Improving the reliability of vegetable seed yields

Dr Alistair Gracie
TIAR

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Improving the Reliability of Vegetable Seed Production

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The purpose of this report is to provide information to the public about vegetable seed production research conducted during the study.

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Table of Contents

MEDIA SUMMARY **3**

TECHNICAL SUMMARY **5**

CHAPTER 1 - INTRODUCTION **7**

SECTION 1 – CARROT SEED PRODUCTION RESEARCH AND EXTENSION **9**

CHAPTER 2 - FACTORS AFFECTING ATTRACTION OF POLLINATORS TO HYBRID CARROT SEED PARENT LINES **9**

INTRODUCTION 9
MATERIALS AND METHODS 10
Trial Site *10*
Plant Material *11*
Trial Design *12*
Cultural Practises *12*
Trial Assessments *13*
Data Analysis *14*
RESULTS AND DISCUSSION 15
Seed Yields *15*
Pollinator Diversity *15*
Effects of Flower Morphotype and Colour on Honeybee Visitation *20*
Umbel Size and Bloom Area *20*
Nectar Abundance *22*
Conclusion *26*

CHAPTER 3 - MANAGEMENT OF FLOWERING TIME IN CARROT **27**

INTRODUCTION 27
Pollinator Activity *27*
Pollen Viability *27*
Managing Hybrid Carrot Seed Yields *27*
MATERIALS AND METHODS 30
Trial Sites *30*
Plant Material and Trial Design *30*
Assessment of Flowering Time and Pollinator Activity *32*
Seed Yield Assessments *32*
RESULTS 33
Climatic Conditions *33*
Effects of Environmental Factors on Pollinator Activity *33*
Effects of Trimming and Paclobutrazol on Flowering Time and Pollinator Activity *36*
Effects of Trimming Treatments on Yield Potential *39*
Effects on Realised Seed Yield *40*
Overview *42*

CHAPTER 4 - DEVELOPMENT OF A WORLD CLASS HYBRID CARROT SEED INDUSTRY IN NORTHERN TASMANIA. APPLIED RESEARCH AND EXTENSION IN ACTION **43**

INTRODUCTION 43

METHODS 44
Project Team *44*
Project Strategy *45*
PROJECT OUTCOMES 45
Problem Identification *45*
Addressing the Problems *45*
Grower and Customer Participation *49*
Extension Activities *50*
EVALUATION 52
INDUSTRY PROSPECTS AND CHALLENGES 53

SECTION 2 – ONION POLLINATION RESEARCH 54

CHAPTER 5 - UNDERSTANDING THE BASIS OF UNRELIABLE HYBRID ONION SEED YIELDS 54

INTRODUCTION 54
MATERIALS AND METHODS 55
Survey Sites *55*
Survey Parameters *55*
Supplemental Hand Pollination Experiments *57*
Seed Yield Determinations *57*
RESULTS AND DISCUSSION 57
Climatic Conditions During The Surveys *57*
Seed Yields from Survey Crops *59*
Flower Abortion *60*
Pollen Viability *60*
Cross Pollination Rates *63*
Effects of Supplemental Hand Pollination *63*
Factors Affecting Pollination *64*
Factors Affecting Pollinator Activity. *67*
Conclusions *71*

CHAPTER 6 - RECOMMENDATIONS 72

Management of Flowering Time in Carrot *72*
Development of a World Class Hybrid Carrot Seed Industry in Tasmania *73*
Understanding the Basis of Unreliable Hybrid Onion Seed Yields *73*
Research Activity *73*

CHAPTER 7 - TECHNOLOGY TRANSFER 74

REFERENCES 77

Media Summary

The Australian carrot and onion seed industries contribute in excess of $10 million annually to the value of Australian horticultural production. Much of this income is derived from production of seed for export markets. Demand for high quality seed production has led to strong interest in expansion of both crops in Australia.

One of the major challenges to realising this opportunity and sustaining future growth of the industries is having the capacity to produce good seed yields, reliably. Yields from onion and carrot seed crops grown in Australia and elsewhere vary widely within and between seasons. This affects the continuity of seed supply to the market and the economics of seed production for the grower.

This project was part of the ongoing research collaboration between the Australian vegetable seed industry, TIAR and seedPurity Pty Ltd to improve standards of vegetable seed production in Australia. In this project we aimed to address some the issues of reliability of seed production by:

- Applying the existing knowledge of pollination biology in carrot to develop and test management practices to: a) improve pollination and seed yields and b) identify key traits for use in breeding and selection of carrot seed parent lines with improved seed production characteristics;

- Adapting and extending the outcomes from past carrot seed production research in Australia to develop Tasmania as a centre for high quality carrot seed production through a series of demonstration trials run in collaboration with key customers, industry stakeholders and their growers;

- Identify the causes of poor seed yields from hybrid onion seed crops in Australia so that future research can be effectively targeted at yield improvement.

Outcomes from this work include:

- Development of cultural practices for management of flowering time in carrot seed crops to maximise pollination;

- Identification of floral traits for use in breeding and selection of carrots to maximise seed yields;

- Extension of previous research outcomes to develop a group of skilled growers and agronomists within the Tasmanian carrot seed industry undertaking best practice carrot seed production;

- Identification of key limiting factors for hybrid onion seed yields.

The outcomes from this project have led to improvements in the reliability of hybrid carrot seed production in Australia and contributed to international and local recognition of Tasmania as a centre for high quality carrot seed production. This recognition has driven expansion of the Tasmanian industry from 50Ha/annum to almost 200Ha/annum in the

lifetime of the project. The impressive yield and quality results achieved by the industry in that time are creating significant opportunities for continued growth. Future attempts to improve onion seed yields will benefit from the outcomes of research in this project which have established the key factors that must be addressed to improve reliability of yields.

Technical Summary

Long term growth and sustainability in Australia's 20 million dollar vegetable seed industry depends on capacity to produce reliable, economical yields of high quality seed. In this project we collaborated with stakeholders in the Australian vegetable seed industry to address issues affecting the reliability of carrot and onion seed crops grown in Australia. The key aims of the project were to increase understanding of yield limitations in hybrid onion and carrot seed crops, test management strategies to improve pollination of carrots and foster the development of a centre for high quality hybrid carrot seed production in Tasmania.

Carrot Seed Research and Extension

Yields of hybrid carrot seed crops grown in south eastern Australia are often limited by inadequate cross pollination. This results from low rates of pollinator activity in hybrid seed crops. In order to improve pollination of hybrid carrot seed crops it is important that traits that determine attractiveness of seed parent lines to pollinators are understood. This information is relevant to breeding activities and crop management strategies aimed at enhancing pollination. In this project we undertook a 2 year survey of flowering traits, insect activity and seeds yield in hybrid seed parent lines. Differences in seed yields of male sterile lines were most closely correlated with honeybee foraging rates, even though honeybees were often a minority species in insect populations at the survey sites. Differences in honeybee visitation to male sterile lines were associated with differences in bloom density, flower morphotype and nectar standing crop volume. Discrimination between morphotypes appeared to be partly linked to differences in nectar production / availability. Nectar and flower samples and pollinator data collected in these surveys are being used in ongoing work to elucidate relationships between flowering traits and pollinator activity in carrot.

In some Australian production locations environmental conditions for pollination (temperature, humidity and incidence of competing forage sources for pollinators) improve towards the end of the normal flowering period of carrot seed crops. In this project trimming treatments were investigated with a view to: a) manipulating flowering time to coincide with favourable environmental conditions for pollination and b) answering industry questions about the best time to trim to address nicking problems in hybrid seed crops. Different trimming times and intensities were tested on a range of temperate carrot seed parent lines. Amongst the treatment combinations tested, trimming crops to a height of 150mm above ground level when the developing inflorescence had extended to between 200 and 300mm above ground level reliably delayed flowering by 10-14 days and least reduced yield potential. Where trimming treatments caused a shift in flowering to more favourable environmental conditions, pollinator activity increased and 25 to 50% improvements in seed yield were observed. The risks of using such treatments were also shown to be significant. If conditions in the later flowering period were no more or less favourable for pollination, significant yield penalties resulted from reductions in inflorescence size caused by trimming.

Tasmania has long been considered to have potential for production of seed of European hybrid carrot varieties. Although interest in developing an industry peaked in the last decade after investment in research and development had led to improvements in production standards in other Australian locations, initial attempts to establish the industry were

undermined by crop failures. In this project researchers from TIAR and seedPurity worked closely with a group of Tasmanian growers, field agronomists and international vegetable breeders to develop the foundations of a successful industry underpinned by best practice derived from Australian carrot seed production research. In 2009, trial crops demonstrating best practice were grown at 5 sites in northern Tasmania. A series of on-farm demonstrations and grower discussions at key periods in the production cycle were used to communicate applied research outcomes to growers and field officers. Following the outstanding yield and quality results achieved in 2009 and a successful season in 2010, the program has grown to include over 160Ha of production and 20 growers in 2011, with good prospects for ongoing expansion. Although the model of extension has changed by necessity to accommodate a larger number of growers, industry and researchers continue to work closely to ensure that Tasmania develops as a premium location for hybrid carrot seed production.

Onion Seed Research

Low or variable seed yields are a common problem for hybrid onion seed producers in Australia and elsewhere. Although attempts have been made to improve seed yields in other production regions, the basis of yield variability is still poorly understood and has not been investigated under Australian production conditions. In this project we undertook a survey of factors limiting seed yields in the Murrumbidgee Irrigation Area (MIA) of New South Wales in 2009 and in southern Tasmania in 2009 and 2010. Although the survey seasons and production locations presented different climatic conditions for onion seed production a common theme of inconsistent yields resulting for variable pollination emerged. Across sites, pollination rates and seed yields were correlated with honeybee activity. Although nectar production influenced honeybee foraging patterns on individual sites, the main cause of poor honeybee activity was competition from alternative forage sources. Higher levels of honeybee activity and improved seed yields were recorded at sites isolated from alternative forage sources. Examination of pollen samples collected from honeybees returning to hives located at the survey sites and ground based vegetation surveys confirmed *Eucalyptus largifloerns* and *E. camaldulensis* as major sources of competing forage in the MIA in 2009. Using the same techniques, a range of agricultural crops, native shrubs and weedy species were identified as alternative forage sources in Tasmania. Research outcomes from this work are being extended in a second project initiated in the MIA in 2010. Results from this work will be used to guide future research and management practices aimed at improving hybrid onion seed yields.

CHAPTER 1

Introduction

The Australian carrot and onion seed industries contribute in excess of $10 million annually to the value of Australian horticultural production[1]. Much of this income is derived from production of seed for export markets.

Through investment in applied research and uptake of outcomes into commercial production, Australian vegetable seed growers have overcome seed quality issues that threatened the viability of the Australian carrot seed industry a decade ago. Australia is now recognised as a producer of high quality seed. In addition to enabling the industry to maintain its market share for open pollinated seed production, this has led to opportunities for expansion and renewed interest in Tasmania as a location for European hybrid carrot seed production. Market research by industry stakeholders indicates that, if the industry is able to capitalise on this interest, it could result in 2–5 million dollars of additional carrot seed production annually in Australia.

Similar opportunities confront the onion seed industry. In the last decade, the global onion crop has increased 30%[2], with a proportional increase in demand for seed. Demand is particularly strong for hybrid seed because of the production advantages hybrid varieties offer vegetable growers and the difficulty of producing hybrid onion seed.

One of the main challenges to realising these opportunities and sustaining future growth is having the capacity to produce good seed yields reliably. Yields from hybrid onion and carrot seed crops grown in Australia and elsewhere vary widely within and between seasons. This affects the continuity of seed supply to the market and the economics of seed production for the grower.

Recent Australian research has established that variation in carrot seed yields is largely determined by the success of pollination. This work has identified critical stages during pollination that limit seed yield and opportunities to improve cultural practices and varietal characteristics for better pollination and seed yields.

Although poor seed yields in hybrid onion seed crops are often attributed to a breakdown in pollination, the basis of these claims is not well understood. The first step to achieving more reliable onion seed production in Australia must be to clearly identify the factors that limit seed yield.

This project is part of an ongoing research collaboration between the Australian vegetable seed industry and TIAR to improve the standards of vegetable seed production in Australia. In this project we aimed to address some of the issues of reliability of seed production by:

1 – Applying the existing knowledge of pollination biology in carrot to develop and test management practices to: a) improve seed yields b) identify key traits for use in breeding and selection of carrot seed parent lines with improved seed production characteristics.

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[1] Source: Seed Industry Association of Australia
[2] Economic Research Centre, United States Department of Agriculture

2 – Adapting and extending the outcomes from past carrot seed research in Australia to develop Tasmania as a centre for high quality hybrid carrot seed production through a set of best practice demonstration trials run in collaboration with the key industry stakeholders and their growers.

3 – Investigating the basis of low seed yields from hybrid onion seed crops.

The outcomes from this work have contributed to improved reliability of carrot seed production in Australia; fostered the development of a Tasmanian hybrid carrot seed industry that is gaining international and local recognition for innovative, high quality production; and generated a clearer understanding of the issues that must be addressed to improve hybrid onion seed yields.

SECTION 1 – CARROT SEED PRODUCTION RESEARCH AND EXTENSION

CHAPTER 2

Factors Affecting Attraction of Pollinators to Hybrid Carrot Seed Parent Lines

Introduction

Seed yields from field grown hybrid carrot seed crops are often limited by inadequate pollination (Spurr, 2003; Erickson *et al.,* 1979). Low rates of cross pollination between parent lines result from a range of factors including competition for pollinators from alternative forage sources and discriminatory foraging behaviour within hybrid seed crops (Erickson and Peterson 1978; Erickson *et al.,* 1979; Funari *et al*., 1994; Delaplane and Mayer 2000; Spurr, 2003). Large variations in attractiveness of hybrid carrot seed parent lines to honeybees and other pollinators have been reported (Spurr, 2007).

Pollinators rely on visual and olfactory stimuli to locate and discriminate between flowers and their rewards (Free, 1993; Pernal and Currie, 2000). Flower form, colour and markings, pollen and nectar abundance and nectar composition and flower aroma have all been demonstrated to contribute to attraction of pollinators to flowers (Delaplane and Mayer, 2000). Although flowering traits that influence pollinator behaviour are well known, there is little information on which are most important for attracting pollinators to carrot flowers. Early cytoplasmic male sterile carrot seed parent lines were unattractive to pollinators and as a result yielded poorly (Erickson and Peterson 1978). Although this was attributed to variations in flower morphology, colour and impaired nectar production resulting from the introduction of CMS (Erickson and Peterson 1978; Erickson *et al.,* 1979), limited data was available to clearly identify which were most important. In more recent times, breeding and selection has led to seed parent lines that are more attractive to pollinators, but the basis of this improved attraction remains unclear.

Amongst traits associated with attraction of pollinators in entemophilous systems, nectar traits (quantity and composition) are often most important (Free, 1993). Honeybees are the dominant and most effective pollinators of hybrid carrot seed crops (Spurr, 2003). Links between nectar abundance and honeybee visitation and foraging efficiency have been documented in many species (Zimmerman, 1988; Free, 1993 and Delaplane and Mayer, 2000). Some studies have shown that pollination by honeybees is more efficient in flower patches that are rich in nectar (Delaplane and Mayer, 2000).

Besides abundance, the most important factor influencing the attractiveness of nectar is its composition (Delaplane and Mayer, 2000). Sugars, glucose, fructose and sucrose, typically account for 10 to 70% of the weight of nectar. Other nectar components include proteins and aroma compounds. Honeybees display a preference for nectars with sugar concentrations of between 30-50% (Waller, 1972 cited in Silva and Dean, 2000) and especially those with

relatively high sucrose content (Silva and Dean, 2000; Delaplane and Mayer, 2000). Wykes (1952b cited in Delaplane and Mayer, 2000) noted that a mixture of equal parts of glucose, sucrose and fructose was more attractive to honeybees than a solution of any single sugar, or a mixture of these sugars in different proportions. Nectars of different plants species are known to vary significantly in both sugar content and ratio of different sugars. These variables are also affected by environmental conditions, genetic variation within species, and pollinator foraging (Kearns and Inouye, 1993; Delaplane and Mayer, 2000).

To date, the main barrier to nectar studies in carrot has been the difficulty associated with collecting very low volumes of nectar from small flowers (gynoecium ~ 1.5mm in diameter). In other species, low volumes of nectar have been successfully collected from small flowers using micro-capillaries, micropipettes, and wicking techniques (Kearns and Inouye, 1993). None of these techniques are effective for collecting carrot nectar except in isolated instances of extremely abundant nectar production (Erickson and Peterson, 1978). Recently, a centrifuge based technique for nectar collection from red clover florets has been modified for use with carrot flowers (Geard and Spurr, 2009).

In this work we examined genetic variation in attractiveness of carrot breeding lines to pollinators and undertook an investigation of the impact of a range of several floral traits on attraction of pollinators to carrot flowers.

Materials and Methods

Trial Site

Plants for screening trials were grown in trial plots at Rijk Zwaan Australia in Musk, Victoria (37°22’03”S, 144°12’08”E, elevation 673m) (Plate 2.1). Long term temperature and rainfall data for the nearest operating Bureau of Meteorology weather station at Macedon approximately 30 km east of the trial site are given in Figure 2.1. Field trials were run in two consecutive seasons, 2009/10 and 2010/11 (hereafter referred to as 2009 and 2010 respectively). Temperature data were logged during flowering at the trial site each season using a portable data logger (Tiny-Tag Ultra 2, Gemini Data Loggers, UK) mounted at canopy height in a Stephenson screen (Gemini Data Loggers, UK). In 2010 the trial was managed to flower approximately 3weeks later than normal to ensure warmer conditions. When the trial flowered from mid January to mid February, temperatures were still milder than those experienced in 2009 (Figure 2.2).

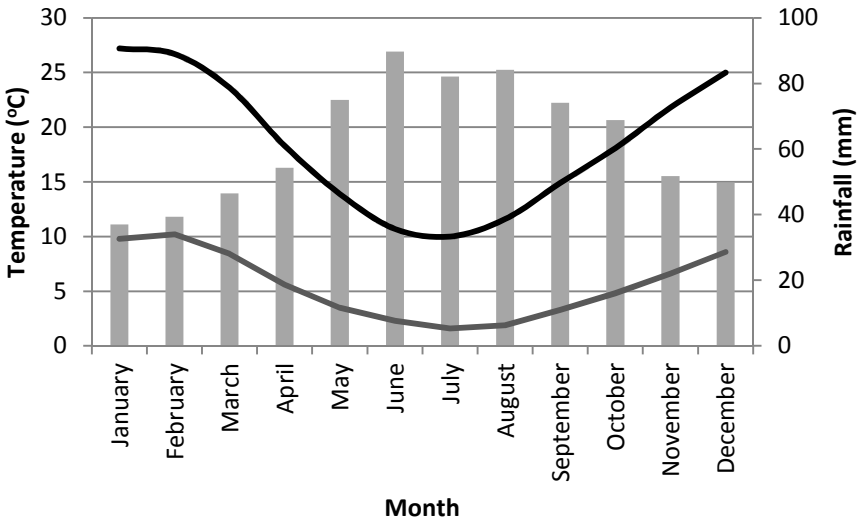


Figure 2.1 – Long term monthly maximum and minimum temperatures (lines) and rainfall data for Macedon, Victoria, approximately 30km east of the trial site. Data are based on long term (1873 to 2011) records from the Macedon Forestry weather station (37.42 °S, 144.56 °E, elevation 505m) Source: Bureau of Meteorology.

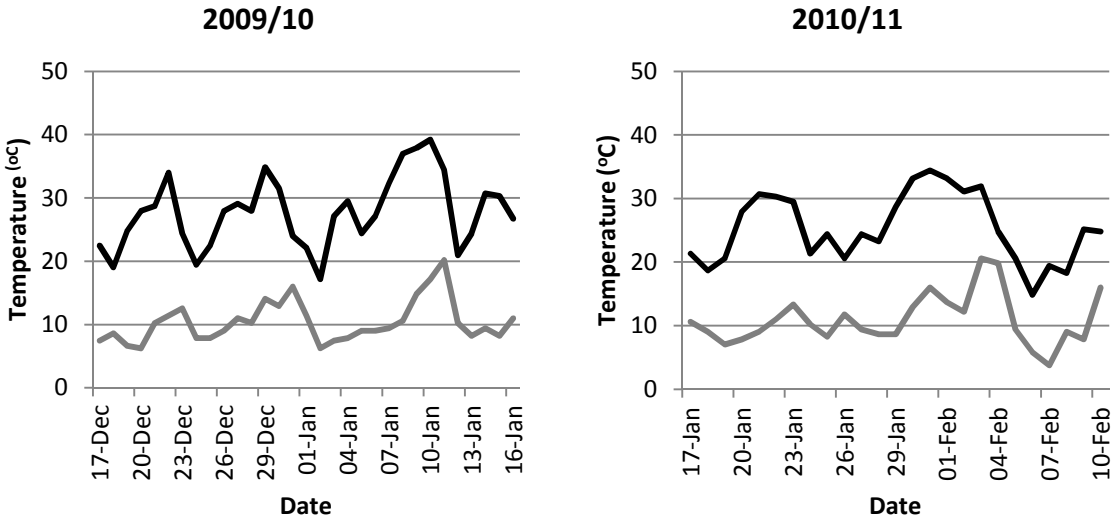


Figure 2.2 - Daily average minimum and maximum temperatures at canopy level during peak bloom in the 2009 and 2010 seasons.

Plant Material

24 hybrid carrot seed parent lines were provided by the Rijk Zwaan breeding department. The lines included 6 pollinator and 18 male sterile lines comprising 6 brown anther, 6 white petaloid and 6 green petaloid lines. All lines were planted in both seasons with the exception of a green petaloid line (PG5) from 2009 which was replaced with an alternative green

petaloid line in 2010 (PG7). Selected lines ranged from those with very good pollination and yield characteristics to lines that were thought to be comparatively unattractive to pollinators.

Trial Design

Each line was grown in 5m long plots in a randomised complete block design with 4 replicates. Each plot consisted of two rows (1 bed) of vernalised stecklings transplanted 3 weeks apart. This was done to ensure a time window for screening when all lines were in peak bloom. A 1:3 (bed) arrangement of pollinator and male sterile lines was maintained throughout the trial to reflect commercial planting ratios.

Cultural Practises

Cultural practices used in this trial followed current commercial production methods for root to seed carrot seed production. Stecklings were grown in a nearby trial plot, lifted in mid-July, graded and selected roots cool stored for subsequent transplanting. To ensure a spread of flowering time lines were transplanted on two dates; September 13 and September 27, 2010. The trial was irrigated using drip tape. During summer the trial was watered twice weekly on average. At early flower, honeybees were introduced to the trial site at a rate equivalent to 16 hives/Ha.

Plate 2.1 - A section of the 2009 carrot trial at Rijk Zwaan Australia.

Trial Assessments

Pollinator Activity

Each plot in the trial was scored for pollinator activity on at least 6 days between 11am and 2pm. This time period was determined in previous studies to best indicate the diversity and level of activity of key pollinators visiting carrot seed crops (Spurr, 2003).

Insect activity scores were based on spot counts of the number of pollinators visiting the plot in 1 minute. Pollinators were classified into Order/Family groupings. Key groupings were: Hymenoptera (honeybees (*Apis mellifera*), native bees and wasps); Diptera (flies and hoverflies) and Coleoptera.

Floral Traits

A number of flowering traits were examined across all lines in the trial plot for correlation with differences in pollinator activity between lines. These were:

1. Flower morphotype. These were grouped as pollinator, brown anther male sterile and petaloid male sterile.

2. Flower colour. Petaloid lines were grouped into white and green groups according to corolla colour.

3. Bloom density. Digital images of each plot were taken from directly above a 0.5m x 0.5m quadrat positioned at canopy height (Plate 2.2). Using image processing software (Fovea Pro, Reinder Graphics and Adobe Photoshop, Adobe) the area of bloom was determined as a percentage of quadrat area. Reliable estimates were only possible for brown anther and pollinator lines. In most plots of petaloid lines there was insufficient contrast between receptive umbels, non-receptive umbels and background colours for reliable estimates to be made.

4. Umbel size. Umbel diameters were recorded for 5 representative secondary umbels in bloom. Secondary umbels were selected because their flowering period coincided with peak bloom in each line and because of their major contribution to seed yields. In 2009, umbel diameter measurements were made on each assessment day. In 2010, umbel diameter measurements were made on 3 days during peak bloom.

Plate 2.2 – A typical image used to determine density of bloom at a given time during flowering. A 500mm x 500mm quadrat (shown) was used to scale each image.

Nectar Volume

Nectar volume measurements were based on the standing crop of nectar harvested from representative, flowering umbels collected immediately after insect spot counts had been made. Ten umbellets with greater than 50% receptive flowers were collected from the second whorl of five secondary umbels in each plot. Umbellets were removed with a set of fine forceps and placed in a 15mm centrifuge tube (Mo Bio Laboratories, California). Sampled umbels were retained so that the average number of umbellets/umbel could be determined for each line. At the completion of each plot, tubes were sealed and placed in ice. At the completion of sampling of each replicate block, samples were returned to the laboratory for processing. In the laboratory, the umbellets from each sample were stacked face down in spin filter tubes (Mo Bio Laboratories, California) and centrifuged at 12000G for 10 minutes to extract nectar. Extracted nectar was collected from the centrifuge tubes in a capillary tube for determination of volume. Standing crop volumes were calculated on a per umbel basis. After measurement, the nectar samples were frozen and stored for future analysis of sugar composition.

Data Analysis

Data analysis was undertaken using the regression and general linear model functions within PASW Statistics (SPSS Inc, Chicago). Fischer's LSD values were calculated for treatment means in data sets analysed with ANOVA.

Results and Discussion

Seed Yields

Significant variation in seed yields from individual male sterile lines were observed in both seasons, ranging from 128 to 770g/plot in 2009 and 53 to 310g/plot in 2010 (Figure 2.3). On a per plant basis, the observed yields varied widely between 1.3 and 19g/plant. Individual lines rankings for seed yield were similar for most lines in both seasons.

Pollinator Diversity

In many families of plants, there are highly specialised relationships between floral morphology and pollination by one or, at most, a few insect species. The carrot, in contrast, has unspecialised flowers that are well adapted to a promiscuous lifestyle. The small, flat form of the flowers and their dense aggregation into umbels means that pollen, nectar and the stigma are readily accessible to most insects. As in other hybrid seed crops though, cross pollination relies on pollinator insects moving between umbels of the two lines; the most efficient pollinators are thorough foragers and move regularly. Insect pollinators that are effective in open pollinated crops may therefore be of relatively small benefit in hybrid seed crops.

The composition of insect visitors to each line in the current trial is shown in Table 2.1. As in previous surveys of carrot (Bohart and Nye, 1960; Abrol, 1997 Spurr, 2003; Spurr, 2007), members of the Hymenoptera, Diptera and Coleoptera were represented. In 2010 a very large population of flies (predominantly *Calliphora* and *Musca*) were present in the trial and Dipterans consequently accounted for 93.6% of observed insects. Despite this apparent bias, other insects such as honeybees (*Apis* mellifera) were present at normal levels within the trial.

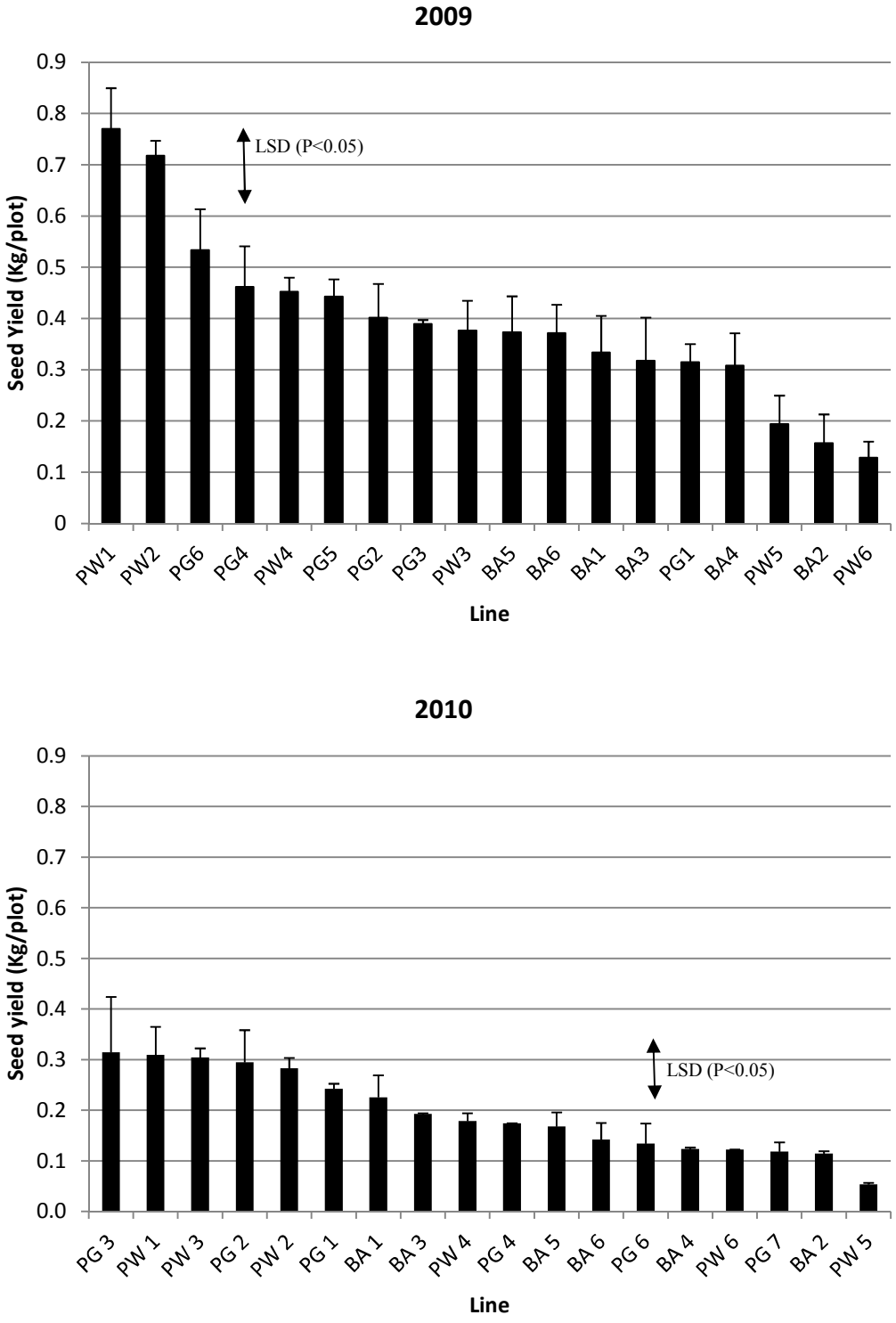


Figure 2.3 – Seed yields from male sterile carrot lines in 2009 and 2010. Least significant difference (P<0.05) for line means in each season are indicated by the vertical bar above the columns. Error bars indicate standard errors (n=4).

Table 2.1 – The composition of insect populations observed within the trials during peak bloom in 2009 and 2010.

Line	Season	Order							
		Hymenoptera				Diptera			Coleoptera
		Honeybees	Native bees	Wasps	Total Hymenoptera	Hoverflies	Flies	Total Diptera	
Brown anther	2009	32.8	6.0	0.0	38.8	23.9	35.4	35.4	1.9
	2010	1.1	0.5	0.1	1.7	0.2	97.5	97.7	0.6
Green Petaloid	2009	44.7	6.4	0.0	51.1	17.6	30.3	47.9	1.0
	2010	2.5	0.4	0.2	3.1	0.6	95.5	96.1	0.9
White Petaloid	2009	38.9	2.6	0.5	42.0	22.1	34.9	57.0	1.0
	2010	2.9	0.5	0.2	3.6	0.5	94.8	95.3	1.2
Pollinator	2009	48.9	3.4	1.0	63.3	29.8	15.8	45.6	1.0
	2010	10.2	1.3	0.0	11.5	0.1	85.3	85.4	3.1
All lines	2009	41.3	4.6	0.4	48.8	23.3	29.1	46.5	1.2
	2010	4.2	0.7	0.1	5.0	0.3	93.3	93.6	1.4

Significant correlations between insect counts and seed yields were only apparent for honeybees (Figure 2.4). Across all male sterile lines, seed yields were significantly correlated with honeybee spot counts within (2009 $P<0.05$,$r^2 =0.31$; 2010 $P<0.05$,$r^2 =0.25$) and across season ($P<0.001$; $r^2=0.48$). Reductions in seed yield in 2010 compared with 2009 were porportional to the reduced honeybee foraging activity observed in the trial in 2010. These observations are consistent with the view that honeybees are the most effective of major pollinators of hybrid carrot seed crops. Although flies were present in very large numbers in 2010 they did not appear to have a significant impact on pollination. This analysis reflects the observed behaviour of flies in the trial, which included long periods of time spent on individual umbels (unless disturbed) with infrequent movement between umbels, and previous observations (Spurr, 2003) that several of the dominant species of flies found in hybrid carrot seed crops carry relatively little pollen on their bodies.

Indivudual line rankings for honeybee visitation rates (spot counts) are shown in Figure 2.5. Seed parent lines varied widely in their attractiveness to honeybees, with mean spot counts for individual lines ranging from 9.5 to 0.5 honeybees per plot in 2009 and 14.7 to 0.7 honeybees per plot in 2010. Most individual line rankings for honeybee spot counts were similar in both seasons (Figure 2.5).

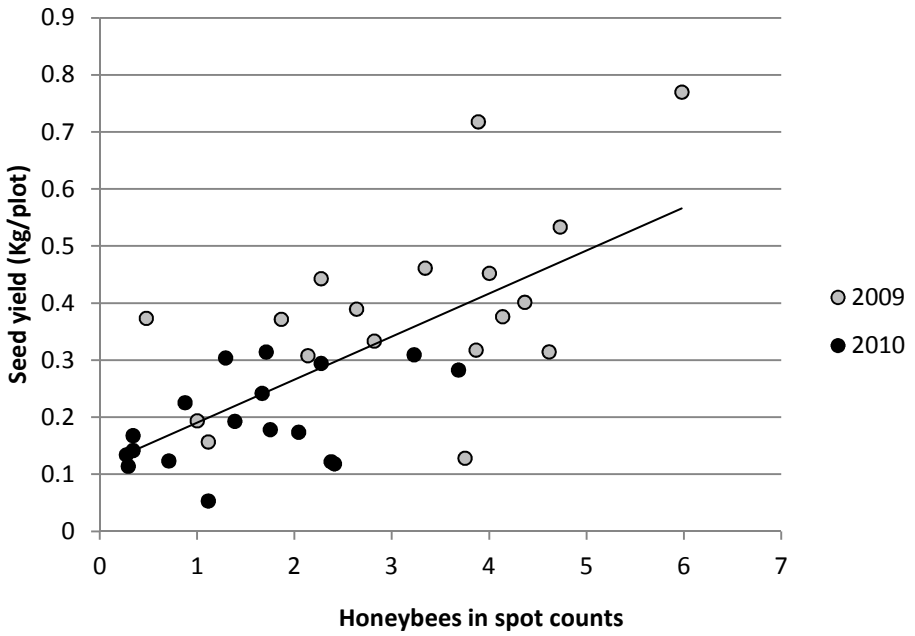


Figure 2.4 – Relationships between honey bee spot counts and seed yields in 2009 and 2010. In both seasons, significant linear relationships were observed (2009; $P<0.05$;$r^2=0.31$; 2010 $P<0.05$ $r^2= 0.25$). The relationship shown is for both seasons data ($P<.001$; $R^2 =0.48$).

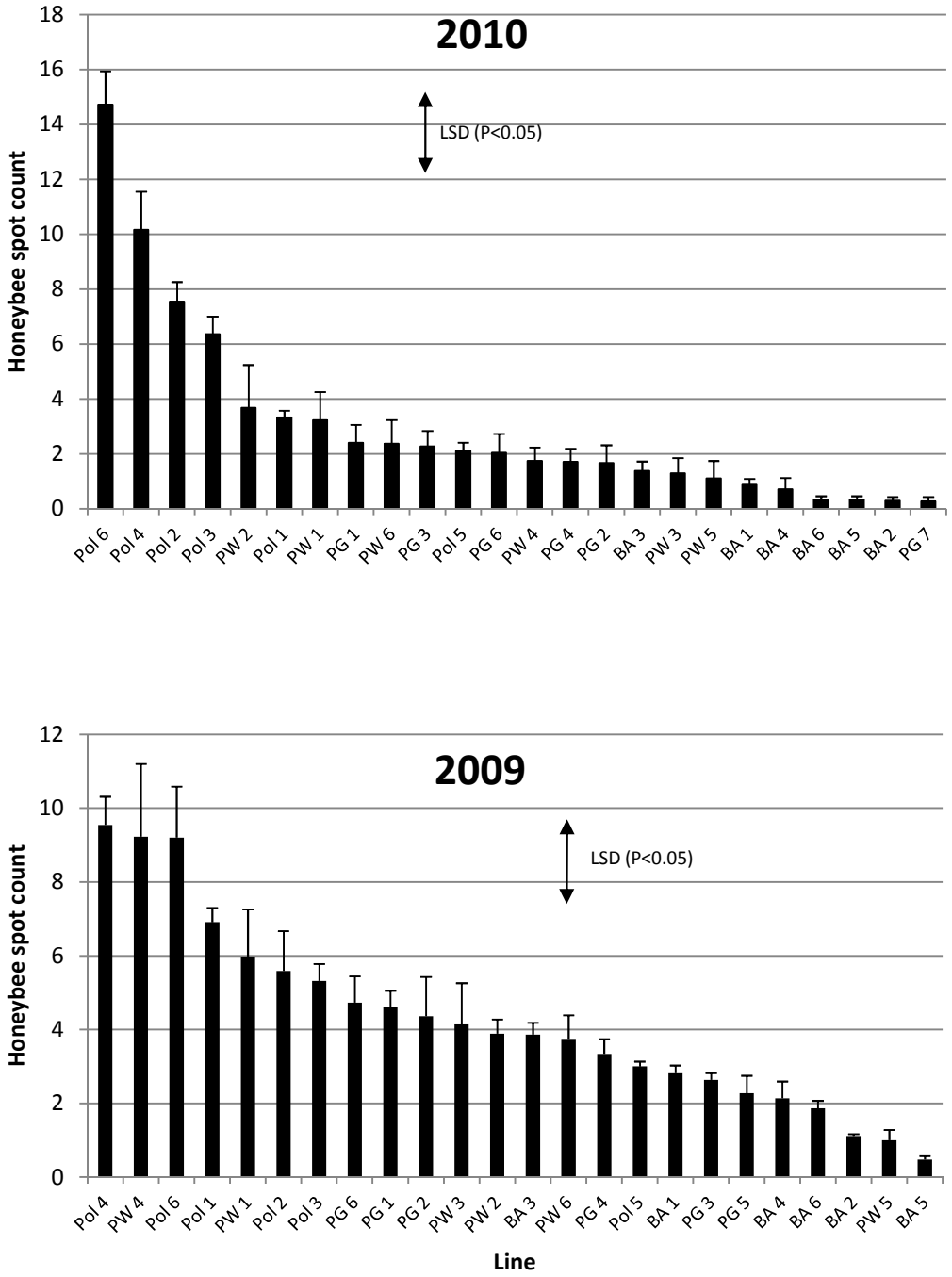


Figure 2.5 – Individual line rankings for attractiveness to honeybees based on spot counts in 2010 (top) and 2009 (bottom). Data points are the means of at least 6 days observation of 4 replicate plots of each line. Error bars indicate standard errors (n=4). Vertical lines above the graphs indicate least significant differences for line means (P<0.05).

Given the importance of honeybees to pollination of hybrid carrots observed in this and previous trials (Spurr, 2003) and in commercial production, the following discussion of factors affecting attractiveness of lines is related specifically to honeybees.

Effects of Flower Morphotype and Colour on Honeybee Visitation

Honeybee foraging preferences were influenced by flower morphotype. Pollinator lines were on average most attractive to honeybees. This result is at least partly because they offer both pollen and nectar rewards. Although the majority of honeybees observed in the trial were nectar feeders, exclusive pollen foraging behaviour was observed. In previous studies of early CMS lines, brown anther morphotypes were reported to be more attractive to pollinators (Galuszka *et al.,* 1989; Erickson and Peterson, 1979). In this study, and an earlier pilot study (Geard *et al.,* 2010) using modern CMS lines, petaloid lines were on average significantly more attractive to pollinators than brown anther lines (Figure 2.6). Within the petaloid lines studied, no effects of corolla colour on foraging preferences were observed. Although honeybee visitation rates to pollinator lines were comparable in both seasons (Figure 2.6), visitation rates to CMS morphotypes in 2010 were less than 50% of 2009 levels. This suggests that factors that determined honeybee visitation rates are less affected by environmental or cultural variation in pollinator lines than male sterile lines.

Umbel Size and Bloom Area

Optimal foraging theory predicts that honeybees will forage efficiently, minimising the amount of energy that is expended (Dafni, 1992). In some cases, larger inflorescences or inflorescences with a larger number of open flowers have greater nectar rewards and honeybees preferentially land on these (Zimmerman, 1988; Delaplane and Mayer, 2000). Average umbel diameters of individual lines at peak bloom ranged from 74 to 104mm. Over this range, only a weak relationship ($P<0.07$) between umbel size and honeybee spot counts was observed. Estimates of bloom area were made for brown anther and petaloid morphotypes. Whilst both morphotype groups had on average similar areas of bloom at peak bloom (42 to 44% of plot area), individual lines varied from 10 to 68%. Across lines, a significant, positive linear relationship between bloom area and honeybee spot counts was observed (Figure 2.7). In both morphotypes relative increase in honeybees in spot counts were directly proportional to the increase in bloom area; that is, increases in honeybee numbers reflected the increase in the resource area rather than a greater attraction to individual flowers.

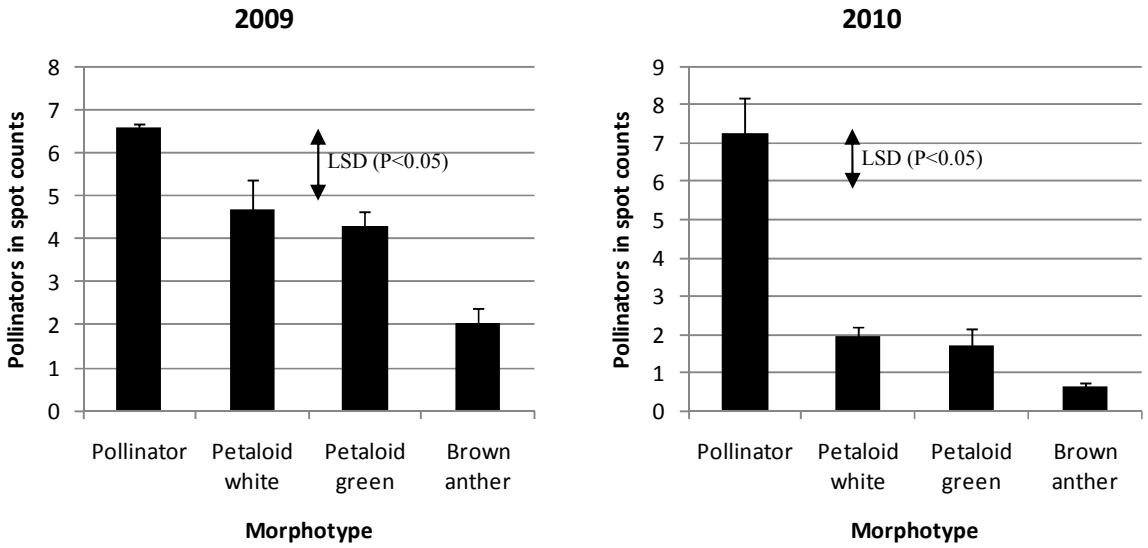


Figure 2.6 – Mean honeybee spot counts recorded for different flower morphotypes within the 2009 and 2010 trials. Data points are the means of observations for 4 replicates of 6 lines of each morphotype taken on at least 6 days during peak bloom. Error bars indicate standard errors (n=4). LSD values (P<0.05) are indicated by the vertical bars above the graphs.

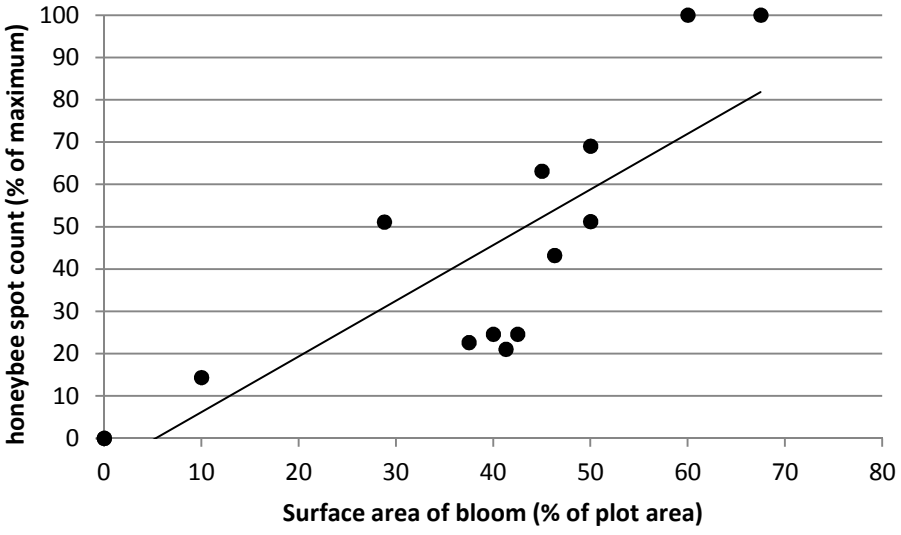


Figure 2.7 – The relationship between surface area of bloom and relative honeybee spot counts (% of maximum recorded) in plots of brown anther and pollinator lines. The relationship is significant (P<0.01; r^2 =0.69).

Nectar Abundance

Investigations of the effects of nectar abundance on insect visitation were based on standing crop volumes of exposed inflorescences, sampled at the time that the insect surveys were undertaken. Although this approach carried a risk of underestimating the capacity of the lines to produce nectar because of its removal by pollinators, it was chosen for 2 reasons: 1) the standing crop that was collected represented the nectar available to insects at the time of the pollinator survey and; 2) netting or bagging causes changes in microclimate around the inflorescence, which may affect nectar production and composition (Kearns and Inouye, 1993).

Large variations between lines in nectar standing crops were observed in both seasons, ranging from 24 to 109µL in 2009 and 1.2 to 57µL in 2010 (Figure 2.9). Although there was variation in individual line rankings, lines were generally either in the upper 50% or the lower 50% for standing crop volumes in both seasons.

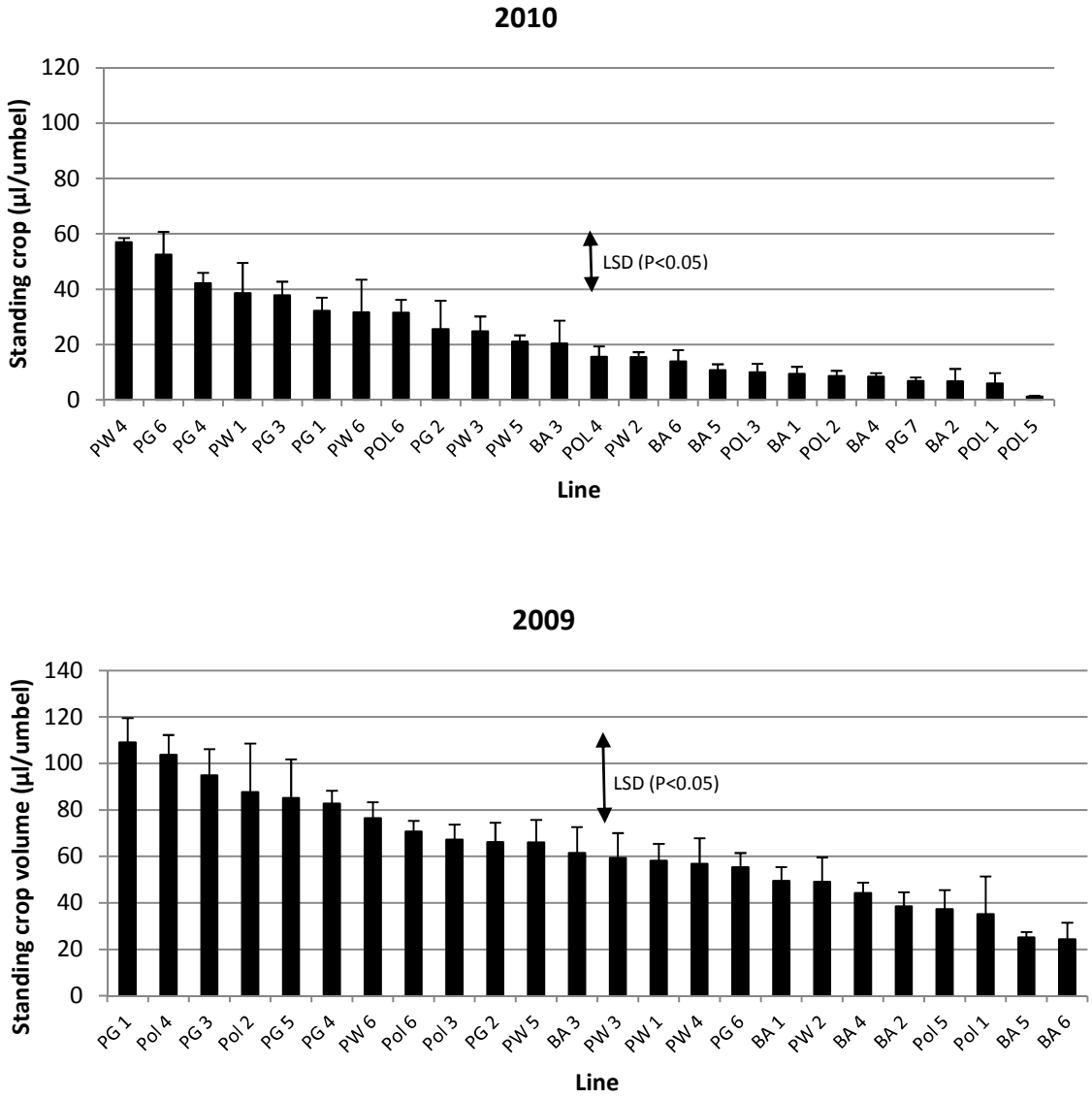


Figure 2.8 – Individual line rankings for nectar standing crop volume in 2010 (top) and 2009 (bottom). Individual data points are the means of 4 replicate samples collected on at least 6 days during peak bloom. Error bars indicate standard errors (n=4). Some line means are significantly different. LSD values (P<0.05) are indicated by the vertical bars above the graphs.

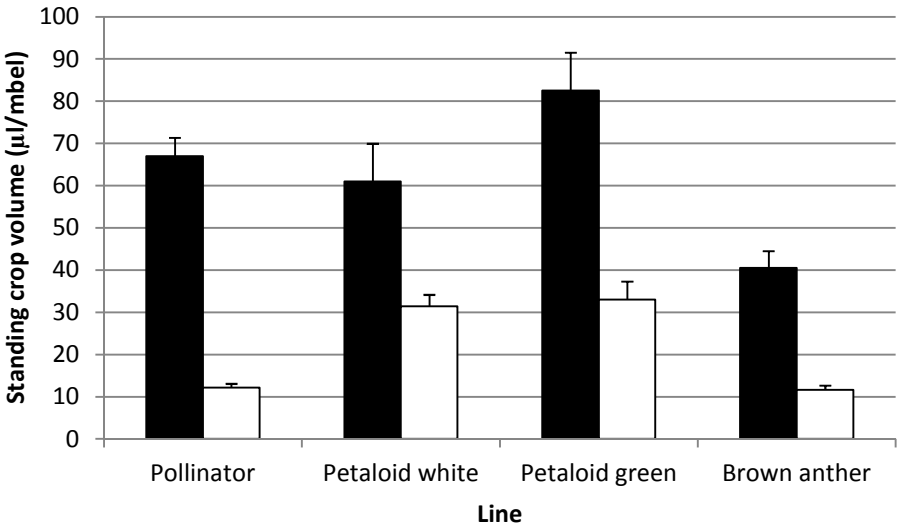


Figure 2.9 – Mean standing crop volumes recorded for different flower morphotypes within the 2009 (■) and 2010 (□) trials. Data points are the means of observations for 4 replicates of 6 lines of each morphotype taken on at least 6 days during peak bloom. Error bars indicate standard errors (n=4).

Significant, positive correlations between nectar standing crop volumes and honeybee visitation rates were observed in both seasons (Figure 2.10). Much closer correlations were observed in 2010 than in 2009. This may be because nectar production was on average lower in 2010 than 2009 (Figure 2.9), causing honeybees to more fully exploit the nectar resources within the trial. It seems likely that some morphotype differences in attractiveness to honeybees are attributable to differences in availability of nectar. In both seasons, significantly smaller average standing crop volumes were observed in brown anther lines compared with lines of other flower morphotypes (Figure 2.9). Impaired nectar production may explain why, on average, brown anther lines were less attractive to honeybees than other morphotypes in both seasons. Petaloid lines had on average standing crop volumes that were comparable to (2009) or significantly larger (2010) than pollinator lines, but were visited less frequently by honeybees. This is partly explained by pollen foraging, but may also be due to additional structures within petaloid flowers concealing nectar or increasing the difficulty of its extraction by pollinators. Samples and digital images collected in this study will be used in a future work to identify potential relationships between flower architecture and pollinator foraging preferences.

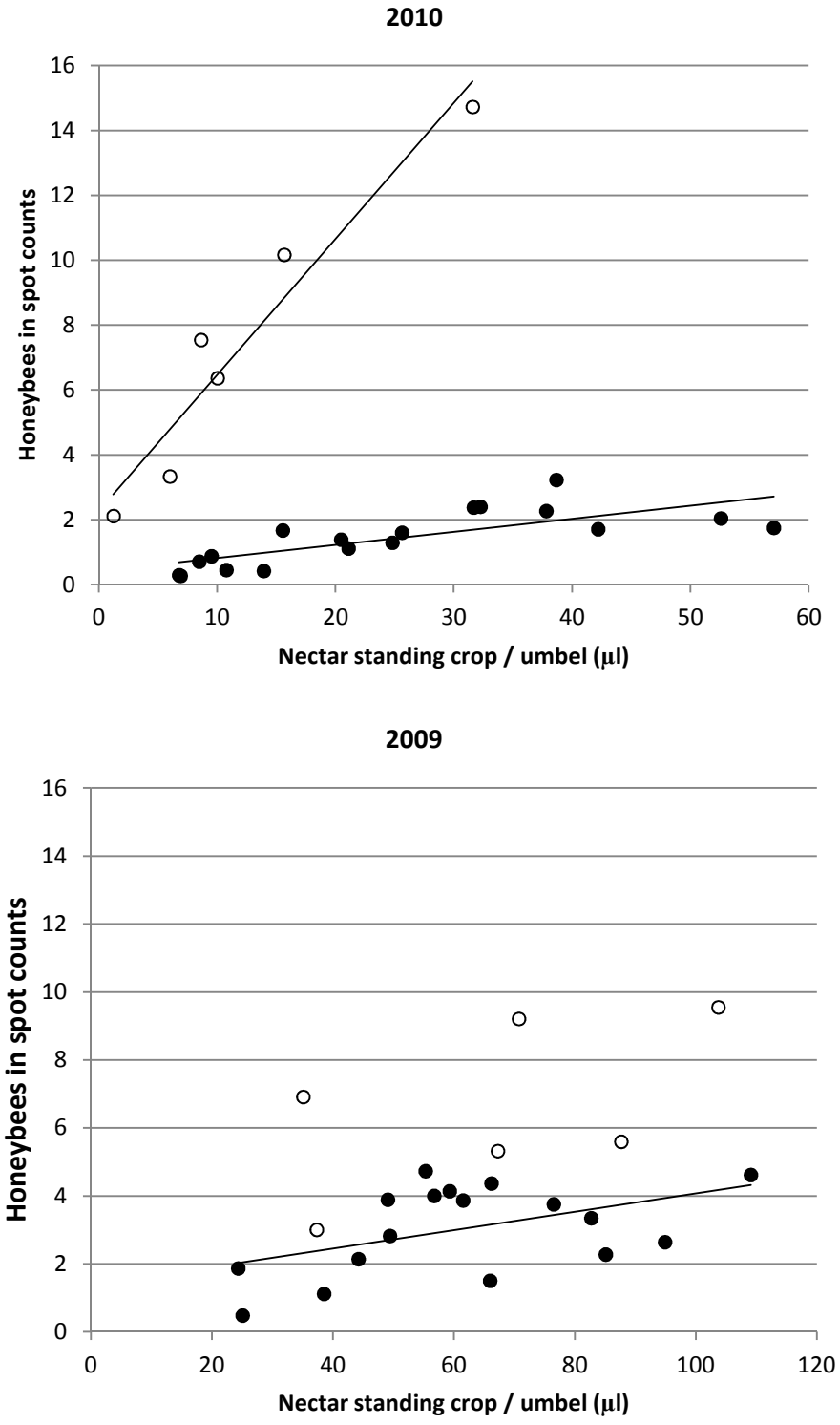


Figure 2.10 – Relationships between nectar standing crop volume and honeybee spot counts in pollinator (○) and male sterile (●) hybrid carrot seed parent lines in 2010 (top) and 2009 (bottom). The relationships are significant for 2010 male sterile lines ($P<0.001$; $r^2=0.56$), 2010 pollinator lines ($P<0.001$; $r^2=0.93$) and 2009 male sterile lines ($P<0.05$; $r^2=0.23$) but not for 2009 pollinator lines.

Conclusion

Improved understanding of the role of nectar and other floral traits in attracting pollinators to carrot inflorescences is important both for breeding efforts and to refine crop management for improved pollination. Other than the work of Erickson *et al.* (1979; 1982) and Erickson and Peterson (1978; 1979) there is little published information that deals specifically with carrot.

Of the insects that are observed to visit hybrid carrot seed parent lines, honeybees appear to be the most important for pollination. In this study, we investigated several traits that were considered likely to influence honeybee foraging behaviour in carrots. Of the variables studied, flower morphotype, bloom density and standing crop volume of nectar were associated with differences in honeybee activity between lines.

The linear relationships between honeybee activity and bloom density or honeybee activity and nectar standing crop volume observed in this work suggest that differences in visitation rates between lines were largely explainable in terms of efficient utilisation of the forage resource within the carrot trial, particularly in 2010/11. Amongst CMS lines, morphotype differences in honeybee visitation rates may also be explained in part by differences in nectar availability. Samples and images collected in this work are being used in a further study of the effects of flower morphotype on nectar production, display and accessibility.

The weaker correlations between nectar standing crop volumes and honeybee activity observed in 2009/10 may reflect an increasing importance of other factors on honeybee foraging choices when nectar is abundant. Future studies will use nectar samples collected in this work to examine the relationships between nectar composition and foraging behaviour.

Although seed production traits are necessarily a secondary consideration in the breeding objective for vegetable crops they are important for deployment of germplasm. The findings of this study highlight potentially important differences in attractiveness of different CMS systems and have identified traits that influence pollinator visitation to individual lines. In addition to their use in breeding, knowledge of the importance of traits such as nectar production could potentially be utilised in commercial hybrid seed production systems to maximise pollinator activity and cross pollination.

CHAPTER 3

Management of Flowering Time in Carrot

Introduction

Research conducted in Tasmania and New Zealand over 3 years showed that, in well grown carrot seed crops, yields are typically 30 to 70% of the crop's potential. This was a result of inadequate pollination (pollen transfer) and poor pollen viability (Spurr, 2003). In a number of historically low yielding lines, the duration of stigmatic receptivity is shorter than in high yielding lines. Where such lines cannot be replaced in commercial production with alternative, higher yielding seed parents, intensive pollination over a shorter time period is required to improve seed set (Brown, 2008).

Climatic conditions at flowering are one of the key determinants of successful cross-pollination of hybrid carrot seed crops for several reasons outlined below:

Pollinator Activity

Pollination in carrots is by insects. In Australia the major pollinators are honeybees, native bees, nectar feeding beetles and various flies. Of these, honeybees are most important (Spurr, 2003). Honeybees forage optimally at temperatures above 19^{o}C (Delaplane and Mayer, 2000). In carrot, honeybee activity is positively correlated to nectar production (see Chapter 2) and both are greatest when temperatures exceed 25^{o}C (Erickson and Peterson, 1979; Spurr and Geard, unpublished).

Pollen Viability

Poor pollen viability is a consistent factor in low yields from carrot seed crops, especially in hybrid seed crops (Spurr, 2003; Brown, 2008).In general, pollen viability before anthesis is high but declines progressively so that at anthesis typically less than 50% of grains are viable (Spurr, 2003; Geard, 2011). Carrot pollen is trinucleate and, typical of this grouping, has a relatively short half life of a few hours (Spurr, 2003) Pollen deterioration before and after anthesis is faster in high humidity conditions and this effect is increased under warm temperatures (>30^{o}C). Under low humidity conditions (<40%RH), pollen longevity is unaffected by temperatures up to 30^{o}C (Spurr, 2003; Geard, 2011). Under field conditions, pollen viability is optimal during periods of warm, low humidity weather.

Managing Hybrid Carrot Seed Yields

In Tasmania and other temperate areas of south eastern Australia, peak bloom of many temperate varieties occurs in a 3 week period from the 3rd week of December. Weather

conditions during this period are often unstable and temperatures relatively low for reliable pollination.

In carrot seed producing regions of Tasmania, maximum daily temperatures in the following 3 weeks are typically 2 to 4 degrees higher, regularly exceeding 25°C (Figure 3.1). Similar trends are evident at Mt Gambier, where the average daily maximum temperature increases by 1.6°C in a 3 week period from 24.1°C compared with 25.7°C (Figure 3.1). At all four sites, the observed temperature increases are also associated with reductions in relative humidity. This suggests that mid to late January is potentially a better time for pollination of carrots than late December to mid January in Tasmania and Mt Gambier.

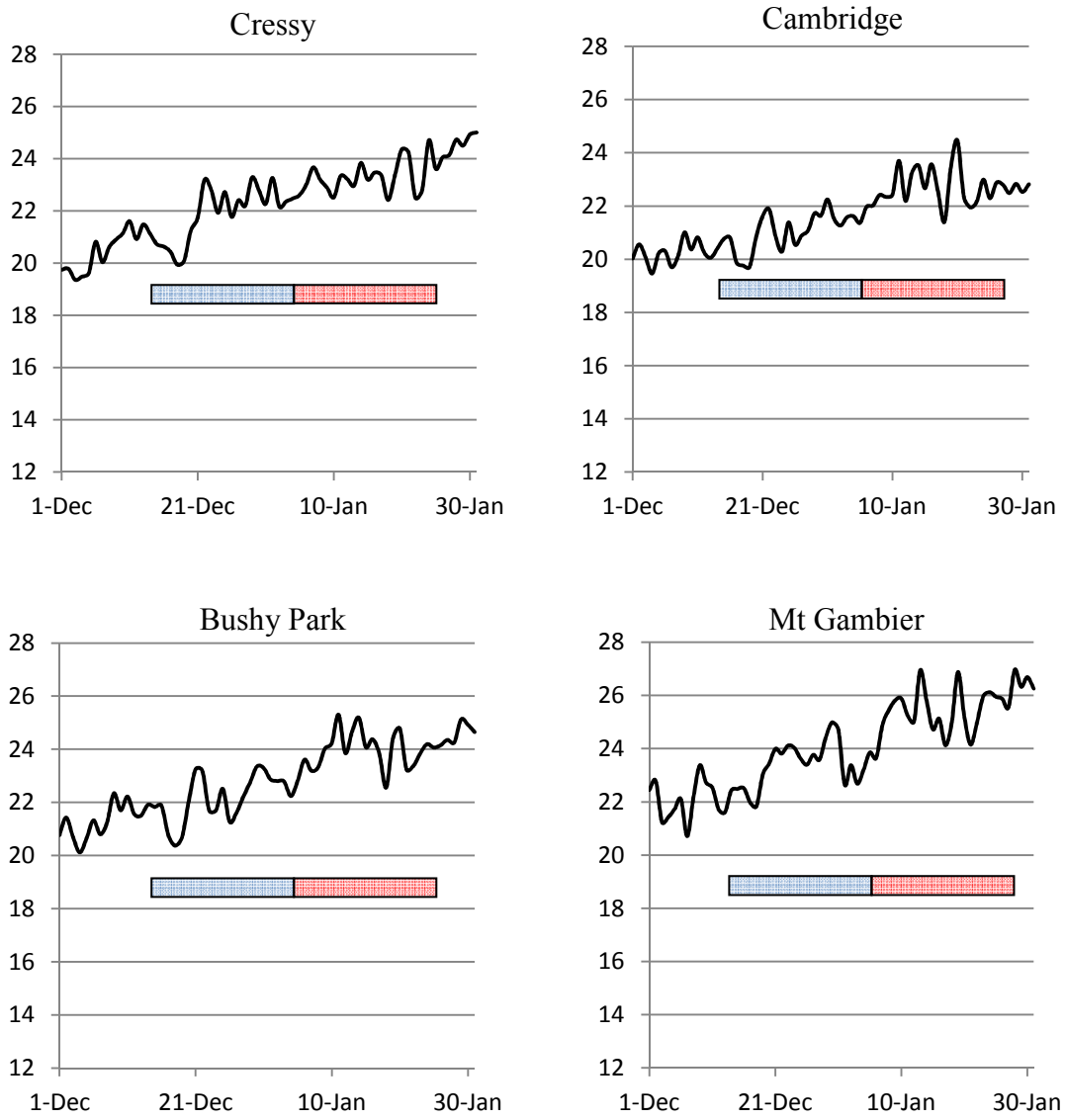


Figure 3.1 – The average daily maximum temperatures (°C) in December and January at Cressy, Cambridge and Bushy Park (Tasmania) and Mt Gambier (South Australia). Data is based on historical averages from the nearest operating Bureau of Meteorology weather stations. Coloured bars indicate the typical 3 week peak flowering period of temperate carrot varieties (▭) and a 3 week period starting 3 weeks later (▭).

Another compelling reason for later flowering of carrot seed crops in Tasmania is that this would prevent peak bloom coinciding with flowering of Prickly box (*Bursaria spinosa*), a widespread native shrub that flowers in late December and competes strongly with carrots for pollinators (Gaffney, unpublished data). Whilst the benefit of delaying flowering has not been conclusively proven, on several occasions we have observed very good seed set in late flowering crops and, within individual crops, improved seed setting on later flowering umbels.

Aside from the direct effects of climate on pollination, successful pollination of hybrid seed crops requires the parent lines to flower synchronously. Often hybrid seed production involves parent lines that differ in flowering phenology, resulting in asynchronous flowering and poor seed yields. There is a need therefore for strategies to manipulate flowering time to improve both the synchrony of flowering in hybrid seed crops and to potentially exploit improved pollination conditions associated with later flowering times.

Temperate carrot varieties have a biennial life cycle. Their developmental sequence is; 1) vegetative phase, 2) vernalisation phase, and 3) photoperiodic phase. After the plant has developed beyond a period of juvenility, flowering is induced by exposure to a certain period of low temperature conditions. Following induction, flower stalk elongation (bolting), flowering and seed production are promoted by long day conditions (Atherton and Basher, 1984). In commercial seed production, the life cycle is reduced to a 13 month period. Crops are sown in summer and complete juvenility before the following winter. Flowers are initiated in winter and flowering and seed maturation occurs in the following summer. Potential for manipulation of flowering time by sowing time is restricted because of a) the need to complete juvenility before winter and; b) photoperiodic regulation of timing of bolting and flowering in initiated plants. In the absence of alternatives, regulation of flowering time is most commonly by way of trimming to remove early flower buds from the developing inflorescences. Such trimming treatments are based on the assumption that trimmed plants compensate for loss of early yield potential by producing more highly branched inflorescences with increased numbers of later flowering, higher order umbels. Although trimming treatments are widely used in commercial production, their effects on flowering and yield potential remain unclear and there is uncertainty about when (if at all) is the best time to trim crops.

Endogenous gibberellins play a key role in regulation of flower induction and progress to flowering in biennial species including carrot (Michaels and Amasino, 2000; Hiller *et al.,* 1979; Samuoliene *et al.,* 2005). For carrot, several authors have reported an inductive effect of exogenous GA_3 on flowering (Dickson and Peterson, 1960; Luchessi, 1983; Bandara and Tanino, 1995), while others have found that GA_3 increased rate of development to flowering or promoted stem elongation (bolting) (Nieuwhof, 1984; Tagliocozzo *et al*., 1992; Galamarini *et al*., 1995; Ghoname *et al.,* 2004). In *Brassica oleraceae*, (also a biennial species), application of paclobutrazol after curd initiation delayed flowering by 1 to 2 weeks (Gracie, 2011). The possibility of using a similar treatment to delay flowering in carrot has not been investigated.

In this work, we examined the effects of trimming and paclobutrazol on flowering, pollination and seed yields in carrot to answer the following questions:

- Can trimming or paclobutrazol application be used to delay flowering?
- What is the impact on yield potential?

- Can delaying flowering improve pollination, and if so is this realised in increased yields

The ultimate aim of the work was to develop practical recommendations for management of flowering time in hybrid carrot seed crops.

Materials and Methods

Trial Sites

Trials were conducted in two consecutive seasons 2009/10 and 2010/11. The 2009/10 trial was located in a commercial open pollinated carrot seed crop at Bushy Park, southern Tasmania (42°41' South, 146°, 57' East). In the second season, treatments were tested on 7 commercially important hybrid parent lines in a purpose designed trial near Richmond, Tasmania (42.83˚S 147.50˚E, 4m). Both trials were grown using current commercial practice for seed to seed carrot crops. Plant densities in both trials were approximately 20 plants per m^2. At flowering, honeybees were introduced at a rate of 10 hives / ha.

Climate data were recorded at each site using portable data loggers (Gemini Tiny Tag) mounted in Stevenson screens. Average daily temperatures and relative humidities for the period leading up to and during flowering at the site of the trial are summarised in Figure 3.2.

Plant Material and Trial Design

The first season's trial was undertaken in a commercial seed crop. Trimming treatments were imposed at 2 times (early and late) and at 3 intensities (light, medium and heavy) (for examples, see Plate 3.1). Trimming times were based on plant development stage as follows: early - trimmed when the main flowering stem had extended to 200mm above ground level on average; late – trimmed when the main flowering stem had extended 400mm above ground level on average. Trimming intensities were light – trimmed to 250mm above ground level; medium - trimmed to 150mm above ground level and; heavy – trimmed to 75mm above ground level. In one treatment a medium trim was applied to the same plants at both the early and late trimming times. Treatments were arranged in a randomised complete block design. Bolting and flowering of the commercial crop were very early (peak bloom in early December). As a consequence, late trimming treatments were applied at a later stage of development than originally intended with the aim of moving flowering time closer to the target period of January.

In year two, 2 trimming treatments and a paclobutrazol treatment were applied to 4 male sterile and two pollinator lines supplied by Bejo Seeds (Table 3.1). The trial was arranged as a split plot design with 4 replicates. Main plots were single rows of each variety, with trimming and chemical treatments imposed in subplots comprising a 5m section of row. A 1m section on each end of all plots was reserved as a buffer zone between plots. Trimming times and intensities were based on results from the 2009 trial. The treatments used were the

early light treatment from 2009 and a less severe late treatment (late light), in which plants were trimmed back to a height of 200mm when their main flowering stem had extended to 300mm.

All trimming treatments were imposed using a petrol line trimmer (Stihl, Australia).

Table 3.1 – Hybrid carrot seed parent lines used in the trimming trial at Richmond, Tasmania

Line No.	Pollinator / Male Sterile	Root Type
1	Male Sterile	Imperator
2	Male Sterile	ABK
3	Male Sterile	Nantes
4	Male Sterile	Flakkee
5	Pollinator	Nantes
6	Pollinator	Berlikumer

Plate 3.1 - Bushy Park trimming trial. a) crop development prior to the early trimming treatment; b) the early-light treatment trimming treatment at this site in which plants were trimmed to just above the second visible stem node, approximately 200mm above ground level; c) a comparison between the early-light and the early-medium trimming treatment; d) plants in the early medium treatment were trimmed to just above the first visible stem node approximately 150mm above ground level and e); a plot trimmed to approximately 75mm above ground level in the early-heavy treatment. Untrimmed plants are shown to the sides and in the background.

Paclobutrazol (Payback®, Cropcare Australasia) was applied at a concentration of 450ppm a.i. on the 1st of October using a 20L chemical knapsack (Silvan, Victoria, Australia) fitted with a fan jet and calibrated to a water rate of 100L/Ha. A wetting agent, Synertrol (Organic Crop Protectants; NSW, Australia) was applied with the paclobutrazol at rate of 2mL/L of water.

Assessment of Flowering Time and Pollinator Activity

The stage of flowering was recorded for all treatment/line combinations at weekly intervals (Bushy Park) or twice weekly (Richmond) from first flower. Flowering was visually assessed using the following scale.

Score	Flowering Stage
1	Start of flowering of first order umbels
50	Start of flowering of 2nd order umbels
100	Maximum 2nd order flowering
150	Start of flowering of 3rd order umbels
200	Completion of first 3 umbels orders

In all plots, more than 90% of bloom was located in the first 3 umbel orders. Plots that were between the developmental stages described in the scale were assigned an intermediate value (25, 75, 125 or 175). All scoring within each trial was done by the same team of 2 scorers working together to ensure that assessments were standardised.

Surveys of pollinator activity were made at the same time plots were scored for flowering. Surveys were conducted between 11am and 2pm, a time period determined in previous studies to best indicate the diversity and level of activity of key pollinators visiting carrot seed crops in Tasmania (Spurr, 2003). The survey data consisted of spot counts of the number of pollinators present in each plot. Pollinators were divided into 2 categories, honeybees (*Apis mellifera*) and others.

Seed Yield Assessments

Plants were harvested by hand when the seed in the first 3 umbel orders had turned brown and loose seed was observed on the surface of first order umbels. 20 plants were harvested from each plot. The numbers of umbels from each plot were recorded and the diameter of 20 representative umbels recorded. After counting and measuring, the umbels were threshed in a modified garden mulcher (Stihl, Virginia Beach, USA), in which the cutting blade had been replaced with a strip of hardened rubber. Hand sieves were used to remove coarse trash and fine dust. Threshed seed lots were placed in zip-sealed pillow slips and dried for 1 week at 25°C in a commercial gas-heated, forced air seed dryer. A laboratory thresher (Wintersteiger, Salt Lake City, USA) was used to de-beard the dried seed. Air-screen cleaning was performed using a laboratory clipper-cleaner (Blount Agri- Industrial, Indiana, USA). The sieve selections were 3.97 to 4.76mm diameter round hole perforated metal top sieves and 1.2 to 1.60mm aperture square nylon mesh bottom sieves, depending on seed size. Air settings were determined by visual assessment of each seed lot. A South Dakota seed blower (Seedburo, Chicago, USA) was used for the final cleaning process, with the settings

determined for individual seed lots on the basis of a visual examination of the seed and trash separation. Clean dry seed lots were stored in sealed plastic bags at 4°C.

Seed yields were determined on samples that had been dried to approximately 10% moisture content.

Results

Climatic Conditions

Maximum daily temperatures in late November and December 2009 were near or above average in southern Tasmania with the long term trend of increasing daily maximum temperatures from December into January apparent (Figure 3.2a). In contrast, the 2010/11 season cool, wet conditions prevailed throughout spring and summer. Daily maximum temperatures did not exceed 25^{o}C for most of the December / January period (Figure 3.2b).

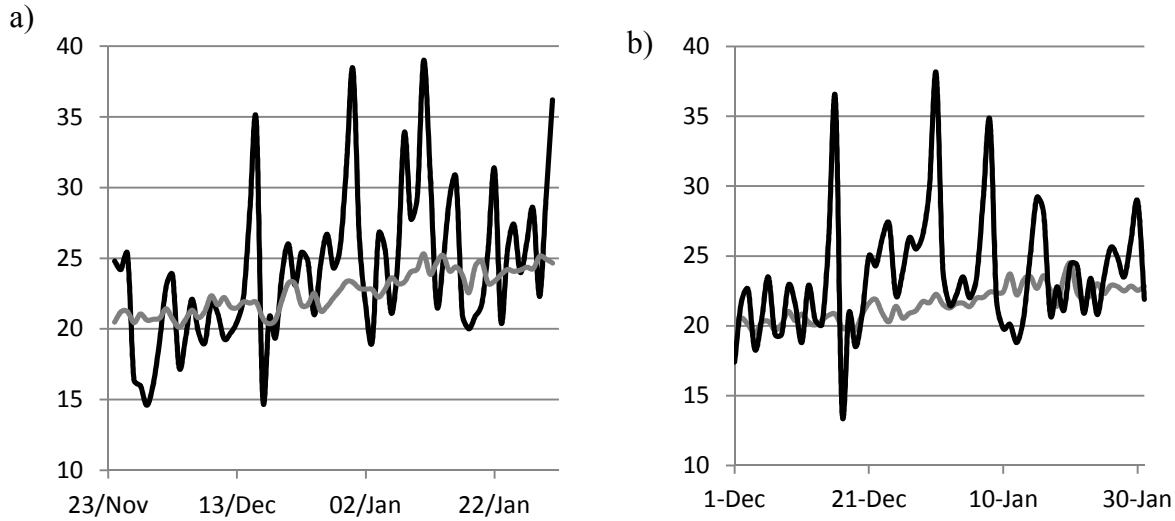


Figure 3.2 – The daily maximum temperatures (black) during trial flowering and long term average daily maximum temperature (grey) at Bushy Park in 2009/10 and b) Richmond 2010/11. Temperatures are based on data recorded by a tiny tag temperature and humidity logger placed at canopy height within the trial, and averages sources from the nearest operating Bureau of Meteorology weather stations at Bushy Park (42.71˚S 146.90˚E, 27m) and Richmond (42.83˚S 147.50˚E, 4m).

Effects of Environmental Factors on Pollinator Activity

Positive correlations between daily maximum temperatures and insect activity (all species) were found in both seasons (Figures 3.3 and 3.4). Other than honeybees, the dominant pollinator species were beetles (*Cantharidae* and *Scarabidae*), wasps and flies (*Calliphoridae and Muscidae*). Amongst the insects observed, honeybees are known to be the most effective pollinators of hybrid carrot seed crops, even when they represent a minority of the insect species present (Spurr, 2003). A positive correlation between temperature and honeybee activity was observed at Bushy Park in 2009/10 (Figure 3.3) but not at Richmond in 2010/11

(Figure 3.4). At Richmond honeybee activity was strongly affected by competition from alternative forage sources, notably prickly box (*Bursaria spinosa*). Due to the wet spring and summer conditions, mass flowering of this species continued until mid January. When prickly box flowering subsided, honeybee numbers within the carrot trial increased dramatically (Figure 3.5).

Much higher numbers of native pollinators were observed in the 2010/11 season than in 2009/10. One reason for this was that the commercial site used in the 2009/10 trial was sprayed with a synthetic pyrethroid insecticide to control Rutherglen bug before scoring commenced. Although honeybees foraging carrot seed crops are unaffected by appropriately applied pyrethroid insecticides, many native insect species are susceptible and their numbers take considerable time to rebuild after spraying (Gaffney, 2011).

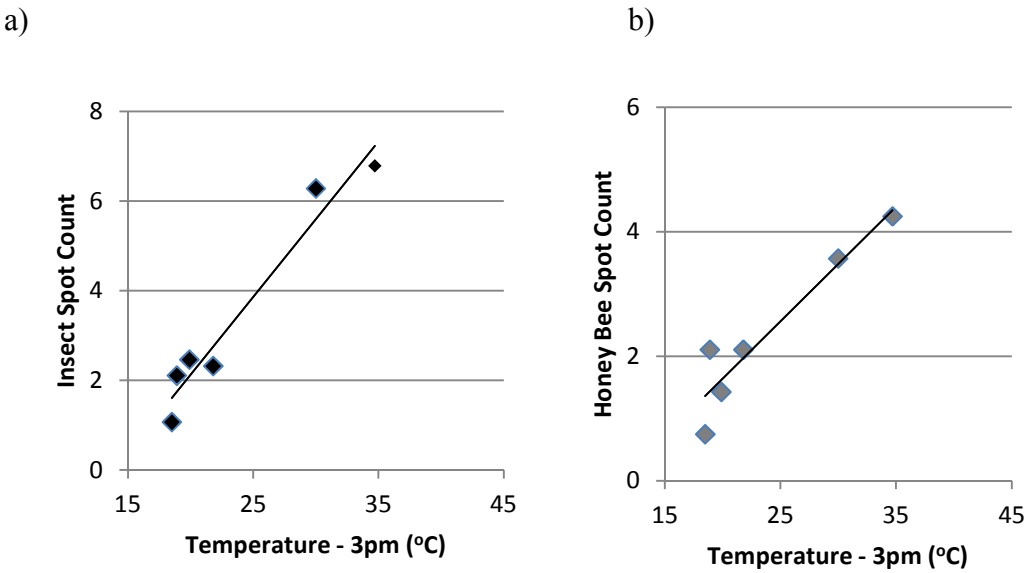
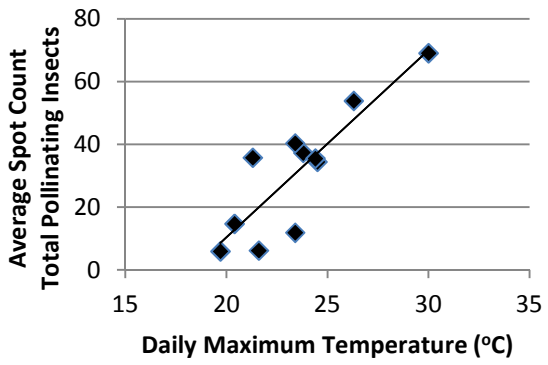


Figure 3.3 - The relationships between daily maximum temperatures and average spot count of a) honeybees and b) total insects during peak bloom in the Bushy Park trial in 2009/10. Both relationships are significant ($P<0.01$), and are represented by the equations a) $y = 0.346x - 4.804$, $R^2 = 0.951$ and b) $y = 0.184x - 2.039$, $R^2 = 0.895$. Data points are based on spot counts of honeybees and total pollinating insects from 21 treated and control plots.

a)



b)

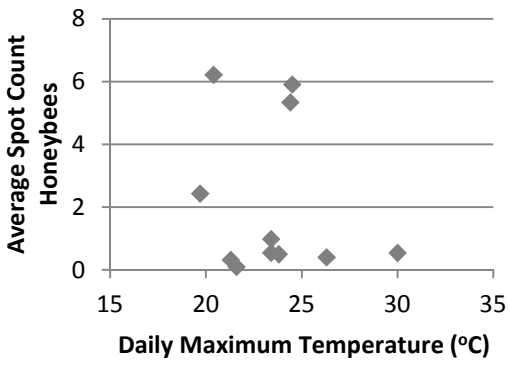


Figure 3.4 – The relationship between the daily maximum temperature and average spot count of a) total pollinating insects and b) honeybees at Richmond in 2010/11. The relationship in a) is signficant ($P<0.01$) and is decribed by the equation $y = 5.967x - 109.0$, $R^2 = 0.746$.

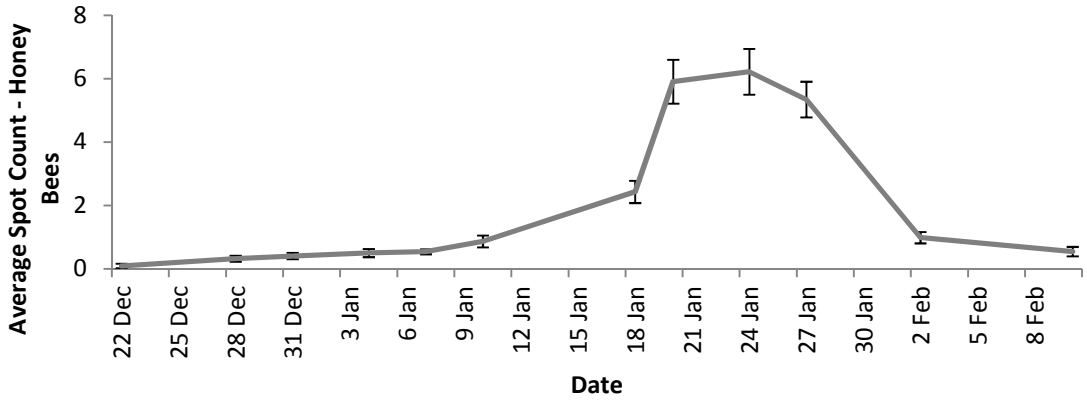


Figure 3.5 – Average honeybee acitivty (per plot) from all plots on observation days occuring at regluar intervals between December 22 to February 8. Error bars indicate standard errors (n=4).

Effects of Trimming and Paclobutrazol on Flowering Time and Pollinator Activity

Trimming delayed flowering in both seasons (Figures 3.6 and 3.8). The extent of delay depended on both the variety and timing and intensity of trimming. In 2009, the trial was undertaken in an early flowering cultivar at Bushy Park. Control plots were in peak bloom between December 5 and 20 (Figure 3.6), approximately 20 days earlier than for most temperate carrot varieties grown in Tasmania. Delays of about 10-14 days to onset of peak bloom were observed in trimmed plots, with the greatest setback observed in plants that received the medium intensity trim twice. These plots did not reach peak bloom until December 23, 18 days after the control. Other trimming treatments resulted in 10 to 15 day delays in peak bloom. The duration of peak bloom was generally between 14 to18 days for treated plots. The exception was for plants trimmed hard early, which flowered unevenly and for an extended period. From a production perspective this is an undesirable result as it leads to uneven maturation of the seed and late harvesting in autumn when weather conditions can make harvesting difficult.

Although the early flowering period of the crop meant that, even with the delays achieved in most treatments, the majority of peak bloom fell outside the target time window, the trimming treatments did move flowering into a warmer period of weather. This was reflected in a clear trend toward increased insect activity (especially honeybees) in most of the late flowering treatments (Figure 3.7). The lowest spot counts of both honeybee and other insects were observed in early flowering control plots.

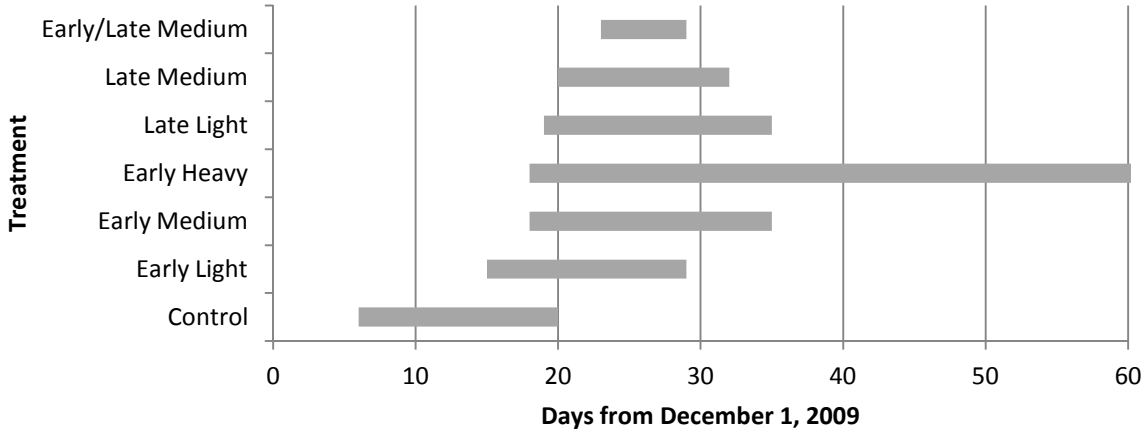


Figure 3.6 – The effect of time and intensity of trimming on the time and duration of peak bloom in a single open pollinated variety grown at Bushy Park in 2009/10. The grey bars represent the average period plants from each treatment were considered to be in peak bloom.

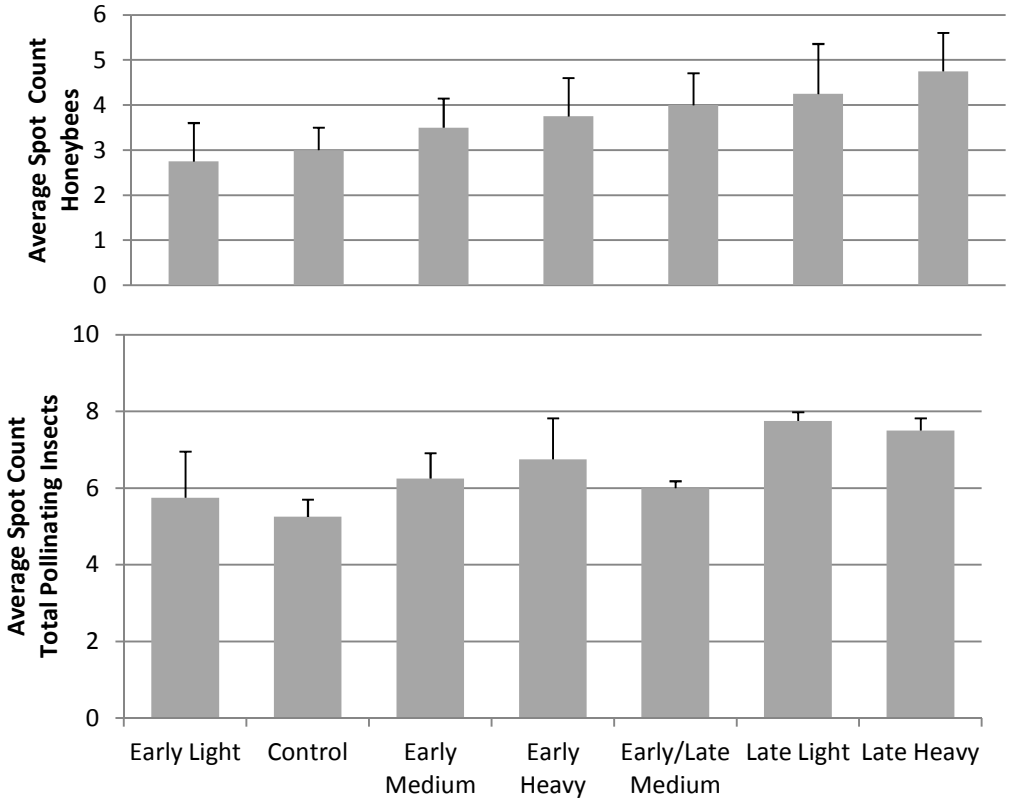


Figure 3.7 – Average spot count honeybees (top) and total pollinating insects (bottom) during peak bloom for each treatment. Error bars indicate standard errors (n=4).

In 2010, the effects of trimming treatments were observed on both pollinator and male sterile lines. Control (untrimmed) plots in this trial reached peak bloom 16 to 31 days later than control plots in 2009/10 (depending on variety) at a time more typical for temperate carrot seed parent lines.

Trimming delayed flowering in all male sterile and pollinator lines by 10-14 days (Figure 3.8). The one exception was for the early light treatment in line 1, which had no effect. Data collected on plant development at the time of trimming suggest that the treatment was applied too early to this line be effective. Treatment with paclobutrazol also delayed flowering by a similar amount to trimming in some lines, but in Line 3 it had no effect. This may be due to a need for greater precision in timing of application to achieve optimal results.

Based on long term averages the extent of delay achieved with trimming and paclobutrazol treatments in 2010 would be sufficient to ensure a significant proportion of the flowering occurred after peak bloom of *B. spinosa* and under more favourable temperature conditions (Figure 3.2). Under the conditions that prevailed in 2010/11, plots of male sterile varieties 1, 2 and 4 and pollinator line 5 had finished or were approaching the end of peak bloom by the time that *B. spinosa* completed flowering and honeybee activity increased (Figures 3.8 and 3.9). Furthermore, because of the consistent, lower than average temperatures for much of the trial, activity of other insects remained at relatively constant levels throughout. Trimmed plots of two later flowering varieties (male sterile line 3 and pollinator 6) did reach peak

bloom later, between the 11th and 15th of January, which coincided with a marked improvement in honeybee activity (Figures 3.8 and 3.9).

In general, trimming resulted in a shorter, more intense period of peak bloom because a) trimming increased uniformity of reproductive development within plots and b) in 2009, trimmed plots flowered under warmer temperatures. Whilst more uniform flowering is generally desirable because it means that the crop matures evenly, it can increase the impact on yield of a period of unfavourable weather during pollination.

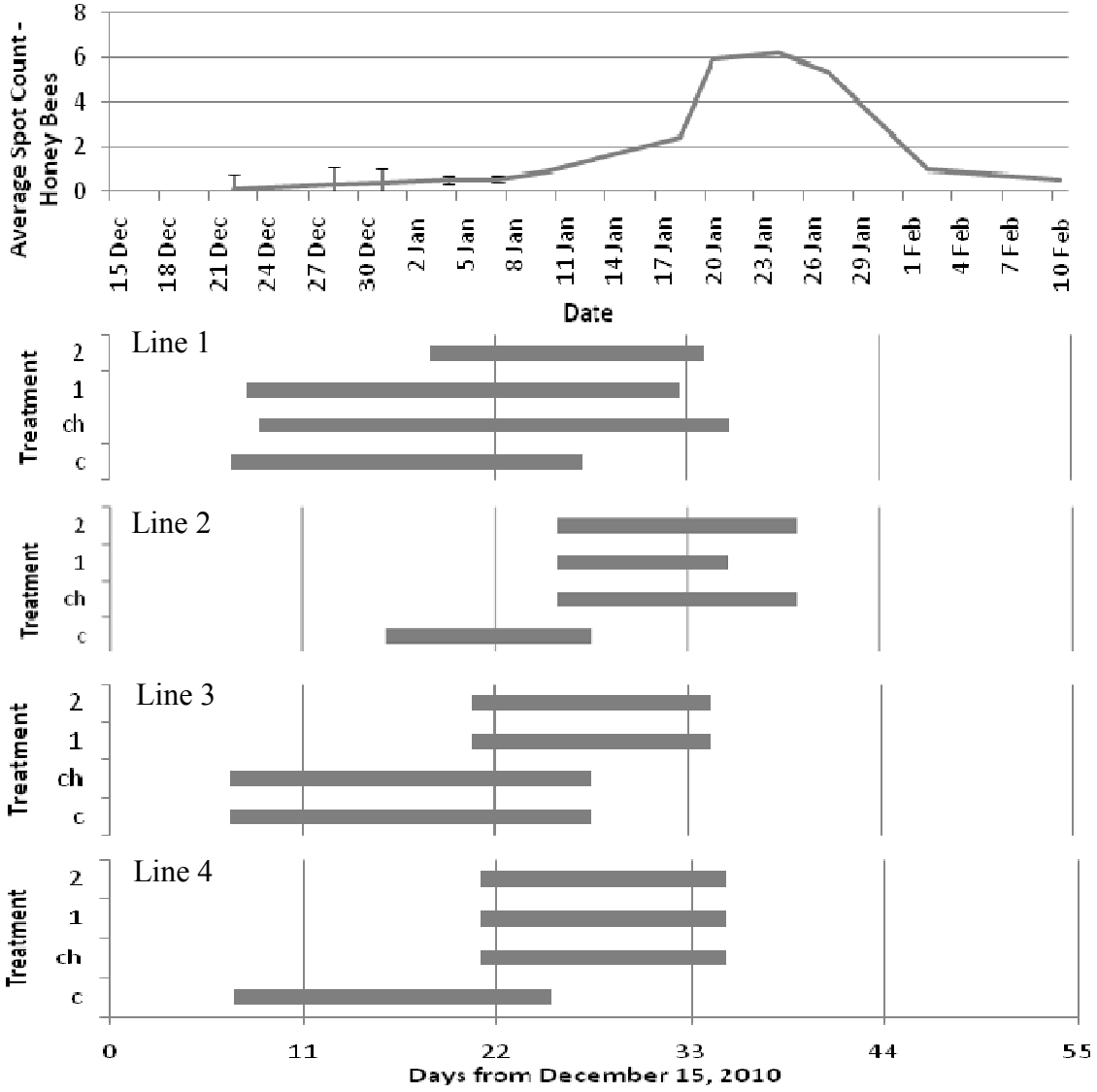


Figure 3.8 – Average honeybee spot counts from observation on survey days from December 15, 2010 to February 10, 2011 (top) compared with the period of peak bloom for treated and control plots of male sterile lines (from top to bottom lines 1, 2, 3 and 4). Trimming treatments are control (c); paclobutrazol (ch); early-light (1) and late-light (2) Line. Peak bloom data are based on the means of 4 replicate plots for each treatment. Honeybee spot count data are based on mean counts from all trial plots on each survey day.

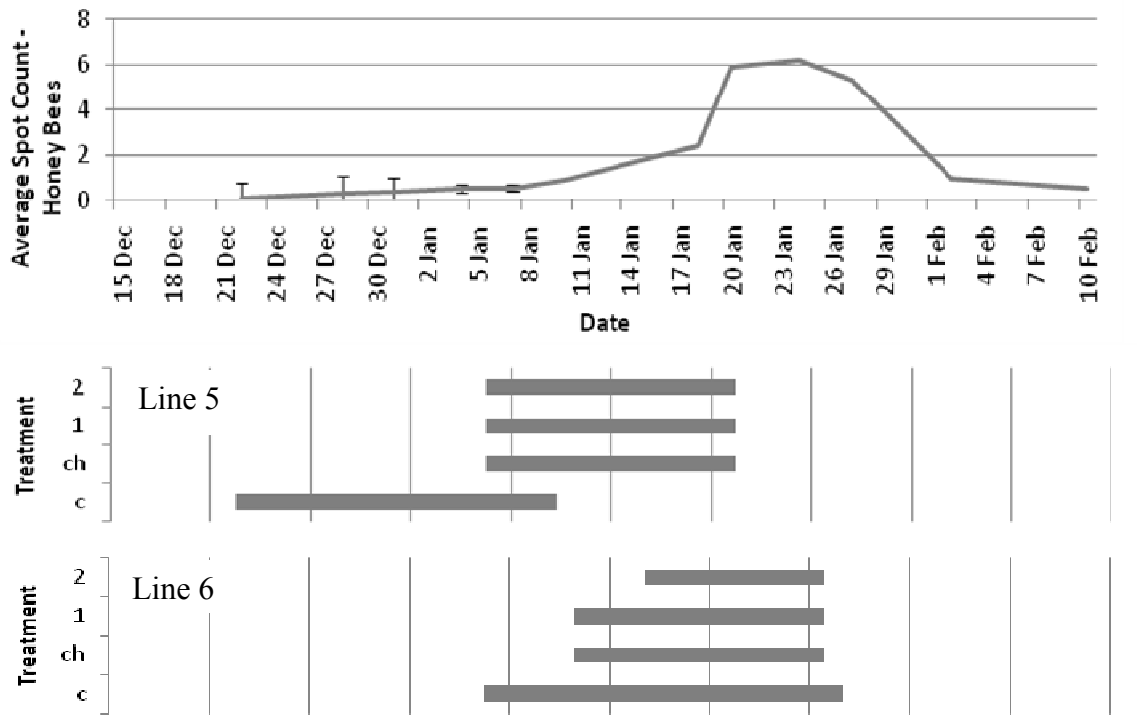


Figure 3.9 – Average honey bee spot counts from observations on survey days from December 15 to February 10, 2009 (top) compared with the period of peak bloom for treated and control plots of male sterile lines 5 (middle) and 6 (bottom). Trimming treatments are control (c); paclobutrazol (ch); early-light (1) and late-light (2) Line. Peak bloom data are based on the means of 4 replicate plots for each treatment. Honeybee spot count data are based on mean counts from all trial plots on each survey day.

Effects of Trimming Treatments on Yield Potential

In 2009 the effects of trimming on yield potential were investigated. Yield potential was measured in terms of the average surface area of umbels borne by plants from each treatment. Trimming caused a decrease in the umbel surface area (500 to 900cm^2 compared with 1500 cm^2 for control plants). Early trimming caused the smallest reductions in umbel surface area, whilst the largest reductions were in the late heavy and double trimming treatments (Figure 3.10).

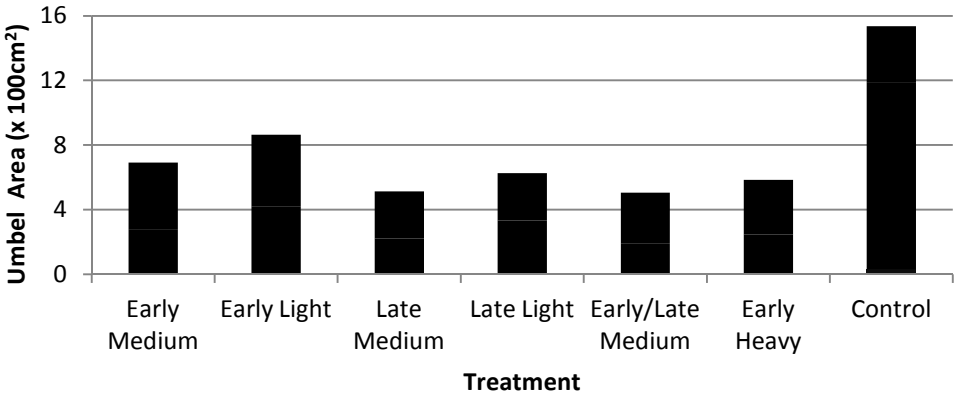


Figure 3.10 - The effect of time and intensity of trimming on mean yield potential (umbel surface area). Some treatment means are significantly different ($P<0.01$)

Effects on Realised Seed Yield

In the 2009 trial at Bushy Park, control (untrimmed) plots yielded on average 9.6g of seed per plant (Figure 3.11). A small but non-significant improvement in seed yield was observed in plots of the early light trimming treatment, which yielded an average of 10.4g / plant. Other trimming treatments significantly decreased seed yields. Late, multiple or heavy trimming treatments were most damaging to seed yields (Figure 3.11), with yields reduced to as little as 52% of control yields.

Trimming treatments in 2010 were based on observations from 2009 to achieve desired levels of delay in flowering with minimal impact on yield potential. Both trimming treatments caused significant ($P<0.01$) yield reductions in lines 1 and 3 of between 3 and 60% because the reduction in yield potential was not compensated by increased pollinator activity. In line 2 flowering was delayed sufficiently by trimming (Figure 3.8) for late improvements in honeybee activity to more than compensate for the loss of yield potential, resulting in significant ($P<0.01$) yield increases of 25% to 50% (early and late treatments) (Figure 3.12).

Treatment with paclobutrazol resulted in seed yields no better or less than those from the control treatments.

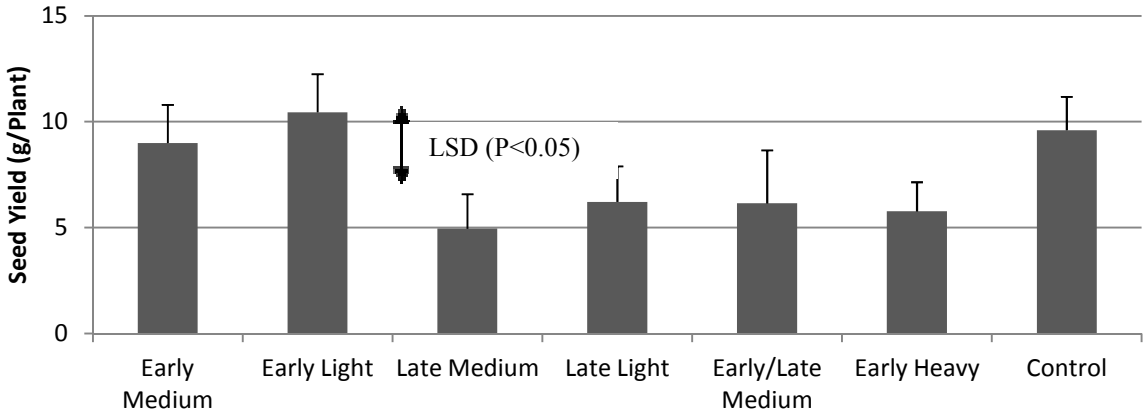


Figure 3.11 –Effects of trimming on mean seed yields (grams/plant) from the 2009 Bushy Park trial. Error bars indicate standard errors (n=4). Some treatment means are significantly different. The LSD (P<0.05) is indicated by the vertical bar on the graph.

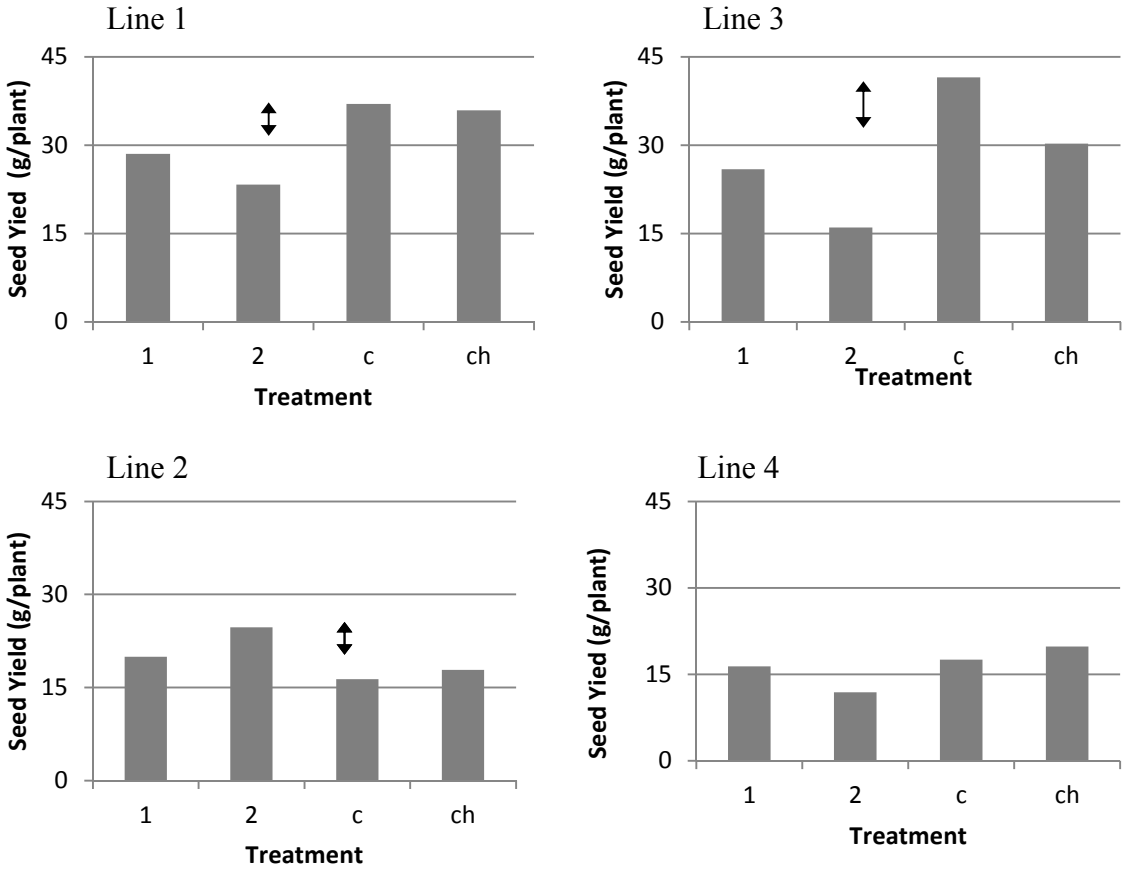


Figure 3.12 –Effects of trimming on mean seed yields (grams/plant) from the 2010/11 Richmond trial. Error bars indicate standard errors (n=4). Some treatment means are significantly different. LSD (P<0.05) values are indicated by the vertical bar on each graph.

Overview

This work was undertaken to establish whether trimming or paclobutrazol treatments could be used to delay flowering and to determine the impacts of such treatments on yield potential, pollination and realised seed yields. Currently seed producers use trimming treatments to correct nicking problems in hybrid crosses and to reduce crop height to prevent lodging. In general treatments are imposed at a late stage of crop development but there is uncertainty about the impact of late trimming on yield potential and realised yields.

Based on our understanding of the pollination biology of carrots we predicted that pollination would be optimal under warm dry conditions and, in Tasmania, would be improved if flowering was spatially or temporally isolated from the bloom period of the native shrub, prickly box (*Bursaria spinosa*). In southern and central Tasmania *B. spinosa* is widespread and abundant throughout most seed growing areas. In these areas it is difficult to find carrot seed production sites that are isolated from it. Based on long term climate data and records from beekeepers for flowering of *B. spinosa* it was determined that a 2 to 3 week delay in peak bloom of most temperate varieties until the 2nd week of January would, on average, improve conditions for pollination in Tasmanian crops. In this work, experiments to confirm this theory were hampered by a) an unexpectedly early flowering variety in 2009 and b) wet, cool conditions in 2010 which meant that *B. spinosa* flowered for longer than normal and anticipated increases in honeybee activity within the trial were delayed until the start of the 4th week of January.

The results obtained in this study confirm that it is feasible to delay flowering in carrot seed crops by 10 to 14 days. Similar delays were achieved for some varieties by applying 450ppm of paclobutrazol at the start of October. Variable responses to paclobutrazol application between varieties could reflect varietal differences in responsiveness or a need for more precise timing of applications to achieve consistent results.

Although trimming treatments that moved flowering to more favourable conditions for pollination did result in improved seed yields, the results of this work clearly highlight the risks associated with this strategy when conditions are no better or worse for pollination in the later flowering window. This risk is increased by the fact that all trimming treatments caused reduced yield potential in terms of the quantity of flowers available for pollination (assessed as umbel surface area). In this regard, trimming treatments that were applied at a later stage of crop development or that removed a greater proportion of the developing inflorescence carried a larger risk of adverse yield effects. Furthermore, some of the later or harder treatments resulted in weak plants and uneven flowering.

Where trimming is used as a management strategy to correct nicking problems in hybrid crops, the results of this work suggest that it is best done early, when plants have a stem extension of approximately 300mm and should not reduce the canopy to below 150mm above ground level. In some cases, more severe treatments may be required to correct extreme differences in nicking but the benefits would have to be carefully weighed against loss of yield potential from the trimmed line.

CHAPTER 4

Development of a World Class Hybrid Carrot Seed Industry in Northern Tasmania. Applied Research and Extension in Action

Introduction

Carrot seed production is an important component of the Australian vegetable seed industry. An estimated 600Ha worth in excess of $A5million is produced annually[3]. Recent investment in research and development and rapid commercialisation of research outcomes by the vegetable seed industry have resulted in significant improvements in the quality of seed produced by Australian growers. This has led to increased interest in Australia as a site for export carrot seed production. Market research by industry stakeholders suggests that this interest could potentially translate into $A2-5million of additional carrot seed production annually for the Australian industry.

There has been interest from international vegetable breeders in Australia as a site for seed crops of European hybrid carrot varieties. Whilst significant improvements have been made in the yield and seed quality achieved in open pollinated crops, attempts to translate these improvements to hybrid production has had limited success. A major limitation to capitalising on potential market opportunities is that hybrid seed production often involves parent lines with strong resistance to flowering; that is, varieties that have been bred to limit incidence of bolting in vegetable crops grown in cool temperate regions. After the plant has developed beyond a period of juvenility, flowering is induced by exposure to a period of low temperature (vernalisation). Resistance to flowering observed in many hybrid parent lines generally manifests as a longer juvenile period, or a requirement for longer durations of chilling and/or lower threshold temperatures for chilling. This means that some locations in Australia that are otherwise appropriate for high quality carrot seed production are unable to provide adequate conditions for natural vernalisation.

Although it is not a historically important carrot seed production area, Tasmania is potentially well suited to growing seed of European hybrid varieties. Important natural and competitive advantages include:

- An ideal climate for induction of flowering and seed maturation. That is, sufficient natural chilling for flower induction and a warm, long summer/autumn period for growth in the juvenile phase and seed maturation;
- Experienced seed growers operating at a scale well suited to small areas of intensive crops such as hybrid carrot;
- Freedom from wild carrot which can cross pollinate seed crops;
- A southern hemisphere location for counter season production for northern hemisphere clients. The majority of established carrot seed production areas are located in the northern hemisphere.

[3] Source: Seed Industry Association of Australia

Although these facts had been recognised by international vegetable breeding companies and the Australian industry, attempts to develop a hybrid carrot seed industry in Tasmania had generally been unsuccessful. This had led to a loss of grower confidence in carrot seed crops and reduced customer confidence in Tasmania as a production location.

Over the last decade, the Australian vegetable seed industry has collaborated with research providers TIAR and seedPurity Pty Ltd and Horticulture Australia to address many of the production issues facing the Australian carrot seed industry. This investment in research and development of innovative production practices had lead to significant breakthroughs that dramatically increased the quality of open pollinated carrot seed grown on the Australian mainland and reignited interest in Australia as a location for high quality carrot seed production.

Amongst industry personnel and researchers at the University of Tasmania's School of Agricultural Science and seedPurity, a firm belief that hybrid carrot seed could be successfully produced in Tasmania remained.

In 2008, South Pacific Seeds initiated this project in collaboration with TIAR and seedPurity in an attempt to adapt recent research outcomes into best practise crop production and secure Tasmania as an internationally recognised centre for hybrid carrot seed production. The project had 3 key aims:

- To build a successful hybrid carrot seed industry in Tasmania;
- To develop a core group of skilled carrot seed growers and agronomists in Tasmania;
- To promote Tasmanian hybrid carrot seed production internationally.

Methods

Project Team

A small project team with the necessary skills was assembled from industry and research personnel. Team members were:

- Tasmanian production personnel: Andrew Jones and Craig Garland, South Pacific Seeds, Tasmania;
- Carrot seed production specialist: Max Dalrymple, South Pacific Seeds, South Australia;
- Researchers: Ang Geard and Cameron Spurr, seedPurity Pty Ltd / TIAR;

As there is an established hybrid carrot seed industry in New Zealand, expertise from this industry was sought. In response, Grant King, a carrot seed production specialist from South Pacific Seeds, New Zealand acted in an advisory role to the project team.

Project Strategy

A strategy to develop the industry was formulated. The key components were to:

- Identify the problems of the past;
- Apply research outcomes and experience to develop solutions;
- Identify and approach growers and international customers to participate;
- Use demonstration crops to train growers and field staff in best practice and build client confidence (start small and build on success);
- Evaluate results and learn from both successes and failures.

Project Outcomes

Problem Identification

In the light of experience in other production locations and knowledge from research, many of the reasons for past crop failures in Tasmania were identified. Key issues included:

1. Sowing date (too late);
2. Poor irrigation practice at establishment;
3. Unfavourable environmental conditions during pollination;
4. Inadequate control of Rutherglen bug;
5. Timing of windrowing.

Each of these factors could be addressed through a combination of application of knowledge and attention to detail.

Addressing the Problems

1. Sowing date

Issues associated with sowing date were addressed in the following way:

- Long term climatic data were used in models of thermal time and chilling requirements for flower initiation in European hybrid varieties developed at TIAR to determine cut off dates for sowing. Predictions were validated with field data collected from past commercial crops and trial plots.
- Ensuring that customers and growers understood the cut off dates for sowing so that stock seed delivery and ground preparation were timely.

2. Irrigation

Recent investment in centre pivot and linear irrigation systems by many farmers in Tasmania provided a significant opportunity to improve irrigation practice during crop establishment.

3. Pollination

Understanding of the pollination biology of carrot has benefited from research conducted over the last decade (Spurr, 2003; Brown and Spurr, 2006). Key findings include the relationships between relative humidity and pollen viability/longevity; effects of temperature on nectar production and pollinator activity (refer to Chapter 2 in this report); the identity and effects of competing forage sources, in Tasmania notably Prickly Box (*Bursaria spinosa*); and impact of pesticide programs on native pollinator populations in carrot seed crops (Gaffney, 2011).

Past attempts to produce hybrid carrot seed in Tasmania had centred on south-eastern Tasmania and the Derwent Valley. In this project we focussed on the central north of Tasmania. The reasoning behind this decision was twofold:

- Climate: Long term climate data indicated that during the critical flowering period (December / January), average daily temperatures are 2 to 3^{o}C warmer (Figure 4.1) and average relative humidity levels up to 10% lower in the central north compared with production locations in the south east.

- Competing forage sources: *Bursaria spinosa,* a small native tree, is widespread and abundant in southern and central Tasmania. It is extremely attractive to honeybees and overlaps in flowering time with carrot seed crops. Sites isolated from *B. spinosa* and other competing forage sources were more readily available in northern Tasmania.

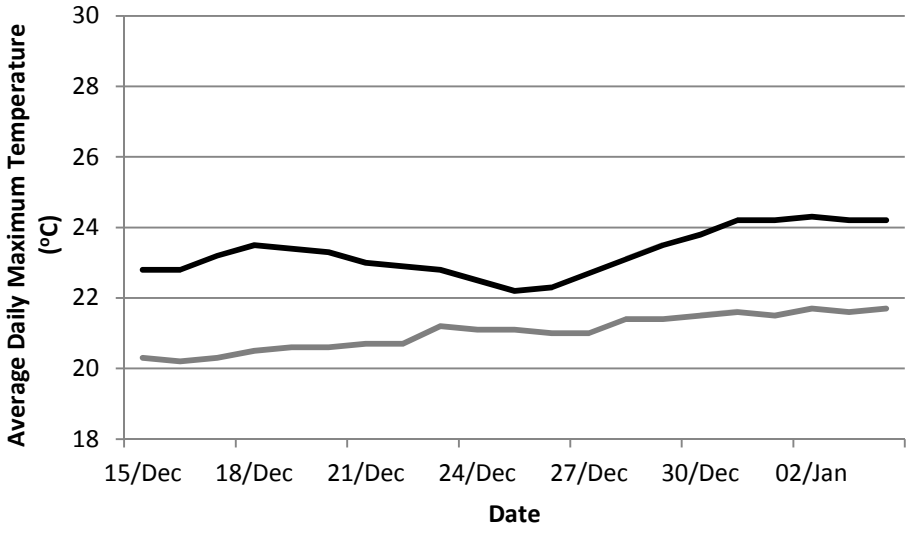


Figure 4.1 – A comparison of the long term average daily maximum temperatures for the nearest operating Bureau of Meteorology weather station to the Coal River Valley region; Hobart Airport (grey line) (42.83°S, 147.5°E, Elevation: 4m) and the Bureau of Meteorology weather station situated at Cressy (black line) (41.72°S, 147.08°E, Elevation: 148 m), in the central north.

4. Managing Rutherglen Bug (Nysius vinitor)

A major factor limiting the success of carrot seed production in Australia prior to 2000 was the difficulty in achieving the standards of seed germination required for export markets. The main problem was identified to be the occurrence of embryoless seeds and seeds with damaged embryos (Plate 4.2). Feeding damage of the migratory insect *Nysius vinitor* (Rutherglen bug) (Plate 4.1) was shown to cause these defects (Spurr, 2003). Rutherglen bugs migrate into crops in large numbers under favourable conditions and rapidly reduce seed germination. Crops remain vulnerable for a relatively long period during seed maturation so effective management strategies are required.

South Pacific Seeds have developed an effective management strategy for Rutherglen bug in open pollinated carrot seed crops grown on the Australian mainland based on: a) routine monitoring of crops and nearby alternative hosts; b) knowledge of the influence of seasonal conditions and weather patterns on the migratory behaviour of the pest and; critical control thresholds developed from data on the damage relationship of *N. Vinitor* in carrot seed crops (Spurr, 2003).

In this project, the Rutherglen bug management strategies were adapted to suit hybrid seed production in Tasmania. Key considerations included the impact of control measures on pollinator populations (Gaffney, 2011) giving the importance of pollination to hybrid carrot seed yields and adjusting control thresholds to reflect the lower average yields of hybrid seed crops.

Plate 4.1 – Female (foreground) and male (background) Rutherglen bug (*N. Vinitor*) feeding on seeds in a carrot umbellet.

5. Timing of Windrowing

Although the knowledge of the effects of Rutherglen bug on carrot seed quality led to a significant improvement in the germination results of seed produced in Australia, there were crops that still failed to achieve the standard of germination required by export markets. The failure of these seed lots could not be explained through the occurrence of embryoless seeds or damaged embryos (Figure 4.2). In 2004 a research project initiated by Bejo Seeds and South Pacific Seeds and funded through the Horticulture Australia project (VG03084) investigated the basis of these crop failures. The key findings and outcomes were:

- Germination failures were attributable to the occurrence of rudimentary embryos. Rudimentary embryos are small, under-developed embryos that are viable but unable to germinate within the 14 days of a standard germination test;

- Unlike other vegetable seed crops, carrot and other Apiaceae are uniquely predisposed to this problem because: embryo development occurs late in seed maturation after the endosperm is deposited (Geard *et al.,* 2004) and; seed maturation is staggered due to successive flowering of different umbel orders and prolonged flowering period;

- High levels of rudimentary embryos are a particular risk in cool seasons, or production environments such as Tasmania that have a comparatively mild climate;

- Markers of crop maturity used in other production locations were unreliable in cool temperate climates.

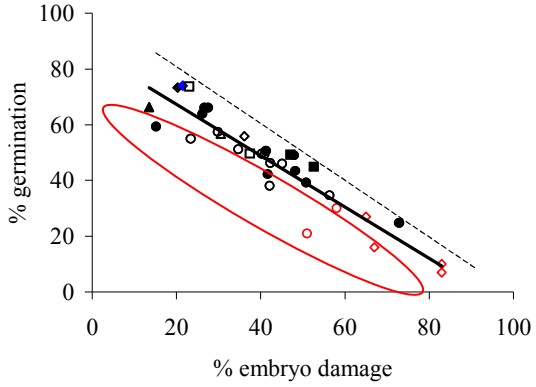


Figure 4.2 – The relationship between the occurrence of embryo damage (embryoless seeds and seeds with embryo damage) and percentage germination for commercial and trial seed crops grown in south eastern Australia between 1998 and 2001. The solid line shows the linear regression y=-0.9207+85.706 ($P<0.001$; $r^2=0.88$) between the two variables. The dashed line represents the threshold where the percentage of embryo damaged seeds in a seed lot would account for all loss of germination. Seed lots in which poor germination was not largely explained by occurrence of embryoless seeds or embryo damage are highlighted within the red oval. (Figure modified from Spurr, 2003).

Following this project, a method for dissecting carrot embryos from seeds was developed into a routine test of crop maturity. In this test, representative seed samples collected from the field are dissected and the embryos examined under a microscope. Embryos are classified as mature, damaged or rudimentary (Plate 4.2) allowing a prediction of crop maturity to be made to support decision making on time of windrowing.

Grower and Customer Participation

Many Tasmanian growers and international vegetable breeders had lost confidence in Tasmania as a production location for carrot seed crops or were aware of past failures. This presented a significant challenge to advancing the project. The project team worked together to develop proposals for both potential customers and growers. These highlighted:

- Investment that the industry had made in improving standards of production on mainland Australia and the results this had delivered;
- The collaborative approach between researchers and industry to developing a Tasmanian industry;
- The natural advantages Tasmania offered for hybrid carrot seed production and the strategic advantages it offered clients in the form of an alternative southern hemisphere production location;

- The emphasis of the project on starting small with a dedicated grower group, attention to detail, skill development and building on success;

- The potential for the industry to grow if initial trials were successful.

Amongst the vegetable breeders approached, 3 agreed to support the project by placing small areas of commercial production in Tasmania in 2009, giving a total production area of approximately 10Ha.

Potential growers with suitable land, infrastructure and experience to be involved in the pilot project were identified and approached. Of 10 growers who were approached, 5 agreed to grow trial crops.

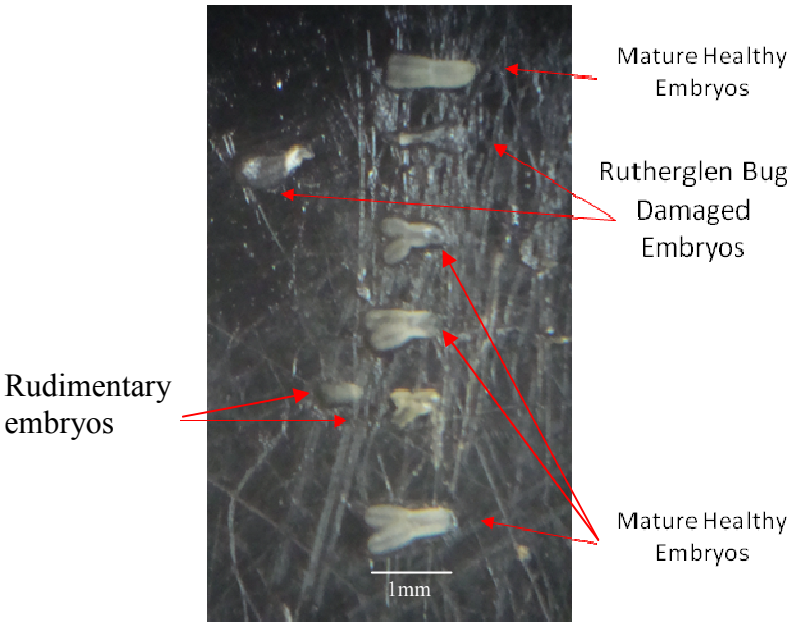


Plate 4.2 – Mature healthy, damaged and rudimentary embryos excised from carrot seeds.

Extension Activities

In 2009, the project team worked very closely with the growers to monitor crops and to ensure they were grown according to best practice. Particular attention was paid to ensuring that the mistakes of the past were avoided and to educating field staff and growers about critical aspects of crop management including flower induction, ensuring synchronous flowering of the parent lines, managing crops and pollinator populations to maximise pollination rates, managing Rutherglen bug and timing of windrowing. The size of the project enabled first year extension activities to be undertaken on site with individual growers and field staff using a 'hands on' approach.

The project team also led a number of visits to demonstration crops from participating and potential customers and potential future growers (Plate 4.3).

Plate 4.3 – Ang Geard from SeedPurity discussing management of Rutherglen bug with growers during flowering in a crop at Whitemore.

Plate 4.4 – A hybrid carrot seed crop grown at Whitemore in 2009. In November 2009 the male sterile line was approximately 2 weeks ahead of the pollinator line. A new trimming protocol developed in this project to manipulate flowering time whilst minimising impacts on yield potential (see Chapter 3) was used to correct the problem. The photo on the left shows the crop during trimming. The trimming resulted in synchronous flowering of both lines in mid-January (photo on right) and a crop that achieved its yield and quality targets.

Evaluation

With the exception of one crop that failed to establish, all demonstration crops met or exceeded industry standards for both yield and quality (Table 4.1). The best yield result achieved fell just short of setting a new record for a variety that has been produced at other sites around the world for at least 2 decades. Images of the 2009/10 crops are shown in Plates 4.4 and 4.5.

Plate 4.5 – Good pollinator activity was observed during flowering in 2010 (left) resulting in good seed set (right).

Feedback from both international customers and growers was extremely positive. All customers involved in 2009/10 contracted an increased area in 2010/11, resulting in a production area of 30Ha. Despite an extremely difficult spring and summer season with record rainfall along the eastern seaboard of Australia, all crops achieved industry quality standards and most were near to or exceeded industry yield targets. As a result, the contracted area in Northern Tasmania has increased to over 160Ha in 2011/12. The number of customers has increased to 6 and the grower base has grown to 20.

The 'hands on' approach to extension employed proved to be effective when working with a small group of growers and field staff. In 2010/11 extension activities were modified to accommodate a larger grower group. These comprised 2 field days at critical stages of crop development in October (flower initiation) and January (flowering) and visits to individual crops/growers by researchers and seed production specialists to address specific production issues (Plate 4.5). In addition a guide to identification and management of Rutherglen bugs was produced and distributed amongst growers.

Each field day included a grower meeting where presentations on research and development activities and cultural practices relevant to the stage of crop development at that time were made. The field days were also structured to provide growers with a forum to discuss production issues with researchers and mainland and international carrot seed production specialists.

Table 4.1 – Results from 2009 Tasmanian hybrid carrot seed demonstration crops. The table summarises the area of crop, the target yield (for total crop area), the actual clean yield achieved and the germination percentage of the cleaned seed-lot. Note 1 additional demonstration crop failed to establish. All other crops met or exceeded customer yield and germination targets.

Crop Number	Crop Area (ha)	Target Yield	Actual Clean Yield	% of Target Yield Achieved	Germination Percentage	Customer acceptance
09/10 A	2.7	1890	3556	188%	90%	y
09/10 B 1	0.8	240	290	121%	88%	y
09/10 B 2	0.8	280	343	123%	83%	y
09/10 C	1.2	420	608	145%	91%	y
09/10 D	2.3	920	918	100%	93%	y

Industry Prospects and Challenges

After two successful seasons, there is considerable international interest in Tasmania as a location for high quality European hybrid carrot seed production. Provided yield and quality standards can be maintained there are very good prospects for future industry growth. The benefits of this growth will not only be to seed production companies and farmers directly involved but also to other sectors through increased demand for labour and agricultural services. This is timely given the recent contraction of some other primary industries in Tasmania. In addition to the challenge of maintaining production standards across a larger program, the industry will need to address key infrastructure issues as it grows.

SECTION 2 – ONION POLLINATION RESEARCH

CHAPTER 5

Understanding the Basis of Unreliable Hybrid Onion Seed Yields

Introduction

In the last decade, international consumption of onions has grown 45% to approximately 70,000,000 tons annually[4]. To meet this increase, the area of the global onion crop has increased 30%[1] and demand for seed has increased proportionally. As an established producer of onion seed, the Australian industry is faced with the challenge of realising the opportunity that this increased demand presents.

One of the main difficulties faced by onion seed producers in Australia and globally is to ensure that economical seed yields can be reliably produced. This challenge is particularly apparent for producers of hybrid onion seed. There have been many examples of hybrid crops failing to produce economical yields throughout the industry in Australia and internationally (Pathak, 2000; Delaplane and Mayer, 2000). Although the reasons for some crop failures are clear, many crops that fail to reach their target seed yields appear healthy and, apart from lack of seed set, well grown.

With the exception of a project investigating flower abortion in a particular group of hybrid onion seed parent lines (HAL Project VN05004; Brown, 2008), the broader issue of unreliable seed yields in hybrid onion seed crops in Australia has received relatively little attention from researchers. In contrast, many overseas studies have investigated ways to improve onion seed yields. A criticism of much of this work is that it attempts to solve the problem without first clearly understanding the basis of poor seed yields. There are studies that have compared the attractiveness of different onion seed parent lines to pollinators (Silva and Dean, 2000), the effectiveness of different pollinator species (Williams *et al.*, 1974; Pathak, 2000); examined the effects of environmental conditions on pollen viability, pollination and seed development (Kho and Baer, 1968; Chang and Struckmeyer, 1976) and tested the effects of parent line arrangements (Williams *et al.*, 1973), growth regulators (Corgan and Montano, 1972; Looper and Wallar, 1982; Bhople *et al.,* 1999), nutrition (Cuocolo and Barbieri, 1988; Tiwari *et al.,* 2002), pollinator attractants (Silva *et al.,* 2003) and numerous other agronomic practices on seed yield. Despite this body of work, there are few examples that focus on identifying what actually happens between flowering and harvest in commercial production conditions to cause the extensive variation in seed set that is observed.

The first step to achieving more reliable onion seed yields in Australia must be to clearly identify the factors that limit seed yields. In this work we undertook a detailed survey of flowering, pollination and seed development in commercial hybrid onion crops grown in two

[4] Source: Economic Research Centre, United States Department of Agriculture

Australian production locations to identify the basis of yield variability. The purpose of this work was to provide a foundation on which future work to improve seed yields can be based.

Materials and Methods

Survey Sites

Onion yield surveys were undertaken in two production locations, in the Murrumbidgee Irrigation Area (MIA) near Griffith, NSW (2009) and southern Tasmania (2009 and 2010). These areas were selected on the basis of importance to the industry (more than 50% of the Australian onion seed crop is produced in the MIA) and, in the case of Tasmania, industry interest in expanding onion seed production. In total, 9 commercial sites comprising 20 hybrid brown and red onion crops (combinations of 17 different hybrid seed parent lines) were surveyed near Griffith in 2009 and two sites comprising 19 hybrid seed parent lines in a variety trial (2009) and two hybrid crosses (combinations of 3 different hybrid seed parent lines) (2010) were surveyed in Tasmania. All crops and trials included in the survey were well grown and healthy at the time of surveying. During peak bloom honeybees were stocked at each site at a rate of approximately 5 hives / Ha.

Survey Parameters

Individual crops were surveyed on a minimum of 5 days during the 2 week period of peak bloom. On each day the following parameters were surveyed at replicated sites within each crop.

Stage of Flowering

Stage of flowering was assessed at each site by recording the number of pollen producing (pollinator lines) and receptive (male sterile lines) inflorescences within 10m sections of row.

Pollinator Activity

Spot counts of pollinators were made on 50m sections of row in commercial crops and 5m sections of row in trials. Spot counts were performed between 9am and 1pm (Griffith) and in the middle of the day (Tasmania) because these times had been identified as the peak periods of pollinator activity. Pollinators were divided into 5 categories, honeybees (*Apis mellifera*), native bees, flies, wasps and others, and counted.

Pollen Viability

Umbels with dehiscent anthers were gently tapped on a sheet of black gloss cardboard. The deposited pollen was easily visible on the cardboard surface and was collected using a fine artist's brush. The pollen was brushed into 1.5ml Eppendorf® centrifuge tubes and placed

over silica beads in a desiccator to dry for 48 hours. After drying the samples were sealed and stored at -18^{o}C. Pollen viability was assessed using the FDA test (Heslop Harison and Heslop Harison, 1970). Prior to testing, samples were re-hydrated for 30 minutes in a covered water bath at 30^{o}C. The test solution was prepared by adding 1ml of fluorescin diacetate dissolved in acetone (2mg/ml) to 10mls of 20% sucrose solution. Samples of the rehydrated pollen were suspended in a drop of FDA/sucrose solution on a microscope slide under a cover slip. After 5 minutes standing under laboratory conditions the slides were examined at 100x magnification using a Leica Leitz DM RBE fluorescence microscope fitted with a 50 Watt HBO mercury vapour lamp, BP 355-425 excitation filter, RKP 455 dichromatic mirror and LP 460 suppression filter (Leica, Heerbrugg, Switzerland). Six randomly selected fields of view (greater than 200 pollen grains in total) were scored for each slide. Brightly fluorescing grains were counted as positive for germination capacity. The results were reported as the percentage of FCR positive grains out of the total number counted.

Nectar Production

10 receptive umbels from each line were sampled on each survey day for measurement of nectar production. 24 hours prior to collection the umbels were covered with a calico bag tied around the stem of the plant to exclude nectar feeding insects. At the time of sampling, 15 receptive flowers were excised from each umbel and packed in 15ml centrifuge tubes lined with a filter tube. Care was taken to ensure that all flowers faced the bottom of the tubes to facilitate nectar extraction. The filter tubes were centrifuged at 12,000g for 10 minutes (time and speed of centrifuging had previously been determined to ensure maximum nectar extraction without contamination of samples with sap). Nectar was collected from the centrifuge tubes and transferred into micro-capillaries for determination of volume. Nectar samples were frozen at -80^{o}C and retained for analysis of composition.

Pollination Rates

Pollination rates were assessed by measuring pollen deposition on the stigmas of male sterile plants. On two occasions during peak bloom 200 flowers with post receptive stigmas were collected from each male sterile line within the surveys and their stigmas excised. The stigmas were mounted in a drop of melted basic fuschin gel (Kearns and Inouye, 1993). The prepared slides were examined under a light microscope at 100x magnification. Individual pollen grains present on the stigmatic surface stained red against a background of unstained stigmatic tissue and could be readily counted.

Alternative Forage Sources

Pollen traps were deployed on bee hives at two sites near Griffith that were poorly pollinated and at sites at Hamilton and Richmond to determine whether alternative forage sources were influencing honeybee behaviour. Pollen balls collected from the traps were separated into different groups according to their dominant pollen type. Individual species of pollen were identified by examination under light and environmental scanning electron microscopes and comparison with descriptions and images provided in the Australasian Pollen and Spore Atlas (Australian National University, Canberra). Pollen identifications were confirmed by cross checking of pollen types against samples collected from candidate plant species. Satellite

image (Google Earth) and ground based surveys of potential alternative forage sources were undertaken over an area of 2km radius around each survey site.

Additional Survey Measurements

In addition to the measurements outlined: plant and umbel densities were recorded at the start of the surveys at each site so that results could be standardised on a per plant or per umbel basis; rates of flower abortion (Brown, 2008) were recorded twice during the surveys; notes were taken on general crop health and; climate data was collected using portable weather stations deployed throughout the survey areas.

Supplemental Hand Pollination Experiments

The effects of supplemental hand pollination were measured in 2 representative hybrid seed crops to gauge whether natural cross pollination rates were adequate. 20 male sterile plants were selected and divided into pairs based on proximity and timing of flowering. A single umbel from 1 plant in each pair was hand pollinated with a brush, using pollen sourced from nearby pollinator plants. The second plant in each pair was left as a control, receiving natural pollination only.

Seed Yield Determinations

Seed yields were determined from replicated samples of 20 representative plants of each male sterile line included in the survey and for plants from the supplemental hand pollination experiments. The umbels were collected at the time of commercial harvest, dried and threshed by hand. Air-screen cleaning was performed using a laboratory sized clipper-cleaner (Blount Agri- Industrial, USA) and air column (SeedBuro, USA).

Results and Discussion

Climatic Conditions During The Surveys

The 2009 and 2010 onion seed growing seasons occurred under markedly different climatic conditions (Figures 5.1 and 5.2). In 2009, drought conditions prevailed in the Griffith area with consistently high daytime temperatures during flowering. The Tasmanian summer was also comparatively warm and dry. Summer conditions in Tasmania is 2010 were characterised by relatively frequent rainfall and cooler than average daytime temperatures during the bloom period for onions.

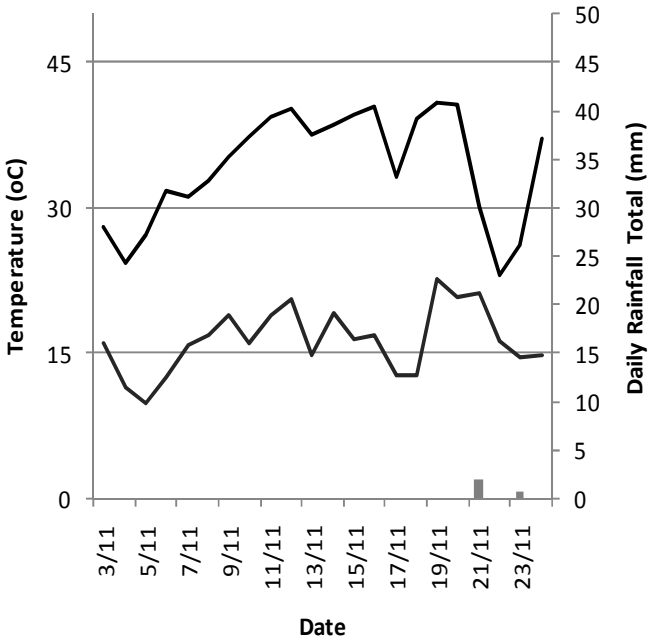
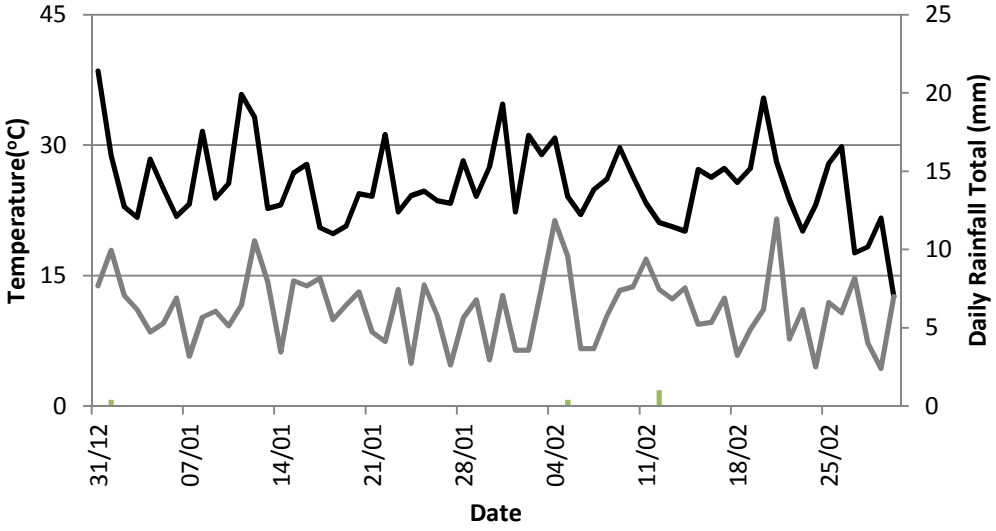


Figure 5.1 - Daily minimum and maximum temperatures and rainfall totals at Griffith in NSW in 2009.

a)



b)

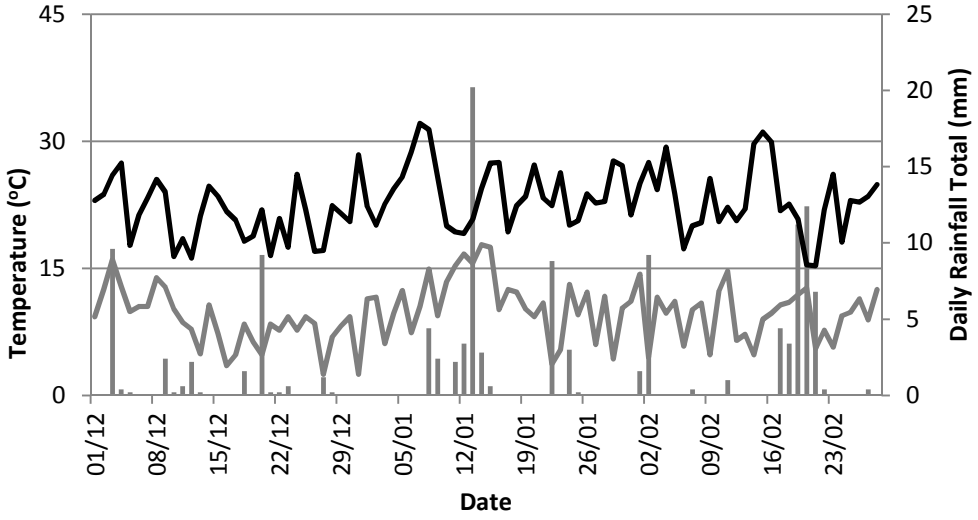


Figure 5.2 - Daily minimum and maximum temperatures and rainfall totals at a) Richmond in 2009/10 and b) Hamilton in 2010/11 during the surveys.

Seed Yields from Survey Crops

In 2009 seed yield data was collected only for crops in the MIA survey as a late outbreak of *Botrytis* at the Tasmanian site adversely affected the trial. Across the MIA and Hamilton survey sites, seed yields were variable, ranging from 0.74 to 4.38g / umbel (Figure 5.3). Accounting for varietal differences, yields ranged from approximately 10 to 100% of industry benchmarks for the varieties in question. This variability is typical of hybrid onion seed

production both in Australia and overseas (Delaplane and Mayer, 2000; Pathak, 2000). A number of potential causes of this variability are discussed below.

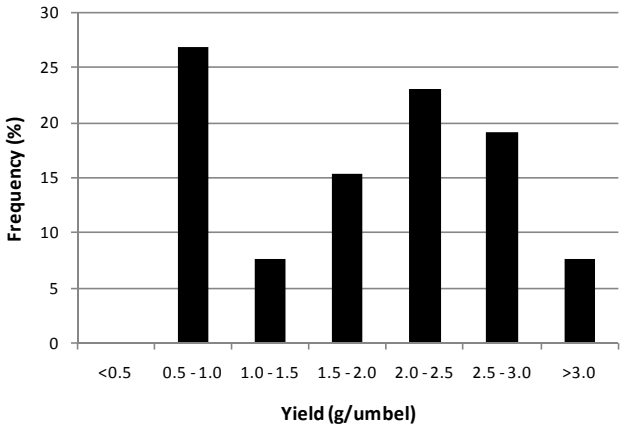


Figure 5.3 - Frequency distribution of seed yields (grams/umbel) from male sterile lines surveyed in Griffith, New South Wales and Hamilton, Tasmania.

Flower Abortion

Pre-anthesis flower abortion in male sterile parent lines has previously been identified as a significant cause of yield variability (Brown, 2008). In this survey we investigated the extent to which flower abortion occurred in other lines. In general, flower abortion rates were low in both the MIA and Tasmania. Out of 20 different male sterile seed parent lines studied, only 3 lines had abortion rates exceeding 10% and of these, only one was severely affected (44% abortion). Two of the affected lines were ones identified by Brown (2008). Although flower abortion is an important yield limitation in certain male sterile lines, these results suggest that it is not a major contributor to widespread yield variability.

Pollen Viability

In order for pollination to be effective the pollen that is used must be viable. Pollen samples collected from the pollinator lines at all survey sites ranged in viability from 42 to 68% at anthesis. Despite the quite different environmental conditions, similar ranges of pollen viability were observed in the MIA and in Tasmania (Figures 5.4 and 5.5). Changes in pollen viability of line O were examined throughout a full day in the MIA on a day when the maximum temperature reached 42^{o}C. Under these conditions pollen viability declined from around 60% at anthesis to around 30% 12 hours later (Figure 5.6). Although viability values appear relatively low, and certainly increase the amount of pollen that has to be delivered to the male sterile line to maximise fertilisation, they are typical of values previously observed in onion seed crops that produced industry benchmark yields (Spurr, unpublished) and

reported in studies with hybrid seed parent lines of onion and other vegetables (Spurr, 2003; Brown and Spurr, 2006; Chang and Struckmeyer, 1976). Therefore, whilst improvements in onion pollen viability would presumably improve yields, pollen viability was unlikey to be the main contributor to yield variability observed in this study.

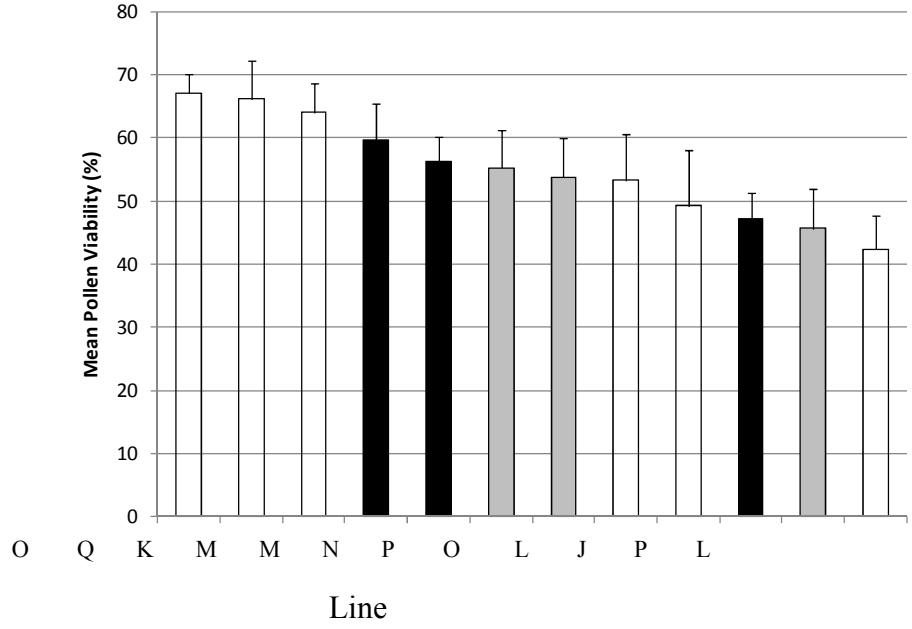


Figure 5.4 – Mean pollen viabilities of 12 pollinator lines surveyed at sites near Griffith in 2009. Values shown are the mean of samples collected on 5 days during peak bloom. Lines that appear twice were scored at two sites. Error bars indicate standard errors (n=5). Flowering time of the lines is indicated by column colour as early (□), medium (▨) and late (■).

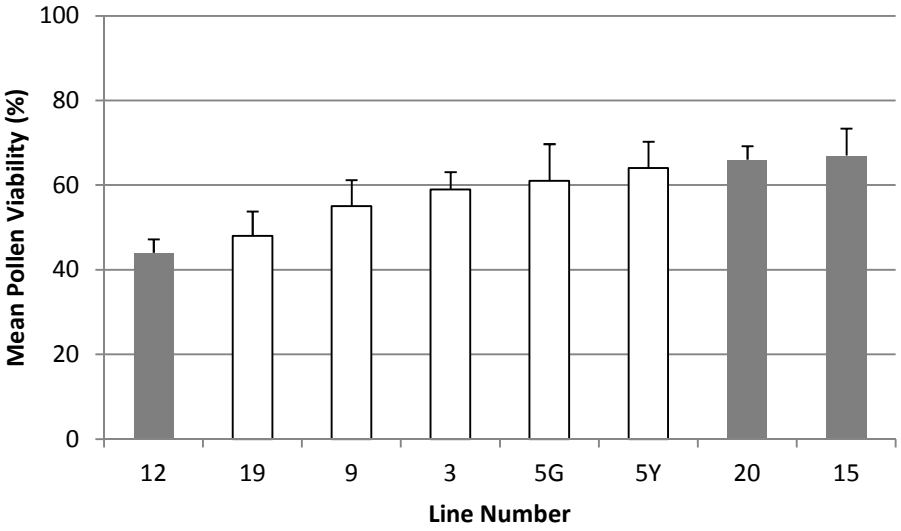


Figure 5.5 –Mean pollen viabilities of 8 onion pollinator lines sampled on 6 days during peak bloom at Richmond, Tasmania. Error bars indicate standard errors (n=5). Flowering time of the lines is indicated by column colour as early (□) and medium (■). Note data for 5G and 5Y are for a common line that segregated into green anther (G) and yellow anther (Y) phenotypes.

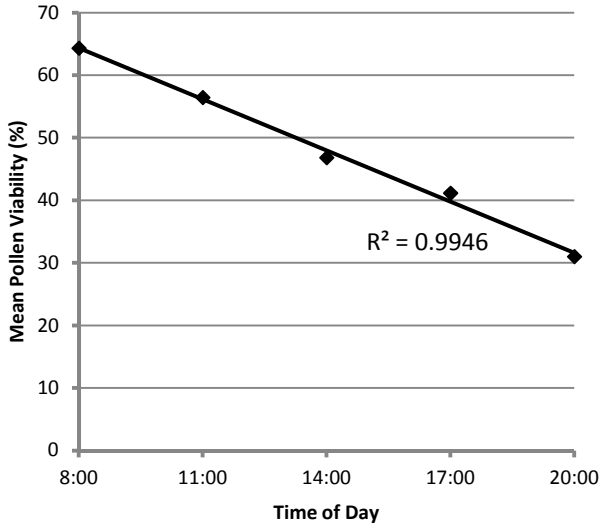


Figure 5.6 – Mean pollen viability values for pollinator line O sampled from 8am to 8pm on the 19th of November, 2010 at a site near Griffith. Data points are the means of 4 replicate samples.

Cross Pollination Rates

A highly significant (P<0.001) correlation between average pollination rates of male sterile lines throughout peak bloom and seed yields was observed at Griffith (Figure 5.7). Pollination rates were variable, ranging from less than 0.2 to greater than 4 viable pollen grains per stigma. Pollination rates of all male sterile lines at the Richmond 2009 trial were very low (<0.2 viable grains per stigma). Pollination rates of the two male sterile lines surveyed at Hamilton in 2010 were 0.5 and 1.3 grains per stigma, which corresponded to seed yields of approximately 0.5 and 1.2g/umbel respectively. Six viable pollen grains are required to realise the full seed setting potential of an onion flower. Although this level of pollination throughout an entire hybrid seed crop is rarely achieved and would probably exceed the capacity of the parent plant to mature seed (Stephenson, 1981), the observed levels of pollination of many male sterile lines are clearly inadequate for optimal seed set. Furthermore, the variability in pollination rates is consistent with the large variability in seed yields throughout the survey.

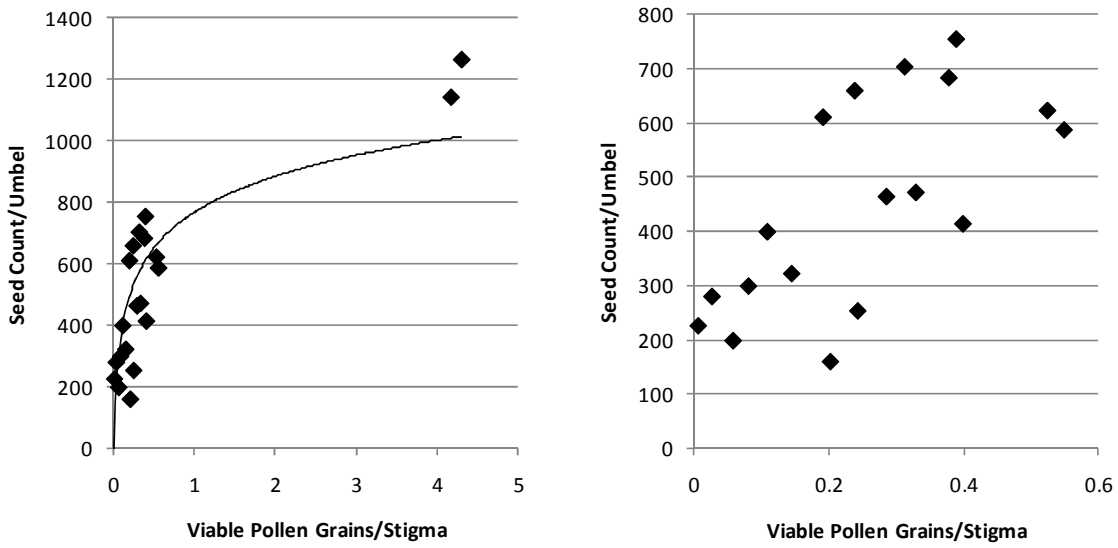


Figure 5.7 – The relationship between average pollination rates of male sterile lines duirng peak bloom and seed yields for hybrid onion crops surveyed near Griffith in 2009. The graph on the right shows a magnified section of the graph to the left for lines with less than 0.6 pollen grains per stigma. Viable pollen grains were estimated for each hybrid cross as the product of pollen deposition rates on the male sterile line and % viability at anthesis of pollen samples collected from the pollinator line. The relationship is described by the equation $y=169\ln(x)+768$ ($P<0.001$; $r^2=0.68$).

Effects of Supplemental Hand Pollination

Hand pollination experiments were performed at two sites in Grififth using hybrid crosses that produced low and moderate commercial seed yields. Although the brush method of hand pollination was not particularly effective, sufficient pollen was delivered to the hand

pollinated umbels to cause significant (P<0.05) increases in seed yield in both lines (Figure 5.8). Seed yields from hand pollinated umbels were 16 to 19% higher than seed yields from insect pollinated umbels. These results provided further evidence that pollination rates at many of Griffith survey sites were inadequate to achieve optimal yields.

Factors Affecting Pollination

Two variables contributed to poor pollination rates. At Griffith, a large number of plants of pollinator line P were late to flower or did not flower, resulting in a shortage of pollen to pollinate corresponding male sterile lines (Figure 5.9). As this pollinator line occurred in 30% of hybrid crosses studied, poor nicking was a significant cause of low yields. In most other hybrid crosses, poor nicking was not a factor.

Where synchronous flowering between hybrid seed parent lines was achieved, visual observations indicated that pollen production (volume) was more than adequate. For these crops, there were significant positive correlations between pollinator (honeybee) visitation rates to onion inflorescences and pollination rates (P<0.001) and seed yields (P<001) (Figures 5.10 and 5.11). Within the Richmond variety trial, pollen availability was not a limiting factor because of the large number of different pollinator lines in close proximity to male sterile lines. Despite this, pollination rates of male sterile lines were negligible. This was presumably because honeybees vary rarely visited the trial despite the location of active hives nearby. At Hamilton, honeybee spot counts of 0.02 and 0.05/umbel, corresponding to pollination rates of 0.5 and 1.3 grains / stigma and seed yields of 0.5 and 1.2 g/umbel respectively were observed.

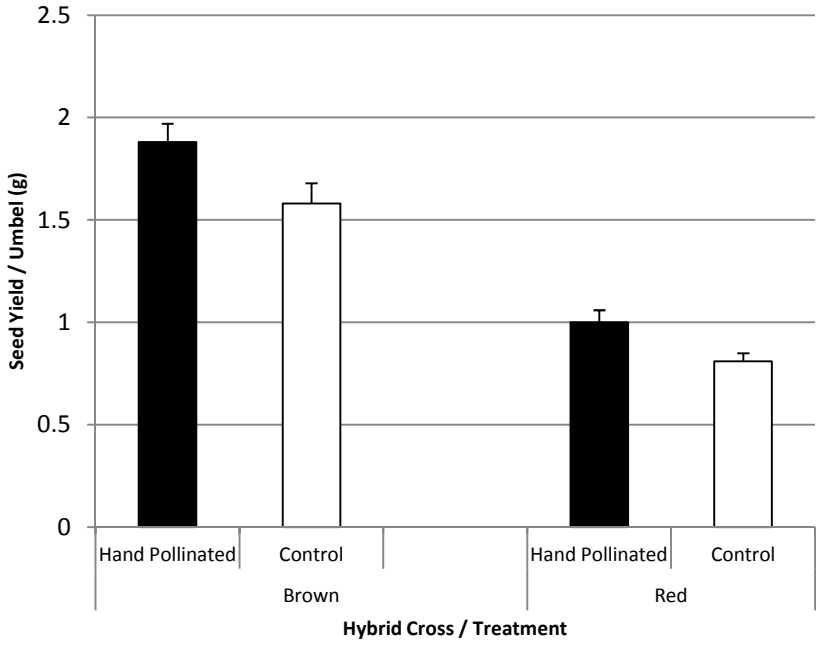


Figure 5.8 – The effect of supplemental hand pollination on seed yields of brown (line D) and red (line H) onion hybrid crosses at Griffith, NSW. Data points are the mean values for 10 plants. Treatment means are significantly different (P<0.05).

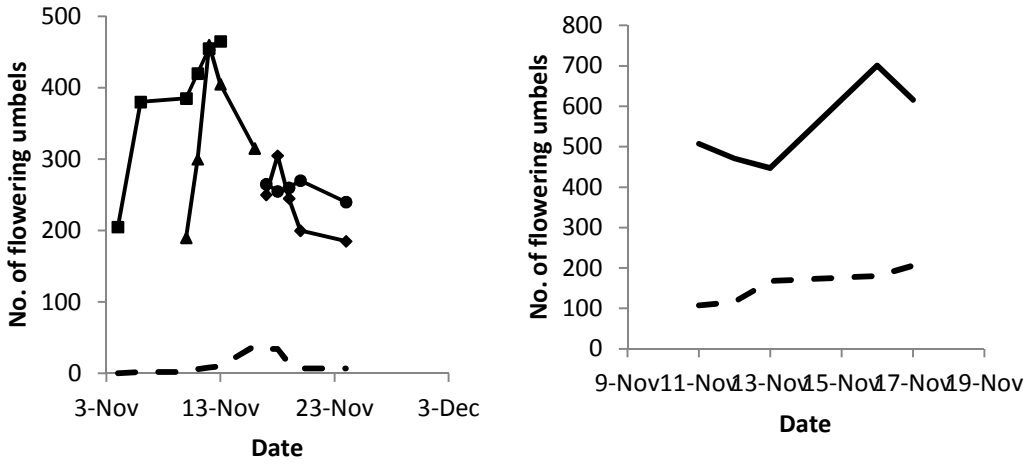


Figure 5.9 - Relative flowering rates of umbels of male sterile (solid lines) and pollinator lines (dashed lines) at sites with pollinator P (left), showing poor nicking with male sterile lines and pollinator O (right), showing synchronous flowering. Both sites had a pollinator to male sterile plant ratio of 1:2. Note the low number of late flowering umbels of pollinator P which resulted in insufficient pollen to pollinate the four male sterile lines at this site (each male sterile line designated by a different shaped symbol).

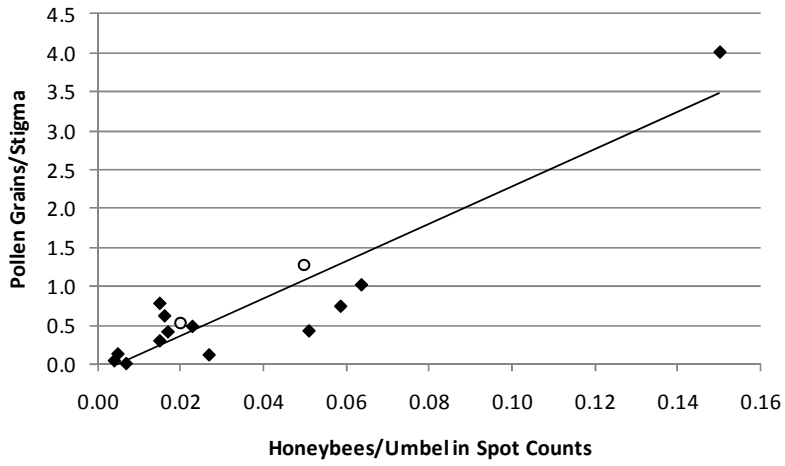


Figure 5.10 – The relationship between honeybee visitation and pollination rates of male sterile lines in hybrid onion seed crops grown at Griffith in 2009. The relationship is described by the equation y=21.03x - 0.12 ($P<0.001$; $r^2=0.79$). Note yield data for lines involving pollinator P has been excluded because poor nicking resulted in a shortage of pollen. Data points for the 2 male sterile lines grown at Hamilton in 2010/11 have been superimposed on the graph (○) but are not included in the correlation.

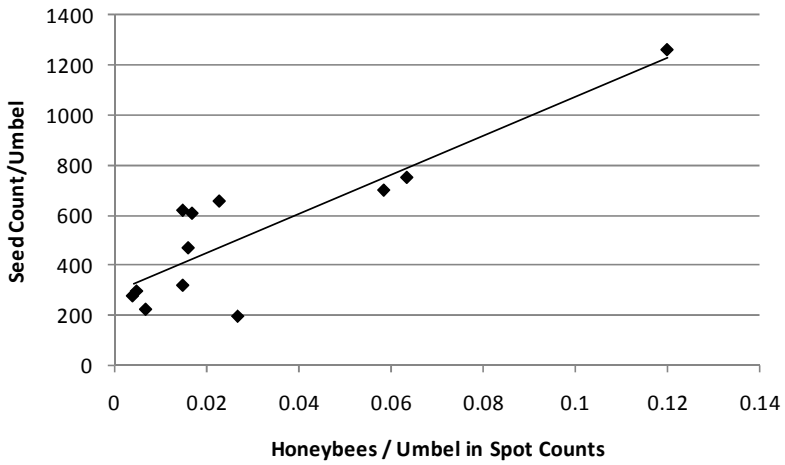


Figure 5.11 – The relationship between honeybee visitation and seed set in hybrid onion seed crops grown at Griffith in 2009. The relationship is described by the equation y=7803.7x +292.1 ($P<0.001$; $r^2= 0.76$). Note yield data for lines involving pollinator P has been excluded because poor nicking resulted in a shortage of pollen.

The dominant pollinator observed on onion inflorescences at sites near Griffith and at Hamilton was the honeybee (*Apis mellifera*), comprising more than 80% of all pollinators at all sites. Generally spot counts of 0 to 0.15 honeybees per umbel on pollinator lines and 0 to 0.12 honeybees per umbel on male sterile lines were observed (Figure 5.12). Much higher activity was observed on pollinator P at Griffith, but this was biased by the low density of flowering umbels during the survey period. Excluding line P, visitation rates observed at Griffith and Hamilton equated to approximately 0.2 to 2 honeybees per metre of row. In previous studies, honeybee spot counts of between 1 and 2.5 bees per metre of row have been documented (Source: Western Australian Department of Agriculture and Food). This indicates that many of the poorly pollinated and, consequently lower yielding, crops in this survey had relatively low honeybee visitation by the standards of other crops in this and previous surveys.

Although honey bee hives were placed near the trial ground at Richmond, few visited the onion trial. Amongst the insects that did visit, most were flies belonging to the genera *Musca* and *Calliphora*. In our experience flies are poor pollinators of hybrid onions under open field conditions, which is consistent with the low rates of pollination observed at Richmond.

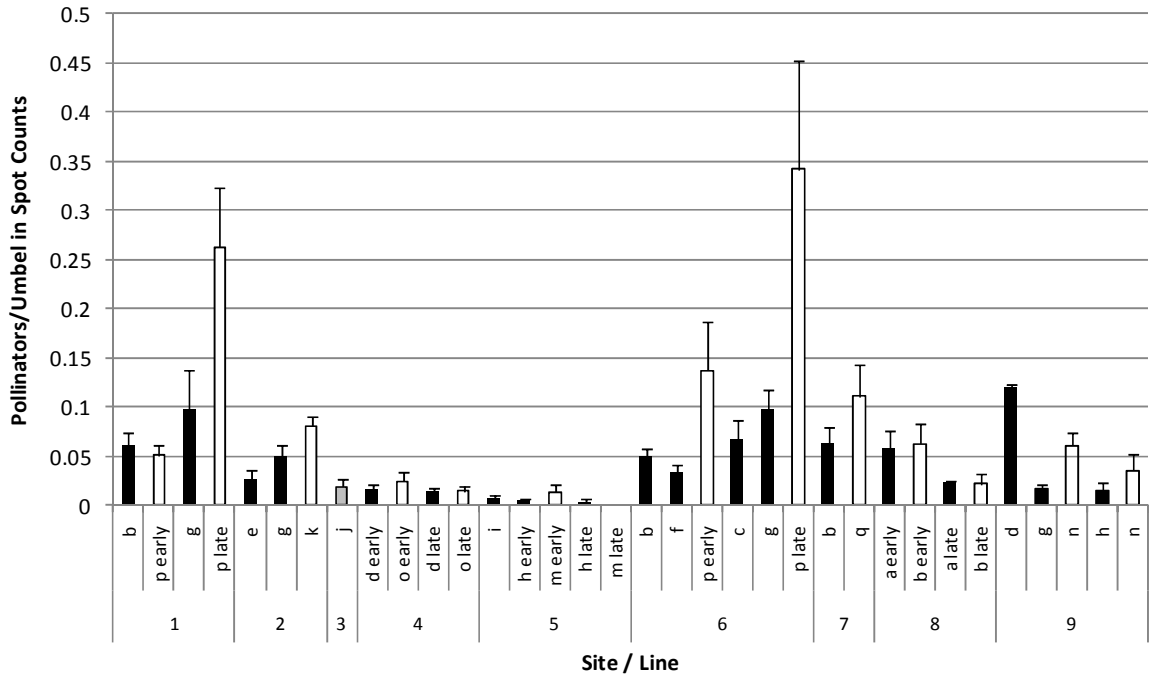


Figure 5.12 – Honeybee spot counts during peak bloom in onion seed parent lines located at 9 sites in Griffith, NSW in 2009. Values are the average of counts made on 5 days. Within the line descriptions early and late indicate seperate plantings of the same line that produced early and late flowering times. Error bars indicate standard errors (n=5).

Factors Affecting Pollinator Activity.

Nectar Production

Within individual sites at Griffith and Hamilton honeybee foraging preferences between lines were correlated with differences in nectar production ($P<0.001$); that is, lines that produced a greater standing crop of nectar had higher levels of visitation (Figures 5.13). At these sites, less attractive lines were not as well pollinated and produced lower seed yields.

Despite this relationship, large site to site variations in pollinator activity could not be explained in terms of nectar production. For example, onion lines at sites C and D at Griffith produced comparable volumes of nectar to lines grown at other sites but had far lower rates of honeybee activity, even though bee hive stocking rates were comparable at all sites (Figure 5.14). In some instances large variations in honeybee visitation to the same line were observed across different sites (for example line g at sites 1, 6 and 9 or line d at sites 4 and 9). Although honeybees were stocked at both Richmond and Hamilton and nectar standing crop volumes were comparable, average honeybee visitation rates at Hamilton were 0.2 to 0.5/m whilst at Richmond very few honeybees visited the trial.

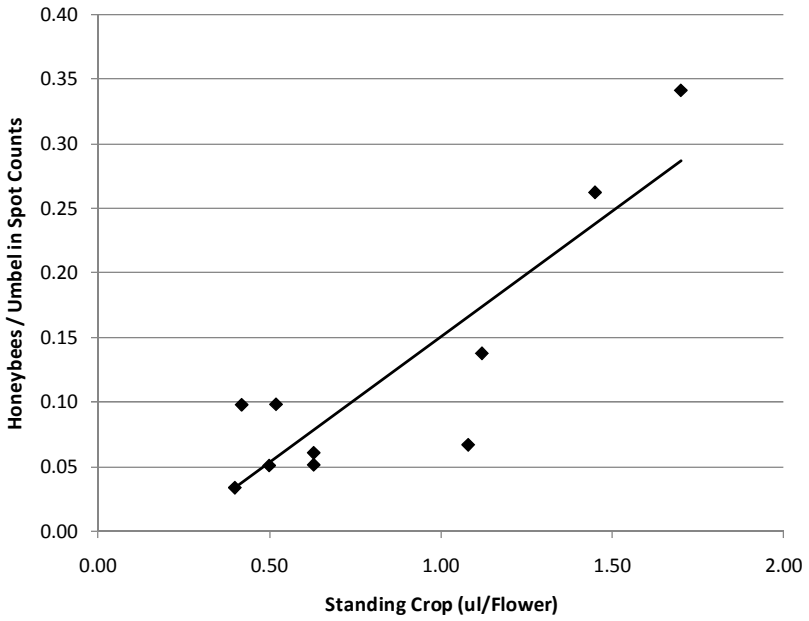


Figure 5.13 – The relationship between nectar volume (standing crop) and honeybee spot counts in hybrid onion seed parent lines grown at one site in Griffith NSW in 2009. The relationship is described by the equation $y = (P<0.01; r^2 = 0.77)$.

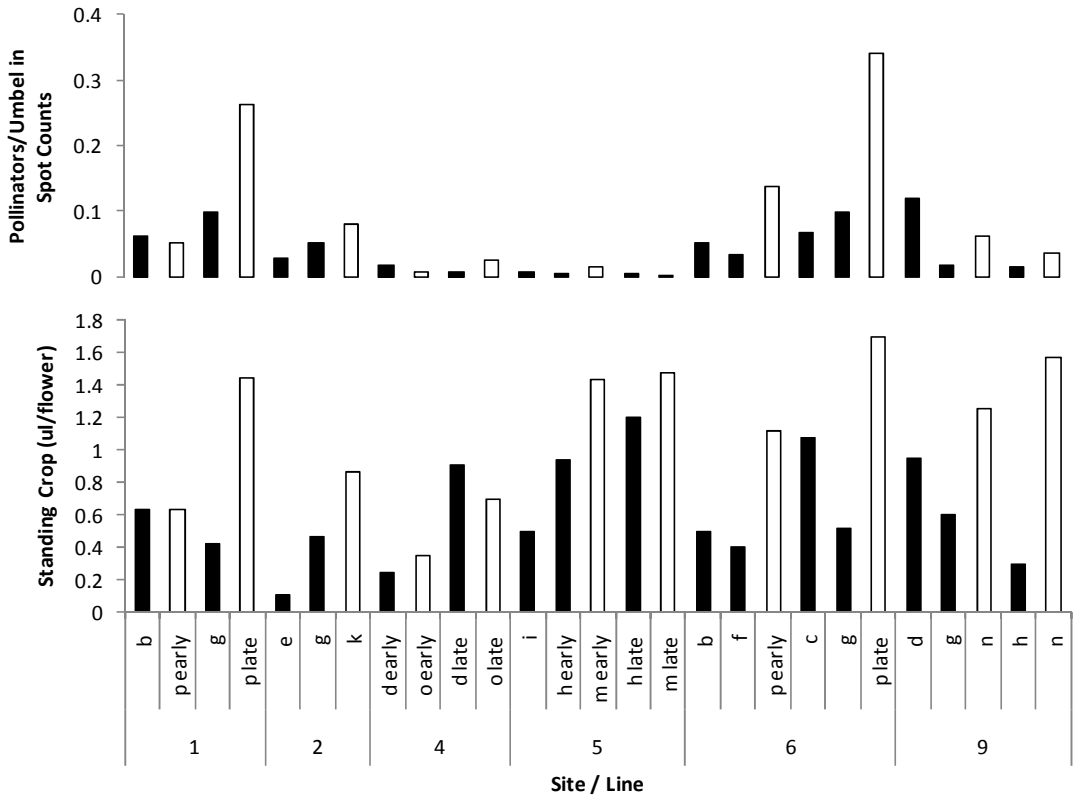


Figure 5.14 – Comparison of nectar standing crop volumes (bottom) and honeybee visitation rates for hybrid seed parent lines grown at 6 sites near Griffith in 2009. Pollinator lines are represented by white columns and male sterile lines by black columns. Within the line descriptions early and late indicate seperate plantings of the same line that produced early and late flowering times.

Alternative Forage Sources

At 2 Griffith sites where honeybee activity within the onion crops was low in 2009 (sites 4 and 5 in Figures 5.12 and 5.14), large yields of *Eucalyptus* pollen were collected from pollen traps mounted to representative bee-hives (Plates 5.1 and 5.2). Examination of satellite images and ground based observations confirmed that these sites were within honeybee foraging distance of remnant bushland and windbreaks containing flowering *E. largiflorens* (site 4) and *E. largiflorens* and *E. camaldulensis* (site 5). Other sites were within foraging range of fewer flowering eucalypts, or in the cases of sites 1, 6 and 7 isolated from eucalypts.

In Tasmania, a diverse range of pollen types were collected from traps mounted on beehives at the Richmond site. The most abundant pollen types included prickly box (*Bursaria spinosa*), fennel (*Foeniculum vulgare*) and several species of *Brassica* (Plate 5.3). All species were found growing wild or in crops within the foraging range of the beehives. In contrast, at Hamilton where honeybee activity within the onion crop was substantially higher, no pollen was collected.

Although pollen traps were deployed at survey sites where honeybees foraged on onions, onion pollen was never collected. This is consistent with observations that onion is not favoured as a source of pollen by bees; in fact those that become dusted with pollen whilst

foraging for nectar are reported to subsequently comb it from their bodies and discard it (Free, 1993).

The results of pollen trapping suggest that a range of species that overlap in flowering time with onion may have a substantial impact on the success of pollination of onion seed crops by drawing honeybees away from the crops.

Plate 5.1 – A pollen trap mounted on the entrance to a beehive.

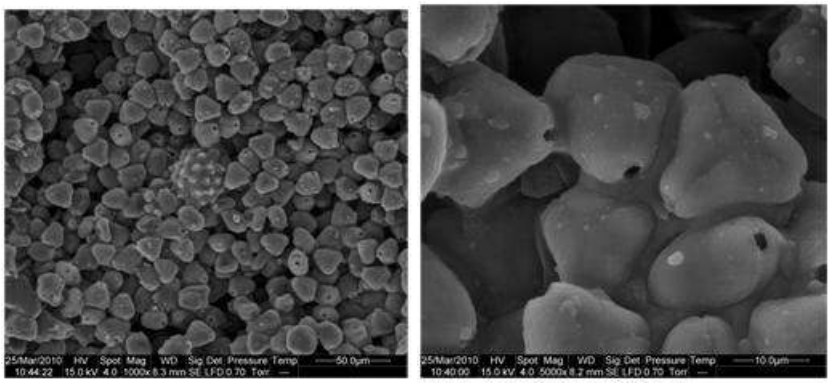


Plate 5.2 – Scanning electron micrographs of pollen collected from a beehive located in an onion seed crop at Griffith in 2009. The dominant species is *Eucalyptus largiflorens.* Diagnostic features of pollen of the *Eucalyptus* genus (shape, size, surface sculpting and number and position of pores) are evident in the enlarged image to the right.

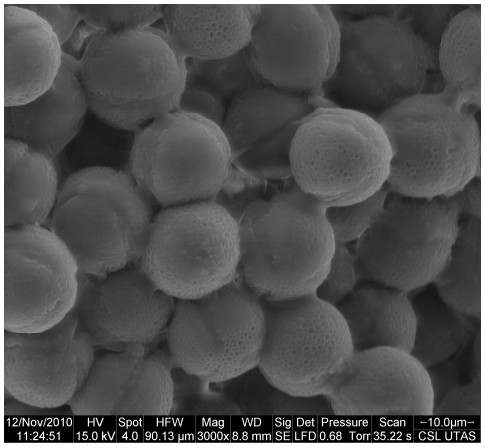


Plate 5.3 Scanning electron micrographs of pollen from B*rassica sp*. collected from pollen traps in the onion seed crop at Richmond in 2009. Diagnostic features of pollen of the *Brassica* genus (shape, size, surface sculpting and number and position of pores) are evident and these grains.

Conclusions

This work was undertaken to identify the basis of unreliable seed yields from hybrid onion seed crops grown in two different production locations in Australia. Survey work was undertaken in the Murrumbidgee Irrigation Area near Griffith in 2009 and in Southern Tasmania in 2009 and 2010. Although the survey locations and seasons varied widely in terms of climatic conditions, a lack of cross pollination was established as the fundamental basis of variable yields throughout the survey. Two key factors contributed to low rates of cross pollination: a) poor nicking of hybrid seed parent lines (isolated to 1 late flowering pollinator line used in a number of hybrid cross combinations in the MIA in 2009) and; b) low rates of honeybee activity within hybrid onion seed crops. Factors that contributed to poor honeybee activity included unfavourable weather conditions (2010) and the occurrence of more attractive, alternative forage sources nearby. In the MIA, *Eucalyptus largiflorens* (Black Box) and *Eucalyptus camaldulensis* were identified as important alternative forage sources affecting honeybee activity in hybrid onion seed crops. It is likely that other weedy or native forage sources may also compete with onion seed crops for pollinators in the MIA but the drought conditions during 2009 restricted their abundance and flowering.

A second finding in this survey was that within individual sites, differences in attractiveness of hybrid onion seed parent lines are correlated with nectar production. Differences in nectar production between lines can lead to discriminatory foraging by honeybees resulting in poor rates of cross pollination of male sterile lines that produce small amounts of nectar. In such circumstances, higher honey bee stocking rates, management strategies aimed at maximising nectar production in low yielding varieties or, where possible, matching of lines on the basis of attractiveness to honeybees may improve pollination.

In 2010/11, South Pacific Seeds initiated an additional research project to build on the work undertaken in this study by developing management strategies to minimise the effects of competing forage sources and increase honeybee activity in hybrid onion seed crops. This project will also involve analysis of nectar samples collected from sites in the MIA in the course of this survey work.

CHAPTER 6

Recommendations

Recent improvements in the standards of hybrid carrot seed production in Australia and increasing demand for onion seed production globally have created significant opportunities for expansion of both crops in Australia. One of the major difficulties to realising these opportunities and sustaining future growth is to be able to produce reliable, economical seed yields. Yields from onion and carrot seed crops grown in Australia and elsewhere vary widely within and between seasons. This project was initiated to address some of the key issues affecting reliability of hybrid carrot and onion seed production in Australia. A number of recommendations have arisen from the project. These are summarised below:

Factors Affecting Attraction of Pollinators to Hybrid Carrot Seed Parent Lines

Although seed production traits are necessarily a secondary consideration in the breeding objective for vegetable crops they are important for deployment of germplasm. This study has highlighted potentially important differences in attractiveness of different CMS systems and identified traits that influence pollinator visitation to individual lines. This work is the first published study to accurately quantify differences in nectar production (standing crop volume) between hybrid carrot seed parent lines and relate these to honeybee foraging preferences. Although hybrid carrot seed parent lines differ markedly in flower morphology, colour and aroma (Erickson and Peterson, 1979), the results of this work demonstrate the over-riding importance of nectar production to honeybee visitation independent of other traits. The results of this work suggest that breeders should focus on nectar production to maximise attractiveness of carrot seed parent lines to honeybees. This information can also be applied in commercial production through adoption of cultural practices to maximise nectar production.

Management of Flowering Time in Carrot

Carrot seed producers have historically used trimming treatments to correct nicking problems in hybrid seed crops or to reduce crop height so as to prevent lodging. Such treatments are generally applied at the start of flowering but there is uncertainty about the best time to trim to minimise effects on yield potential and the extent of delay in flowering that can be achieved. To the best of our knowledge, trimming treatments have not been used previously applied to carrot seed crops to move flowering time to a more favourable period for pollination.

The results obtained in this study confirm that trimming between bolting and flowering delays flowering in carrot seed crops by 10 to 14 days. In Tasmania, historical climate records indicate that, on average, this would shift peak bloom into a period of more favourable conditions for pollination. In this work trimming treatments that resulted in plants flowering under more favourable conditions did improve seed yields, but the significant risks of reduced yields when conditions were no better or in fact worse for pollination in the later flowering window were also clearly demonstrated. The potential magnitude of negative effects is increased by the fact that all trimming treatments resulted in reduced inflorescence size. In this regard, trimming treatments that were applied at a later stage of crop

development or that removed a greater proportion of the developing inflorescence carried a larger risk of adverse yield effects. For these reasons any decision to trim crops for the purpose of shifting flowering to a period of more favourable environmental conditions should be carefully considered.

The results of this work suggest that the current practice of trimming crop near the onset of flowering to correct nicking problems can be improved upon. Trimming appears to be best done soon after bolting when the flowering stems of the advanced line have extended to no more than 300mm above ground level, and should not reduce the canopy to below 150mm above ground level. Ideally the treatment would coincide with the onset of stem extension in the late line. In some cases, more severe (later) treatments may be required to correct extreme differences in nicking but the benefits would have to be carefully weighed against loss of yield potential from the trimmed line.

Development of a World Class Hybrid Carrot Seed Industry in Tasmania

The success of demonstration crops and international interest in Tasmania as a centre for hybrid carrot seed crops documented in this report highlights the potential for the development of a significant hybrid carrot seed industry in Tasmania. Sustainable expansion of the industry depends on maintenance of the high production standards underpinned by research. The research extension model used in this work was effective and should be considered for similar activities in the future.

Understanding the Basis of Unreliable Hybrid Onion Seed Yields

A common theme of seed yields limited by inadequate pollination emerged from the surveys conducted in this project. In many instances pollination rates were affected by competition for honeybees from alternative forage sources. Although the MIA surveys established that *Eucalyptus largiflorens* and *E. camaldulensis* were a significant source of competition, other potential sources of competition were restricted by the drought conditions that prevailed in 2009. Future research should focus on understanding the range of alternative forage sources and developing production protocols to mitigate their effects. At the time of writing, a follow up project that aims to address these issues is underway in the MIA.

Research Activity

This project represents a continuation of agricultural and forestry seed research at TIAR which is responsive to industry identified problems and undertakes industry specific projects. During the course of this project a number of leading national and international seed companies have engaged in collaborative research projects with TIAR, continuing the diverse range of opportunities for seed research and strengthening the ties between the Australian and International vegetable seed industries. This activity has seen a continuation of postgraduate, honours, undergraduate and exchange students participating in seed research, leading to an accumulation of expertise and interest in this field. It is recommended that this activity should be maintained and supported into the future.

CHAPTER 7

Technology Transfer

This project has delivered a number of research and extension outcomes for the Australian vegetable seed industry. The industry partners have been involved at all stages of the project and kept informed of research outcomes as they have arisen. In several instances, project outcomes have already been, or are being incorporated into commercial production and research. For example:

- Extension activities aimed at ensuring that Tasmanian hybrid carrot seed crops are produced using best cultural practice derived from research targeted at addressing key issues for cool temperate carrot seed production in Australia have contributed to the growth of this industry and its emerging international reputation for high quality production. The issues that have been addressed include crop establishment (time of sowing and irrigation practice), management of crops and honeybee populations for optimum pollination, management of Rutherglen Bug and determining optimum timing of windrowing. By adopting the practices promoted in this project to address these issues, Tasmanian growers have achieved excellent yields and seed quality compared against international benchmarks set by breeding companies in 2 consecutive seasons (2009-10 and 2010-11). Prior to this work, most carrot seed crops grown in Tasmania failed to meet these standards. The positive results from this project have increased interest from leading carrot breeders in contacting carrot seed production in Tasmania and from farmers in growing these crops. As a direct result, the industry in Northern Tasmania has expanded from 10Ha of trial crops with 5 growers and 3 customers in 2009 to 160Ha of production involving 20 growers and 6 customers in 2011.

- Findings on factors affecting hybrid onion seed yields are being used in the MIA to target research and crop husbandry practices towards: a) understanding and managing competing forage sources (site selection, matching of crops to sites) and managing honeybee colonies and stocking rates to maximise pollinator activity. South Pacific Seeds have initiated planning for a subsequent research project that will focus on addressing the yield limiting factors identified in this work. At this stage it is too early to see the direct benefits of this work to onion seed producers as the issues identified in this work must now be addressed.

- Information on traits that influence attractiveness of carrot seed parent lines to honeybees are being adopted into the research, development and breeding activities of Rijk Zwaan.

Additional evidence that the research outputs are valued by industry lies in the commitment of Australian and International industry partners to continued investment in seed research activity at the School of Agricultural Science / TIAR.

Other technology transfer activities in this project include:

Conferences / Research Days

Geard, A. M., Spurr, C. J., Gracie, A. J. and Jones, A. D. (2011) Development of a world class hybrid carrot seed industry in Tasmania – applied research and extension in action. Accepted for presentation at the ASHS Lorne, Victoria, September, 2011.

Geard, A. M. and Spurr, C.J. (2010) Presentation at Northern Tasmanian carrot seed growers field day. South Pacific Seeds, Longford.

Spurr, C. J. and Geard, A. M. (2011) Presentation at Northern Tasmanian carrot seed growers field day. South Pacific Seeds, Longford.

Spurr, C. J. and Geard, A. M. Invited presentation to the South Pacific Seeds onion growers, Griffith, NSW. March, 2010.

Spurr, C. J., Geard, A. M. and Gracie A. J. (2010) Invited presentation to the South Pacific Seeds Production Conference, Myrtleford, Victoria.

Visiting Students / Exchanges

Through her involvement in the project Ms Leah Hannah, Research Manager for Rijk Zwaan Australia enrolled in an honours research project investigating factors affecting attractiveness of carrot seed parent lines to pollinator insects

3 undergraduate students, Julian Gutter, Martijn Doornbusch and Casper Roxburgh from the Netherlands were sponsored by Rijk Zwaan to visit Australia and participated in research training within this project to satisfy the research training requirements of their degrees. One of these students is currently planning to return to Australia to undertake a PhD at the University of Tasmania, School of Agricultural Science.

Other Output

Annual progress reports were prepared and distributed to the industry partners.

An information sheet on Rutherglen bug identification and management in carrot seed crops was prepared and distributed to Tasmanian carrot seed growers and field agronomists.

The project has involved a large amount of formal and informal communication between the researchers and industry partners. This has included:

- Regular visits to all 3 industry partners in Australia by the researchers;
- Discussions with growers;
- Visitation of industry partners (including Rijk Zwaan Netherlands research managers) to TIAR and seedPurity;
- Demonstration of techniques (nectar extraction, carrot embryo testing and pollen viability testing) to industry production and research personnel;

- Visits from international carrot seed customers from France and the Netherlands to inspect the Tasmanian carrot seed demonstration trials and meet researchers at TIAR and seedPurity.

This project has provided a platform for the development of other seed research activities within the School of Agricultural Science at the University of Tasmania. There are 5 current and recently completed candidatures in honours (3) and PhD (2) research in seed science at the School of Agricultural Science, University of Tasmania across a range of industry and government funded projects. This activity has lead to at least 10 journal, conference and industry publications and presentations in the last 2 years.

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