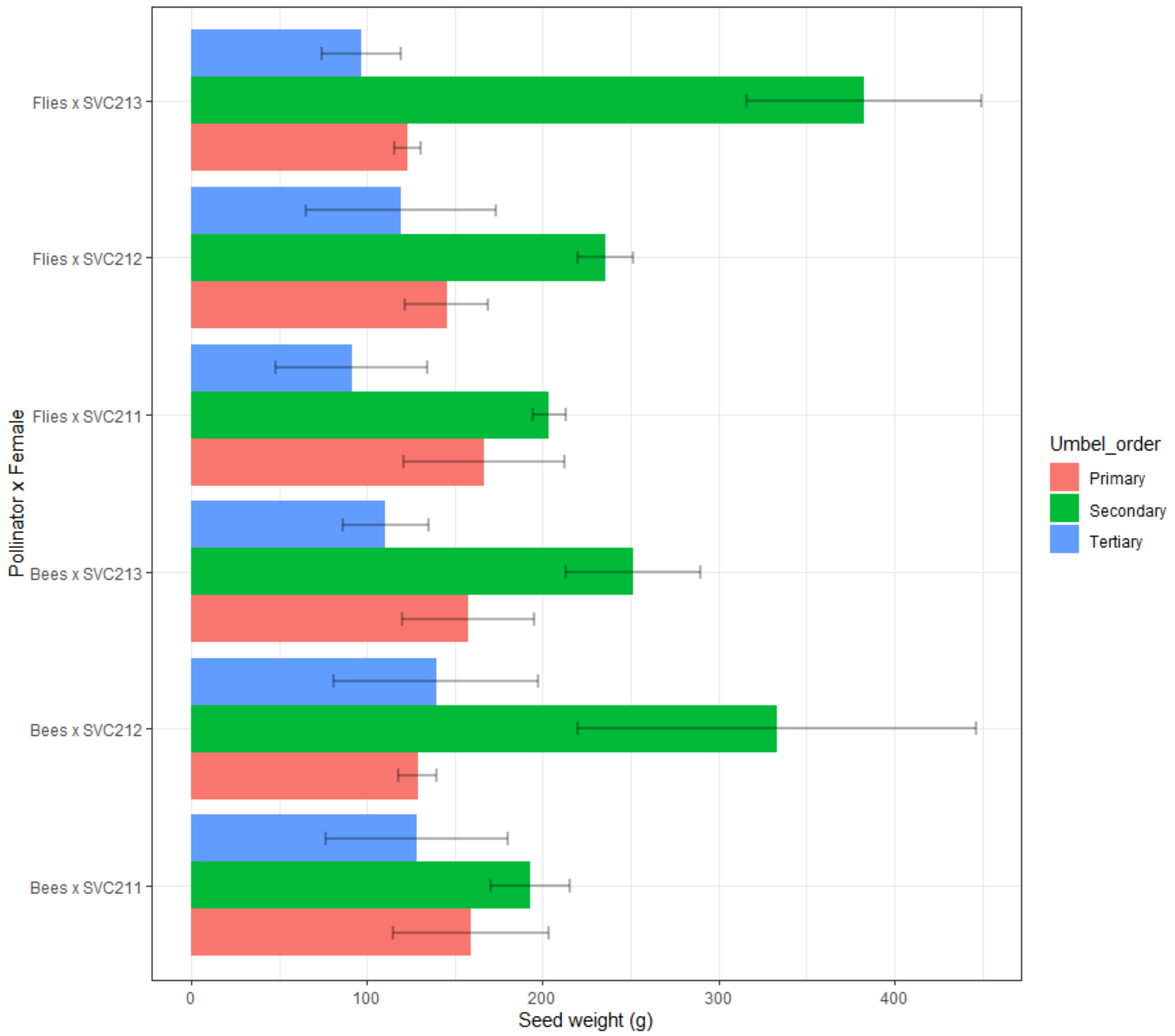

Figure 11: Significant three factor interaction effects on seed weight in grams per plot across all umbel order harvests.

Figure 11 presents significant high order (three factor) interaction effects on seed weight. The seed weight of harvests from SVC212 pollinated by Flies in the primary umbel category was higher than when the same female was pollinated by Bees. The reverse was true for secondary umbel harvests where SVC212 pollinated by Flies recorded lower seed weight than when pollinated by Bees. The combination Flies x SVC213 had lower seed weight than Bees x SVC213 in the primary umbel category whereas in the secondary umbel category it was the Flies x SVC213 which performed better than Bees x SVC213.

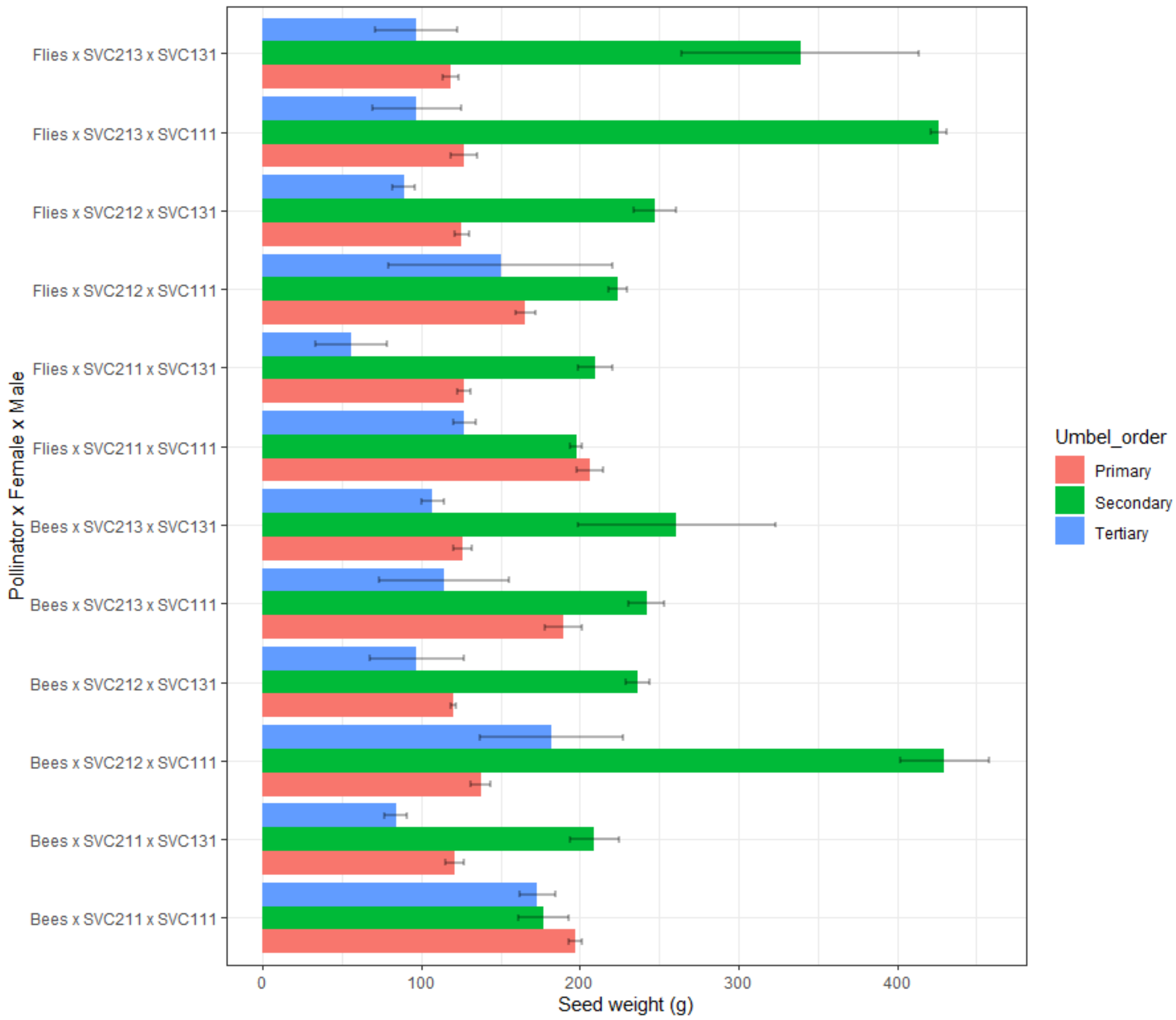

Figure 12: Significant four factor interaction effects on seed weight in grams per plot across all umbel order harvests.

In Figure 12, some combinations of female and male performed differently when different pollinators were used, and in the different umbel categories. For example, Flies x SVC213 x SVC111 recorded lower seed weight than the Bees x SVC213 x SVC111 in the primary umbel category; however, the reverse was true in the secondary umbel category. Another example is the Flies x SVC212 x SVC111 which performed better than Bees x SVC212 x SVC111 in respect of seed weight in the primary order harvests while the reverse was true in the secondary umbel harvests.

CHAPTER 5

DISCUSSION

The study has shown there is variability among the carrot inbred lines in respect to weight of umbels, seed weight, and germination percentage. Observed that the effect of the deployment of pollinator species (*Apis mellifera* and *Chrysomya choropyga*) among the carrot inbred lines for each different female inbred line was significantly high in the first umbels than the second and third umbels. The results suggest that trait qualities of hybrid carrot inbred lines were being influenced by the two male fertile donors (SVC 111 and SVC 131). The overall traits (weight of umbel, seed weight, and germination percentage) were highly significant for all interaction and main effects.

The data indicated that the honeybees were not significantly different from the fly pollinators in all the quantitative traits (weight of umbels, seed weight and germination percentage) and in all different stages of umbel flowering. The weight of umbels showed the impact of morphologically different inbred lines, with the weight ranging between 500 g and 210 g across all female lines in all cages. The influence arising from the variation in morphology was reported by other researchers (Brittain et al., 2013). Hence, a significant difference ($p \leq 0.001$) was observed for seed weight among the female inbred lines in all replications and cages.

The results are also in agreement with the work by Howlett (2012) who indicated that flies could be utilized and were successful known contributors to pollination. Relatively high seed weight was observed from the first umbel (125 g) for the flies and female (SVC 211), and interaction of Flies x SVC 211 x SVC 111 was 210 g. The seed germination percentage for the first umbel was not significantly different from the second umbels but significantly higher than that of third umbels, ranging between 85.5% and 52%. The seed germination percentage of the flies' treatment was very high by 78%, indicating that non-bee insects can contribute to good quality carrot seed as reported by Larson *et al* (2001). Similar results were reported by Brittain *et al*. (2013) who reported that flies complement bees as pollinators.

There were clear differences in pollinator visitations between both inbred lines and umbel stages. Traditionally, honeybees are the dominant pollinators of carrots and their performance is essential as reported by Vicens and Bosch (2000). The study has shown the important role of flies in flowers visitations throughout the day to the extent that they appeared dominant over the honeybees (Rader *et al.* 2013). For instance, in the early morning more than four flies could be observed active in each umbel. In addition, there were significant differences between the pollinator visitations on the male and female lines under the different cages. The preference for foraging could also be seen in the differences observed for seed weight and seed germination in the different umbels for CMS lines, where these were low in the third umbels. However, this could indicate that crop fluctuation of flowering affects the deployment of pollinators (Erickson *et al*. 1979).

There was no significant difference among manageable honeybees' in their contribution in all phases of umbels flowering, even though their activity is still in question. This outcome reinforces the importance of having alternative pollinators (such as flies) to assist in successful pollinations, whilst the future management of honeybees is being reimagined. This finding agrees with Howlett et al. (2015) who explained that the importance of alternatives insect (flies) visitation may help in the understanding of their significant influence in improving successful pollinations. The traits' quality mean amongst all the umbels (1st, 2nd, 3rd) showed significant differences for all interactions, except for seed set and seed yield where the pollinators were not comparably enough to observe differences.

The interaction effect was also significantly different for seed weight and germination percentage but was not high in the third umbels, resulting in no significant difference (Table 8). The treatment factor combination with significant interaction indicates the extent of the visitation to different parts within a flower, inflorescence, or other flowers within the same plant (high versus low flower) as a result of pollinator deployment. Therefore, these flowers entice pollinators by variation in morphology, colour, and scent. The study results suggest that inbred lines and the trait's quality should be considered when deploying the sterile male lines' pollinators and variability.

Weather conditions influence insect pollinator flower visitations as has been confirmed by many researchers (Vicens and Bosch, 2000; Kevan and Baker, 1983; Abrol, 2006; Eric *et al.* 2010), and temperature and humidity are some of the major factors that affect insect pollinator behavior and foraging patterns. However, though temperature and humidity data were recorded in this study, no data were collected to study the foraging behavior of the insect pollinators at varying temperature and humidity levels. Future researchers to study the effectiveness of different pollinators should gather enough data so that the species can be compared at varying levels of weather factors especially temperature and humidity. This is quite important especially with the expected climate changes; elevated temperatures, for example, may negatively impact some pollinator species, thus affecting pollinator foraging and pollination effectiveness in the future (Gaffney *et al.* 2018). Figure 4 shows that when temperatures are low, the relative humidity percentage is high and vice versa. The study temperature data showed that morning temperatures were low, and afternoon were high, especially for November month. For example, on 18 November 2019, temperature was 15^{o}C and relative humidity (RH) was 68.5% in the morning and afternoon it was 28^{o}C, with RH of 36%. Consequently, pollinators' activity took place earlier, and there was a significant interaction of the pollinators with the female and male inbred lines. Therefore, as indicated in Figure 5, flies had the advantage of frequent and more visitation over bees during their foraging.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

In summary, the results of this study have shown that flies were the primary pollinators in the secondary umbels while honeybees were more common during the entire pollination period. The activity of the two pollinators varied by traits (weight of umbels, seed weight and germination percentage) and umbels order (1^{st}, 2^{nd}, 3^{rd}), indicating that their effectiveness is comparable, and they could thus be deployed together. The results also indicated where both pollinators foraged more and visited the most during the pollination period. The deployment of pollinators was predominant in the male fertile inbred line (SVC 111) over the male fertile (SVC 131) with high significant difference in all the umbels order and the traits. In addition, differences were observed between the pollinators in how the pollen was collected from the male inbred lines, with extremely low preference of the SVC 131 line. However, further investigations of pollen quality are needed to check the impact on fertilizing the ovary of the male sterile line during cross pollination.

There were highly significant differences between the female inbred lines (cytoplasmic male sterile), whereby female lines SVC 212 and SVC 213 showed better and higher significant performance than male sterile line SVC 211. Whereas, the germination percentage of SVC 211 and SVC 213 showed no significant difference, the two lines were significantly different from the female inbred line SVC 212. The overall results indicated no significant difference in the three factor interaction effects for all the traits except for seed weight, which was highly significant. While, the significant difference in the four factor interaction effects that include pollinators, parental inbred lines and umbel orders implies that bees and flies can be deployed together during pollination to accomplish a good seed set and quality.

Based on the overall results, it is important to select lines (both male sterile and male fertile) with favourable traits as poor traits might result in inadequate pollination. Managed honeybees are important pollinators throughout the world, but their foraging behaviour is influenced by environmental factors. The results have shown that flies can be comparable to bees for effective pollination .

The findings will benefit the commercial hybrid carrot production and provide significant opportunities for plant breeders, seed industry, and growers to improve pollinations in seed production, increase awareness of factors that influence the breeding lines, seed yield and qualities. The challenge of Calliphorides flies is still great to maintain a competitive advantage over the manageable pollinators (*Apis mellifera*), as their potential management option is still not clearly understood. However, from this study, simultaneous deployment of the two-pollinator species should be possible and is therefore recommended to accomplish successful pollination.

6.2 Summary of research finding

a). Assessment of different male sterility inbred line using two pollinator species deployment.

- ❖ The ANOVA showed no significant difference between the deployment of pollinator species for all the quantitative traits of the female inbred lines (cytoplasmic male sterile).
- ❖ The foraging activity and visitation in all female inbred lines showed that Calliphorides flies performed much better compared to honeybees through primary, secondary, and tertiary flowering stage of umbels.
- ❖ The Primary umbels of the main effect showed that SVC 211 was significantly different in weight of umbels and seed weight compared to SVC 212 and SVC 213. However, there were no significant differences between the pollinators. This means that their foraging activity was high, and their visitation was both good for pollination. However, as influenced by morphological traits in the female inbred lines, slight differences were observed in the number of visitations.

- Significant four factor interaction effects that includes pollinators, parental inbred lines and umbel orders implied that bees and flies can be deployed together during pollination to accomplish a good seed set and quality.

6.3 General implication and the way forward

- The significant three and four factor interaction showed significant difference in pollinators. The performance results indicate that high variation exist trough male fertile flower so selection should be made for good deployment and stability through pollination service.

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