

**Identification and Management of Factors Limiting  
Hybrid Carrot Seed Production in Australia**

by

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## Summary

Worldwide, carrot seed production is a highly dynamic industry, with vegetable breeders contracting the production of proprietary varieties to areas where technology and climate combine to give the most reliable yields of high quality seed. Australia has had a significant share of the world market for carrot seed production since 1984, servicing customers in Asia, Europe and the United States. Since the mid 1990s, requirements for increased quality and reliability of production, coupled with improvements in the production standards of other carrot seed exporters, have threatened Australia's market share. The failure of a significant number of Australian crops to meet the current minimum production standard of 85% seed germination required by export markets has been the major issue faced by the industry during this period. In addition, producers have experienced difficulty in consistently achieving satisfactory yields of seed of some hybrid varieties.

The present study was undertaken to address both of the issues facing Australian producers, with an emphasis on achieving improvements in the production of seed of European hybrid carrot varieties. Preliminary work established that the problems of low germination and unreliable yields were largely unrelated, leading to two research themes based on cause and management of low germination and unreliable seed yield.

Low germination of Australian seed lots was closely correlated to the occurrence of seeds without embryos or with embryos exhibiting extensive physical damage. Surveys of carrot fields and caging trials in South Australia and Tasmania established that both conditions resulted from feeding of the endemic insect, *Nysius vinitor* Bergroth (Hemiptera: Lygaeidae) on developing carrot seeds. Adult *N. vinitor* were found to infest carrot seed crops in a series of migratory flights from shortly before flowering until harvest. In Tasmania evidence was collected that suggested that a significant proportion of migrants originate from nearby areas of weedy host species. During peak periods of migration populations of up to 17 insects per carrot plant were observed. Field based

caging trials showed that loss of germination due to *N. vinitor* feeding could occur from flowering through to harvest. For individual male sterile plants, daily reductions in seed germination of 0.04 to 0.11% per insect were observed during this period.

Because of the need to respond quickly to *N. vinitor* infestation to minimise germination loss, work was undertaken to develop protocols for monitoring the insect in carrot seed crops. In trial plots, sticky traps detected migrations of *N. vinitor* into carrot fields, with the number of insects caught closely correlated to average population densities on the plants. In commercial crops *N. vinitor* population densities were non-randomly distributed across the fields, with gradients associated with the prevailing wind directions during the periods of migration. Thus, the positioning of the sticky traps was shown to be important for reliable detection of *N. vinitor* as well as accurate estimation of population size.

The issue of unreliable seed yield was examined in two hybrid crosses, No. 22, a 3-way (F1 male sterile line) Nantes hybrid with brown anther cytoplasmic male sterility (CMS) and WO030, an F1 Nantes hybrid with petaloid CMS. Two alternative hypotheses; source limitation (assimilate) and pollination limitation, were tested to explain the occurrence of low seed yields. Shading, umbel removal and leaf trimming treatments applied to both hybrids over two seasons did not significantly affect seed yield, seed size or seed quality. Supplemental hand pollination treatments increased seed yield by up to 284%, providing evidence that seed yield was strongly limited by inadequate pollination in No. 22, and to a lesser extent in WO030. In both hybrids, pollination was restricted by variable pollen viability at anthesis, low pollen longevity under field conditions and low rates of pollen transfer to the male sterile line.

Commercial hybrid carrot seed production is based on the strip method of hybrid seed production in which rows of the pollinator line are alternated with the male sterile line. Within the strip method, varying the ratio of pollinator and male sterile lines between 1:4 and 4:4 and distance between adjacent male beds between 2.4 to 7.2m had no effect on pollination or seed yields of No. 22. Surveys of insect pollinator visitation, pollen loads



and foraging patterns explained these results. Honeybees, *Apis mellifera ligustica* Linnaeus (Hymenoptera: Apidae) and two Dipteran species *Calliphora ruficornis* Macquart (Diptera: Calliphoridae) and *Eristalis tenax* Linnaeus (Diptera: Syrphidae) effected most pollination in carrot seed plots. All three species showed a strong tendency for directional foraging within a single row of plants, thereby restricting the opportunities for pollination of the male sterile line. These findings suggest that the strip method of production may be incompatible with the foraging behaviour of some important pollinators of hybrid carrots and hence the attainment of optimum seed yields in some varieties.

This thesis identifies significant opportunities for improvement of the quality and reliability of hybrid carrot seed production in Australia. Management protocols for *N. vinitor* derived from the research have contributed to an increase in the percentage of commercial carrot seed crops achieving the germination standard required for export from below 70% prior to 2000 to 100% in 2003. Studies of yield variability identify inadequate pollination as a contributing factor. Efforts to improve pollen viability and pollen transfer in commercial production may contribute to greater reliability of hybrid seed yields.

## **Acknowledgements**

I would like to sincerely thank my supervisors, Drs. Neville Mendham and Philip Brown for their support and guidance throughout this project and enthusiasm to help at all times.

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In the early stages of this project I was able to undertake a study tour of vegetable seed production in the Pacific North West of the United States and attend the 28<sup>th</sup> International Carrot Conference in Washington State through the financial support of Horticulture Australia and SPS. I am particularly indebted to Phil Hancock, Managing Director of SPS, who organised many of the contacts and provided the introductions that paved my way on this excellent learning experience.

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# Chapter 1

## General Introduction

*This chapter is written in three sections. The first provides a brief historical background to the Australian carrot seed industry within the context of the worldwide industry. The status of the Australian industry during the late 1990s, at the time of commencement of this project, and the development of the problems that were its genesis are described. The second section explains the concepts of reproductive development and seed production that are fundamental to the research that was undertaken. The final section of the chapter describes the focus and broad objectives of the research and outlines the structuring of this thesis.*

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### **1.1 - The Carrot Seed Industry**

#### **Worldwide Carrot Seed Production**

Worldwide, in excess of 3000 ha of carrot seed crops are produced annually (Schreiber and Ritchie, 1995; Simon, 2000). A large proportion of this is contracted or 'in house' production of proprietary varieties for vegetable breeding companies. The United States (Washington, Oregon, Northern California and Idaho) and southern France currently dominate the seed production industry. Other significant producers include Australia, New Zealand, Italy, Israel, Japan and Chile. Based on the figures provided by Schreiber and Ritchie (1995) and Simon (2000) hybrid seed accounts for approximately 60% of carrot seed production.

## **The Australian Carrot Seed Industry**

### ***Background***<sup>1</sup>

Commercial carrot seed production has occurred in Australia since the 1940s. In the initial years, production focussed on domestic seed of open pollinated varieties in the areas around Swan Hill in northern Victoria (Latitude 35.3° south) and Griffith in southern New South Wales (Latitude 34.2° south). In this era, the minimum germination standard for carrot seed for domestic sale within Australia was 60%, and little difficulty was experienced in achieving this. In response to market demands for higher germinating seed during the 1970s, carrot seed production commenced in south-eastern South Australia. The rationale for this was that a cooler production climate might improve seed germination. Production in South Australia proved successful; carrot seed germination results between 75 and 80% were regularly achieved and the industry developed in the area surrounding Mount Gambier (Latitude 37.8° south).

Export of Australian carrot seed commenced in 1984, with contracts to supply seed companies in the United States, who were experiencing difficulty with the reliability of domestic production. The success of early crops for the United States formed the basis of a developing industry, which grew to include markets in both Europe and Asia during the next 12 years. Australian carrot seed production peaked during this era, but no reliable statistics on the industry size appear to have been kept.

The market share of the Australian export industry first came under serious threat during the mid 1990s. At this time, there was a significant improvement in the standard of carrot seed production in the United States and, worldwide, export markets began to demand higher quality carrot seed, typically with 85% minimum germination. Although there were periodic successes, Australian producers generally failed to

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<sup>1</sup> Background information on the Australian carrot seed industry provided by Mr Max Dalrymple, Production Manager, South Pacific Seeds, South Australia.

achieve this standard of germination on a consistent basis. The difficulties experienced by Australian producers at the time were enhanced by a worldwide shift towards hybrid carrot seed varieties, which were harder to produce and often gave unreliable seed yields in addition to problems with seed germination. During the period that Australian producers have experienced difficulties, a carrot seed industry based on the Canterbury Plains region of New Zealand (Latitude 43.8° south) has been successful in producing export carrot seed of a high germination standard. In order to maintain market share, a number of stakeholders in the Australian industry have spread their operations between Australia and New Zealand, with a resultant loss of production from the Australian industry.

#### ***Status of the Australian Carrot Seed Industry in 1999***

Despite declining exports to the United States and Europe and difficulties in maintaining Asian markets, carrot seed was still a major crop for Australian vegetable seed growers in 1999. In excess of 800 ha of carrot seed crops were grown in Australia in 1999, with the seed having a market value of approximately \$AU 5.4 million (Jones, 1999). A large proportion of this production was for export markets. Mount Gambier and the surrounding areas of south-east South Australia remained the most significant region for carrot seed production in Australia, but crops were also produced near Griffith in New South Wales and, to a lesser extent in southern Tasmania (Latitude 42° South).

## **1.2 - Reproductive Biology of the Carrot**

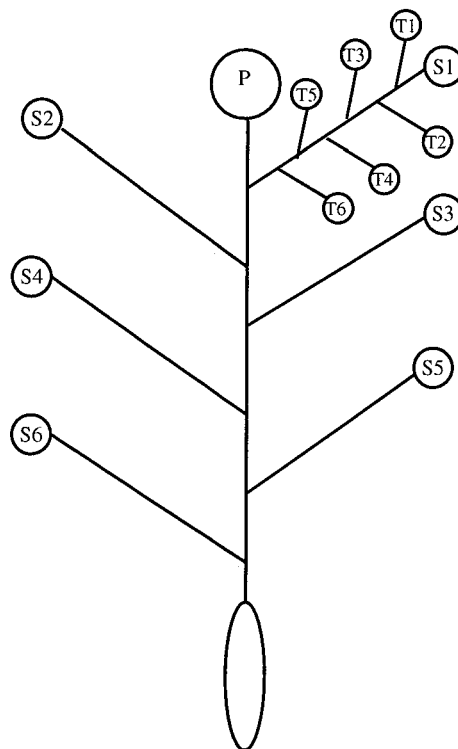
### **Initiation of Flowering – Juvenility, Vernalisation and Photoperiodic Requirements**

Carrot varieties originating in Asia tend to be annuals when grown under long days and do not have a vernalisation requirement for flowering. Varieties that have been developed in temperate areas of Europe and North America are biennials. 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). The specific conditions required for each phase of development are variety dependent. For temperate carrots, juvenility typically ends after the plant has initiated 8–12 leaves (Atherton *et al.*, 1990) and vernalisation requires 2 to 10 weeks exposure to low temperatures between 0 and 10°C (Dickson and Peterson, 1958). Development to flowering is more rapid with increasing thermal times of vernalisation. Short photoperiods (12hr) or darkness during vernalisation result in an earlier and a more promotive effect on flowering than long days (16hr), suggesting that temperate carrots are short–long day plants (Atherton and Basher, 1984). Plants which have been induced to flower, but are not yet undergoing stem elongation, can be de-vernalised by several days of high temperatures (28–35°C) (Hiller and Kelly, 1985).

Floral initiation in carrot involves a morphological shift from the relatively flat apical meristem producing leaves, to an uplifted conical meristem capable of producing stem elongation and an inflorescence (Hiller *et al.*, 1979). Depending on the variety, the seed stalk in carrots may elongate to a height of 60–180 cm, forming a highly branched hispid inflorescence having large nodes and hollow internodes.

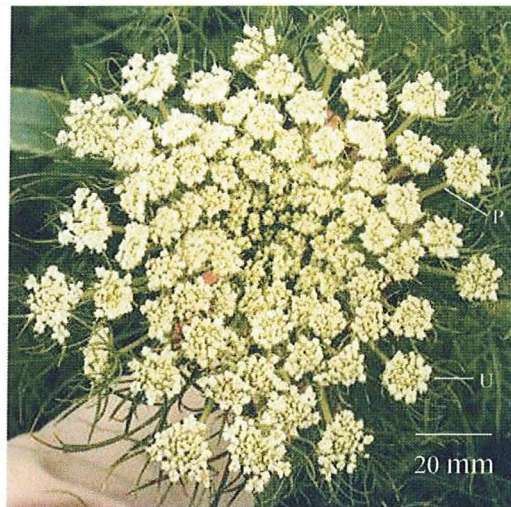
## Flowering and Pollination

The carrot inflorescence is a series of compound umbels borne terminally on the seed-stalk branches. The central main stem terminates in the primary or first order 'king' umbel, which is typically 100 to 150mm in diameter. Secondary lateral branches form in successive order from the top with each terminating in a smaller compound umbel (second order). The system of branching usually continues for up to 4 orders, with successively smaller umbels (Hawthorn *et al.*, 1961) (Figure 1.1). Generally, greater than 90% of the seeds are borne on the first three umbel orders (Hawthorn *et al.*, 1961; Hiller and Kelly, 1985).



**Figure 1.1** – Schematic diagram of a flowering carrot plant showing the general arrangement of umbels; P = Primary order (King umbel), S1 – S6 = 1<sup>st</sup> to 6<sup>th</sup> secondary order umbels, and T1 to T6 = 1<sup>st</sup> to 6<sup>th</sup> tertiary order umbels on the first secondary branch. Fourth and higher order umbels may also occur. Diagram not to scale.

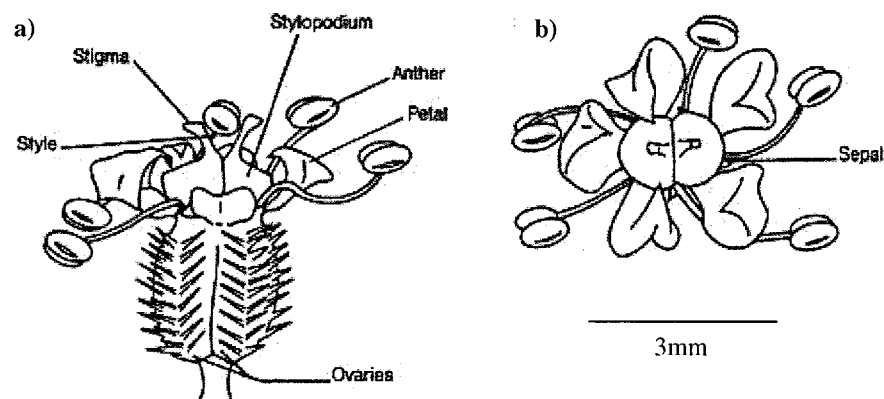
The umbel structure is shown in Figure 1.2. The flowers occur in umbellets where pedicels of each flower radiate from a common point. The umbellets in turn arise on pedicel rays originating from the apex of the inflorescence stalk. The umbellets are more or less arranged within the umbel in a series of concentric circles or whorls. The surface of the umbels is flat, but may become cupped as the plant matures.



**Figure 1.2** – Surface view of a carrot umbel in the early stages of flowering. Individual flowers are clustered in umbellets (U) arising from pedicel rays (P) originating from the inflorescence stalk. Flowers within the outer whorl of umbellets have commenced anther dehiscence.

Floral development is centripetal. Flowering commences on the primary umbel, with the process beginning about a week later on successive umbel orders. The flowering period of individual umbels is usually 7 to 10 days (Hawthorn *et al.*, 1961; Hiller and Kelly, 1985). Depending on the seed production area, variety, cultural practices and environmental conditions, a plant can flower for 4 to 6 weeks (Hiller and Kelly, 1985). As in other Apiaceae, the different phases of reproductive development, flowering, seed setting and seed development occur simultaneously on individual plants in different umbel orders.

Carrot flowers are usually perfect, small and white or occasionally greenish white or pale yellow. The flowers consist of 5 petals alternating with 5 stamens and an entire calyx (Figure 1.3). The flower has one pistil of 2 united carpels, with 2 styles and an inferior, bilocular ovary. Each locule contains a single functional ovule. Individual carrot flowers are protandrous (Koul *et al.*, 1993). A tendency to produce male flowers with no ovaries occurs with increasing frequency in higher order umbels (Braak and Kho, 1958).



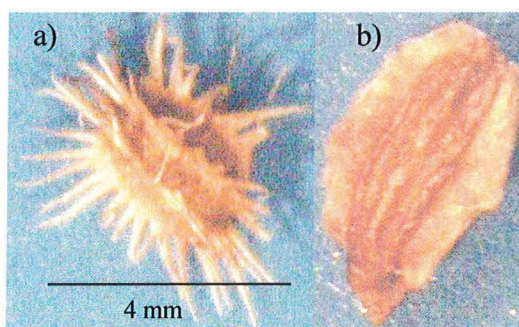
**Figure 1.3** – Carrot flower anatomy: a) side view, b) top view. (Figure reproduced from Rubatzky *et al.*, 1999).

Carrot flowers are entomophilous (Koul *et al.*, 1993). Nectar is secreted from disk shaped nectaries located on the upper surface of the ovaries (Erickson *et al.*, 1982). Bohart and Nye (1960) observed 334 insect species, notably from genera in the Hymenoptera (bees and wasps), Diptera (flies) and Coleoptera (beetles), visiting carrot flowers and determined that when there was an adequate supply of pollinating insects, both seed yield and seed quality were high. Where natural insect pollinator populations were low, there was an advantage in placing honeybee colonies adjacent to the carrot fields.



## The Carrot Seed

The fruit that develops following fertilisation is a schizocarp consisting of two mericarps, each mericarp being an achene or true seed. Upon drying the paired mericarps are easily separated. Mature seeds (Figure 1.4) are flattened on the surface facing the septum of the ovary. The opposite side has five longitudinal ribs, with spines protruding from some ribs. These are removed by abrasion during commercial milling and cleaning to facilitate seed handling. Seed size variation is common within individual plants and between varieties and can range from less than 0.5 to greater than 2mg (Rubatzky *et al.*, 1999).



**Figure 1.4** –Mature carrot seed a); and with spines removed after debearding b).

The carrot seed consists of the testa or seed coat encasing the endosperm and embryo. The endosperm comprises cells containing starch, lipid and protein energy reserves used in germination and early seedling development. The carrot embryo is elongate, with two broadly linear cotyledons that are as long as the radicle, but no plumule (Borthwick, 1931b). It is located near the distal end of the seed and surrounded by the endosperm tissue. In contrast to many seeds, the majority of embryo development occurs late in seed maturation after the endosperm reserves have been laid down and the seed has attained maximum dry weight (Gray *et al.*, 1984). At maturity, the embryo

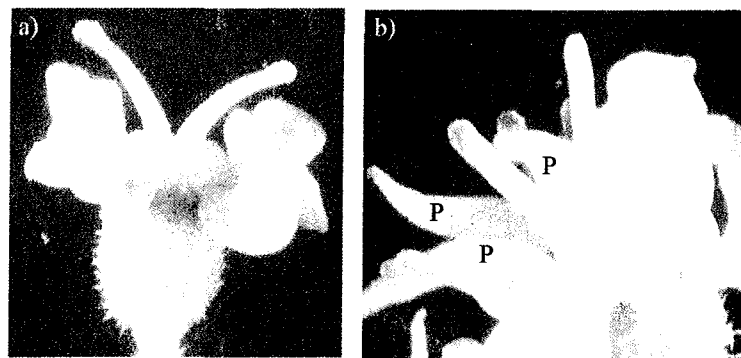
is the equivalent of between 2 and 3% of the endosperm volume within the seed (Gray *et al.*, 1984).

## **Hybrid Systems**

Hybrid varieties of carrots have the advantages of producing vigorous plants with relatively uniform roots. These factors are primarily responsible for the gradual substitution of open pollinated varieties with hybrids within Europe and North America (Rubatzky *et al.*, 1999; Simon, 2000). This trend is particularly evident for fresh market production, and is expected to continue in the future (Rubatzky *et al.*, 1999). Carrot hybrids are usually three way crosses, consisting of a single cross F1 hybrid male sterile seed parent crossed with an inbred male fertile line, since the F1 male sterile often results in higher seed production than an inbred male sterile parent (Erickson and Peterson, 1979). Single cross hybrids are more uniform than three way crosses, and they do not require an additional year to produce the F1 seed parent stock. Thus if seed productivity of a single cross is adequate, it is used in preference.

Production of hybrid carrot varieties is based on two distinct genetic-cytoplasmic types of male sterility (CMS). Brown anther sterility (Figure 1.5a) first reported by Welch and Grimball (1947), results in anther degradation and abortion of pollen at the microspore stage of development. In the petaloid form of sterility (Figure 1.5b) discovered by Munger (1953 cited in Erickson and Peterson, 1979) the 5 anthers develop as petaloid structures and are incapable of producing pollen. The final morphology of the petaloid structures varies from petal-like to filamentous (Eisa and Wallace, 1969). Whilst the flowers of brown anther male sterile lines retain the white colouration typical of open pollinated carrot flowers, petaloid flowers vary in colour from white to bright green (Erickson and Peterson, 1979). In contrast to other CMS hybrid crops such as onions and maize, restoration and maintenance of CMS in carrot is under complex genetic control and has not been fully elucidated.

Brown anther CMS has been shown to be phenotypically unstable in certain production environments (Hansche and Gabelman, 1963), resulting in production of male fertile flowers in the secondary and higher order umbels of male sterile plants. This problem is particularly evident in the United States, where almost all hybrid carrot seed production is based on the petaloid form of CMS (Rubatzky *et al.*, 1999). In contrast, carrot hybrids produced in Europe and Asia more often use brown anther CMS since male fertile flowers are not observed (Bonnet, 1985 cited in Rubatzky *et al.*, 1999). The basis of differences in the stability of brown anther and petaloid CMS expression has not been explained.



**Figure 1.5** – Male sterile flower forms used in commercial carrot seed production: The brown anther form a) lacks developed anther structures. Although not seen in this photograph, abortive anthers are often visible. The petaloid form b) produces 5 petaloid structures (P) in place of the anthers, 3 of which can be seen in this photograph.

Hybrid seed is more expensive to produce than open pollinated seed because: a portion of the seed crop (typically 20–40%) consists of male fertile plants which are destroyed after flowering to prevent contamination of the hybrid seed line with inbred seed at harvest; the inbred parent lines lack vigour and are more difficult to grow; and the male sterile plants typically produce less seed than open pollinated plants.

### **1.3 – Cultural Practices for Carrot Seed Production**

Although temperate carrots have a biennial growth pattern, carrot seed is grown over about 12 months. To prevent inter-varietal contamination during pollination, crops of different varieties of carrot seed are usually separated by at least two kilometres (Fulton, 1999). Two distinct methods of production, root to seed and seed to seed, are employed. In the root to seed method, stock seed is sown in the late summer in steckling (seedling) beds and the stecklings transplanted into fields for carrot seed production in the following spring. Depending on local winter conditions, the stecklings may be vernalised *in situ* or lifted in the late autumn and vernalised in cool stores. In the seed to seed method, the stock seed is sown into the carrot seed field in late summer; the roots over-winter in the field and flower and produce seed in the following summer. Compared with the root to seed method, seed to seed production is less expensive and enables greater planting densities, but is more reliant on high quality stock seed because there are fewer opportunities for roguing of the crop. Seed to seed production requires a production environment in which plant juvenility is overcome prior to a period of cold of sufficient low temperature and duration to satisfy the cultivar's vernalisation requirement. This is particularly important when choosing production locations for carrot seed crops with large vernalisation requirements, and also where the requirements of two parent lines have to be considered to enable synchronised flowering (nicking). This limitation has been overcome to some extent through the use of growth regulators such as GA<sub>3</sub> to induce bolting and flowering in environments where natural vernalisation is insufficient (Nieuwhof, 1984; Bandara and Tanino, 1995).

Hybrid carrot seed production is usually based on the strip method of hybrid seed production in which the male fertile and male sterile plants are arranged in alternating beds across the field. Male fertile to male sterile plant ratios of 1:2 to 1:4 are typically used, with the male sterile beds consisting of 2 to 8 rows of plants (George, 1985; Rubatzky *et al.*, 1999).

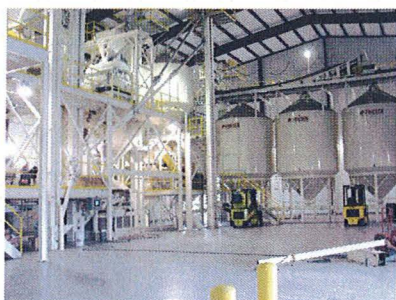
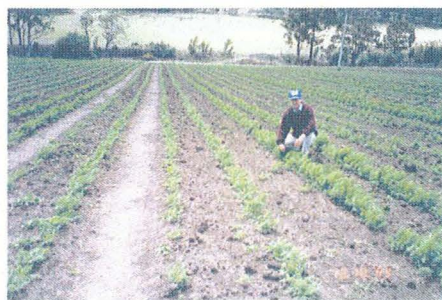
Although a wide range of naturally occurring insects pollinate carrots (Bohart and Nye, 1960), most producers introduce honeybees into carrot seed crops at flowering. In hybrid crops flowering is usually allowed to proceed for 4 to 6 weeks. After this time the male fertile line is removed by slashing to avoid contamination of the hybrid seed line with inbred seed during harvesting.

In small-scale commercial production of high value stock seed, individual carrot umbels are cut and harvested by hand as they ripen (Shinohara, 1984). On a larger scale, a single mechanised harvesting is performed. Generally, the crop is cut and laid in windrows when seed on the primary umbel is starting to shatter (George, 1985). Because the umbels are brittle at this stage, windrowing is performed early or late in the day when there is dew on the seed heads. After drying in the windrows, the seed is threshed and separated from the remainder of the plant debris by a combine harvester. It is also preferable that combining is done early or late in the day to reduce seed losses.

Prior to further cleaning and grading operations, the carrot seed spines are removed by a debearder to improve seed flow and reduce seed lot volume. Further cleaning and grading of the carrot seed to a marketable standard is achieved using aspirated screens, indent cylinders and gravity separators. Industry standards for purity and germination of carrot seed lots vary between varieties and with the target market. For export carrot seed crops grown in Australia, minimum standards of 99.9% purity and 85% germination generally apply (Fulton, 1999).

Carrot seed yields vary widely between varieties, production environments and with differing cultural practices. For the United States Rubatzky *et al.* (1999) estimated that most open pollinated crops produce 800–900 kg/ha of seed with hybrid crops typically yielding 200–700 kg/ha. In 1995 gross returns from open pollinated and hybrid carrot seed crops grown in Washington State, USA were estimated to be US\$1980–2640 and \$3960–5500/ha respectively (Schreiber and Ritchie, 1995).





**Figure 1.6** – Carrot seed production using the “seed to seed” method. Clockwise from top left: sowing a hybrid seed crop; a hybrid seed crop at the vegetative stage (note the size difference of the male fertile and male sterile lines; the planting arrangement is 4 male sterile (smaller plants) rows to 2 male fertile rows); inspecting a crop at an early stage of flowering; a hybrid seed crop at a late stage of flowering; separation and removal of the male line from a hybrid seed crop at the end of flowering with a mechanical slasher; harvesting of dried windrows of carrot seed with combine harvesters; a commercial seed cleaning plant used to de-beard, clean and grade carrot seed.

## ***1.4 - Research Impetus and Project Focus***

This project was initiated by a major stakeholder in the Australian carrot seed industry, South Pacific Seeds P/L, to address production issues contributing to the decline of the Australian export carrot seed industry. The project focused primarily on hybrid carrot seed production because of the emphasis on hybrid varieties in the market place and the greater difficulty experienced in successfully producing seed of these. Specifically, European Nantes varieties were studied based on the rationale that there was potential for expansion of production of European varieties in south eastern Australia if they could be produced successfully. Two broad objectives for the project were defined. The first (and major) was to identify the cause of low seed germination and based on an understanding of this, develop management strategies to improve the germination of Australian carrot seed. The second objective was to further the understanding of factors affecting seed yield in hybrid varieties with a view to applying this knowledge through improved management for more reliable seed yields.

## ***1.5 – Outline of Thesis Structure***

This project is reported in 8 chapters. This, the first chapter, provides a general background to the Australian carrot seed industry, introduces the concepts of reproductive development and seed production in carrot and outlines the project focus and thesis structure. Chapter 2 comprises the research methods within the project that are common to several thesis chapters. Details of the plant material and long term average climatic conditions at the trial sites used in the research are also provided in this chapter. Chapter 3 outlines preliminary research comparing the results of variety trials at two Australian production locations, Mount Gambier, South Australia and Cambridge, Tasmania with a site near Winchmore on the Canterbury Plains of New Zealand. Data from these trials and commercial production records were used to establish that the germination and yield problems within the Australian industry are largely unrelated. The subsequent chapters are divided between two research areas,

cause and management of low germination (Chapter 4) and cause and management of low seed yield (Chapters 5 to 7).

Chapter 4 tests the hypothesis that feeding damage of an endemic Australian insect, Rutherglen bug (*Nysius vinitor*) causes loss of germination in Australian carrot seed. Data collected to facilitate the management of this insect in carrot seed crops are presented.

As a precursor to seed yield studies, Chapter 5 outlines the development of methodologies for collection, storage and viability testing of carrot pollen. This work was undertaken because methods suitable for carrot or other Apiaceae could not be found in the literature. Research examining low yields in hybrid carrot seed production is presented in Chapter 6, with an examination of two alternative hypotheses for low seed set; pollination limitation of seed yield and mother plant resource limitation of seed yield. Chapter 7 reports studies of the basis for low rates of pollen transfer within Tasmanian hybrid carrot seed crops.

Chapter 8 draws together the major conclusions from the research undertaken and, based on these, outlines a series of recommendations for carrot seed production in Australia.



## Chapter 2

### General Materials and Methods

*The bulk of the experimental component of this thesis consists of research on the cause and management of low germination of Australian carrot seed crops and the roles of pollination and pollen viability in hybrid seed yield determination. Both fields of investigation were based largely on field trials established using the seed to seed method of production. Within the pollen viability studies, several trials were conducted using samples sourced from glasshouse grown plants produced from stecklings.*

*This chapter describes the plant material and cultural practices that were used within the field and glasshouse trials. In addition, methods of pollen sampling, measurement of pollination and harvesting, handling and viability testing of seed that are common to more than one research chapter are outlined. The basic statistical procedures used throughout the thesis are also described. Further details of particular methods and descriptions of methods unique to individual research chapters are given within the materials and methods sections of the relevant chapters.*

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#### **2.1 - Plant Material**

Seed lines were sourced from the vegetable breeding companies Vilmorin (La Menitre, France), Nickerson Zwaan (Made, Netherlands), Rijk Zwaan (De Lier, Netherlands) and Seminis (Woodland, USA). Initial trials included a range of inbred and F1 hybrid parents, and open pollinated lines (for details, see Table 3.1, Chapter 3). Two Nantes type hybrid

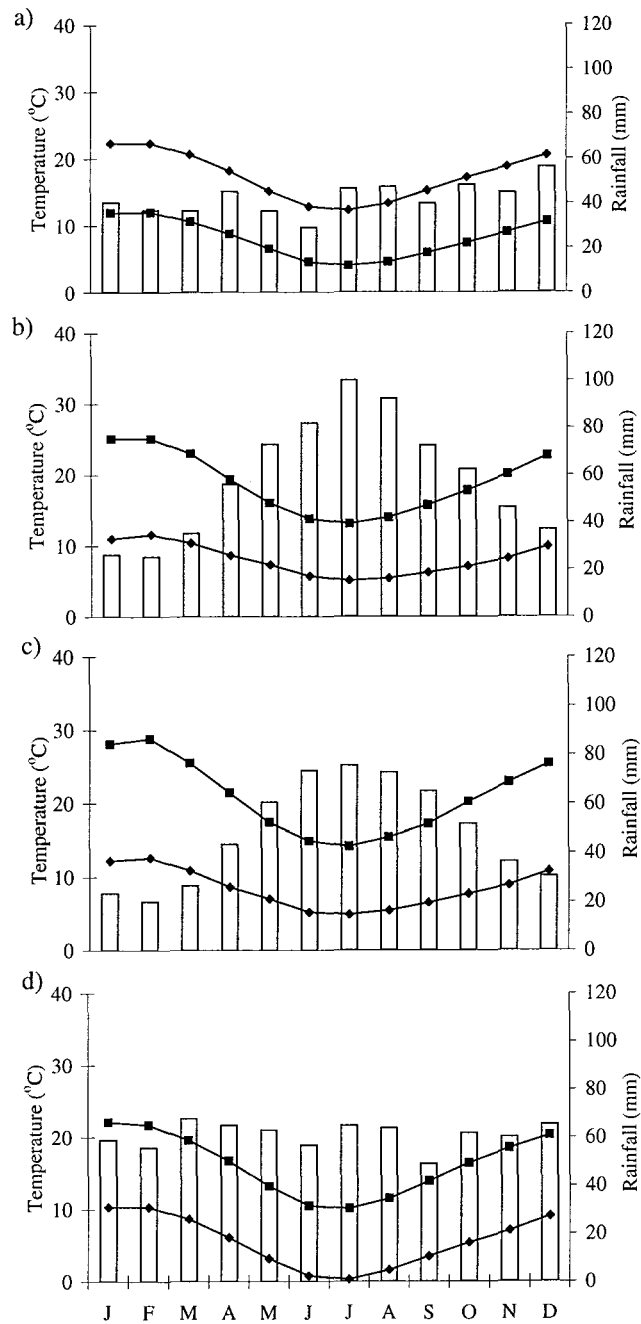
crosses, Rijk Zwaan WO030 and Vilmorin No. 22 were selected for the main body of research. The male sterile parent within the hybrid WO030, is a petaloid inbred line. The male sterile parent within the hybrid No. 22 is a brown anther F1 line. Both male fertile lines are inbred. The reasons for selection of these lines were; a) commercial interest in their production in south-eastern Australia, b) they represent the two major CMS systems in use (petaloid and brown anther) and c) the yield and germination of hybrid seed from the two male sterile lines was typical of a range of lines examined in preliminary trials

## ***2.2 - Field Experiments***

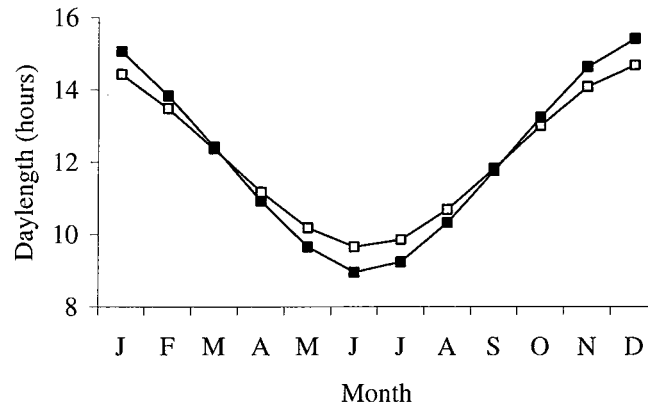
The locations of all experimental sites used in this project are shown on the map (Figure 2.1). In Tasmania, field trials were conducted in the lower Coal River Valley at Cambridge (42.48°S, 147.25°E), Richmond (42.45°S, 147.24°E) and Tea Tree (42.43°S, 147.20°E). Several Tasmanian field experiments were replicated at commercial production locations near Naracoorte (37.0°S, 140.7°E), Millicent (37.6°S, 140.4°E) and Mount Gambier (37.7°S, 140.8°E) in South Australia and near Winchmore (43.5°S, 171.5°E) in the South Island of New Zealand. Long-term temperature and rainfall data for the nearest operating weather stations to each site are given in Figure 2.2. Monthly daylight hours (calculated from sunrise to sunset) for Winchmore and Naracoorte, which encompass the range of latitudes of the trial sites, are given in Figure 2.3.



**Figure 2.1** –Australian field trial sites were located in the Lower Coal River Valley, Tasmania (42.5°S; 147.2°E); Millicent, South Australia (37.6°S; 140.4°E); Mount Gambier, South Australia (37.7°S; 140.8°E); and Naracoorte, South Australia (37.0°S; 140.7°E). A site near Winchmore (43.5°S; 171.5°E) in the South Island of New Zealand was also used. Glasshouse experiments were conducted in Hobart, Tasmania (42.9°S; 147.3°E). Map source: General Libraries, The University of Texas at Austin (2003).



**Figure 2.2** – Long-term mean monthly minimum (◆) and maximum (■) temperature and rainfall data (□) for a) Hobart Airport (42.8°S; 147.5°E), b) Mt Gambier Airport (37.7°S; 140.8°E) c) Winchmore (43.5°S, 171.5°E), and d) Naracoorte (37.0°S; 140.7°E) weather stations. Sources: Bureau of Meteorology Australia and South Pacific Seeds' production site climate notes. Note: the Mt. Gambier Airport weather station is the closest weather station to both the Mt. Gambier and Millicent trial sites.



**Figure 2.3** – The number of daylight hours on the 15<sup>th</sup> day of each month of the year at Naracoorte (□), South Australia (37.0°S, 140.7°E) and Winchmore (■), New Zealand (43.5°S, 171.5°E). Source: Geoscience Australia (2000)

Field experiments were conducted in commercial crops or in purpose designed experimental plots. Commercial carrot seed crops were sown with a Stanhay Singulaire<sup>®</sup> 785 air seeder (Stanhay, Newmarket, UK). Target plant densities ranged between 12 and 25 plants/m<sup>2</sup>. Experimental plots were sown on raised beds, 1.6m wide with two rows per bed, 0.8m apart, using a Hege 90<sup>®</sup> (Hege Maschinen, Waldenburg, Germany) two row cone seeder, or by hand seeding. The target density was 24 plants/m<sup>2</sup>, with the plots thinned to 12 plants/m<sup>2</sup> after emergence. Other than sowing technique, the cultural practices used in all field trials were based on current commercial practice (for details see Table 2.1).

At the onset of flowering, honeybees were introduced at a density of 6 hives/ha to supplement natural pollinator levels. These were removed after the completion of flowering of the tertiary umbels of the male sterile line. After the removal of the beehives, the male fertile line was removed with a tractor-mounted slasher or garden line trimmer.

**Table 2.1** – Summary of the cultural practices used to grow trial plots of hybrid carrot seed studied in this thesis. Note: 1) Herbicide rates varied with soil type. Rates quoted are those used for brown soils on dolerite at Cambridge, Tasmania; 2) Chemical application varied slightly between seasons and locations according to the presence or absence of particular weeds, pests or diseases; 3) All irrigation systems used were of the overhead type; 4) Rutherglen bug controls were applied to trial plots in the 2000-01 and 2001-02 seasons after it became apparent that it was necessary to control this insect.

Month	Cultural Practice	Comment
February-March	Irrigate, work ground 14 days later	For weed control / land preparation
	Incorporate fertiliser	9:13:17 (NPK), 300-500kg/ha
	Form beds	1.6m wide beds
	Seeding	Target density 12-20 plants/m <sup>2</sup> , 400–800mm row width
	Irrigation	25mm directly after seeding, as required thereafter until the end of January
	Pre-emergence herbicide application	Stomp® a.i. pendimethalin, 2L/ha
April–May	Control of grass weeds	One of Verdict® a.i. haloxyfop, Fusilade® a.i. fluazifop-P or Sertin® a.i. sethoxydim at 80ml, 500ml and 500ml respectively
	Control of red legged earth mite	Dimethoate® a.i. dimethoate, 400ml/ha
	Control of centre grubs	Ambush® a.i. permethrin, 100ml/ha
	Control of broadleaf weeds	Linuron® a.i. linuron, 1kg/ha and Gesagard® a.i. prometryn, 500ml/ha
September	Spot spraying of remaining weeds	150ml/15L Roundup® a.i. glyphosate
December	Introduce honeybees	5 hives/ha at start of flowering
	Commence Rutherglen bug control	Dominex® a.i. alpha-cypermethrin, 250ml/ha. Control until harvest
		Dimethoate® a.i. dimethoate 400ml/ha used after flowering
January	Remove honeybees	At end of flowering
	Remove male fertile rows	At end of flowering
	Control of <i>Alternaria</i> and <i>Cercospora</i> fungal diseases	Mancozeb® a.i. mancozeb, 2kg/ha or Score® a.i. difenoconazole, 1L/ha
February-March	Windrow	When first 2 umbel orders are mature
	Harvest	7-14 days after windrowing

## **2.3 - Glasshouse Experiments**

Glasshouse experiments were undertaken at the University of Tasmania's Horticultural Research Centre in Hobart (42.9°S, 147.3°E) using the root to seed method. Stecklings were grown in a temperature-controlled glasshouse (15 to 25°C) under natural light and daylength conditions. Seeds for stecklings were sown between February and June in 35L Easilift<sup>®</sup> planter bags filled with a potting mix consisting of a 7:3:1 mixture of pine bark:sand:peat. Each 50L of potting mix contained 300g of Osmocote Plus<sup>®</sup> slow release fertiliser with micronutrients, 90g of dolomite, 25g of iron sulphate and 75g of lime. In each pot, a hexagonal planting pattern was used, with 2 seeds sown at each point. After emergence, the seedlings were thinned to provide 6 evenly spaced plants per pot. Water was provided on a daily basis via overhead sprinkler irrigation.

The stecklings were harvested after 3 months, when they had a root collar diameter of approximately 30mm. After lifting, the tops of the stecklings were removed 30mm above the shoot apex. The trimmed stecklings were packed in moist sand in polystyrene boxes and vernalised in a cool store at 4°C for 10 weeks.

Following removal from cool storage, visibly disease free stecklings were selected for transplanting and dipped in a fungicide mix consisting of Benlate<sup>®</sup> (a.i. benomyl) and Kocide<sup>®</sup> (a.i. copper hydroxide). The stecklings were transplanted into 35L Easilift<sup>®</sup> planter bags, filled with the potting mix described above, at a density of 4 per bag. After transplanting, the planter bags were moved to a temperature-controlled glasshouse where the daylength was extended to 16 hours using a bank of 100 watt incandescent globes. To prevent devernalisation, the plants were initially grown at 15°C for two weeks, with the temperature subsequently increased to 25°C. Irrigation was by Octa-Drippers<sup>®</sup> located at the potting mix surface in each planter bag. Irrigation rates were set to maintain non-stressed growing conditions and gradually increased as the plants developed. Flowering commenced approximately three months after transplanting.

## ***2.4 - Pollen Collection, Storage and Viability Testing***

Pollen was collected from male fertile plants in both glasshouse and field based experiments. 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 for immediate hand pollination or storage. Pollen for storage was brushed into 1.5ml Eppendorf® centrifuge tubes and placed over silica beads in a desiccator. Specific details of the pollen collection methods for individual experiments are provided in the Materials and Methods sections of the relevant chapters. A lack of published methods for the storage and viability testing of pollen of carrot or other members of the Apiaceae meant that existing techniques had to be tested and modified for use in this work. Details of this method development are provided in Chapter 5.

## ***2.5 - Examination of Pollen Deposition on the Stigmas of Male Sterile Flowers.***

Pollen deposition on the stigmas of male sterile plants was measured in several experiments to confirm the efficacy of hand pollination treatments and assess the level of natural pollination. The method used was adapted from Beattie (1971a; cited in Kearns and Inouye, 1993). Basic fuchsin gel was prepared as follows. 40g of hydrated gelatin was melted in a microwave oven and mixed with 60ml of glycerol. Basic fuchsin powder was gradually added to the mixture until a transparent mid-red gel, matching a colour standard determined in preliminary trials to differentiate pollen and stigmatic tissue of carrot, was obtained. The resulting mixture was filtered through glass wool and stored at 4°C.

The petals of flowers selected for examination of pollen deposition were excised to expose the gynoecia. These were mounted on a microscope slide in a drop of melted basic fuchsin gel. 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 easily counted.

## ***2.6 - Seed Harvesting, Preparation and Storage.***

Plants were harvested by hand when the seed in the first three umbel orders had turned brown and loose seed was observed on the surface of the primary umbel. After removal, the umbels were threshed with a modified garden mulcher (Stihl, Virginia Beach, USA), in which the cutting blade had been replaced with a strip of hardened rubber, or for larger experiments, with a small plot thresher (DPIWE, Launceston, Australia). Hand sieves were used to remove coarse trash and fine dust. Threshed seed lots were placed in hessian bags and dried for 1 week at 25°C in a commercial gas-heated, forced air seed dryer. After drying, seed moisture contents, determined by the low constant temperature method (ISTA, 1993) were typically 10% (w/w). A laboratory thresher (Wintersteiger, Salt Lake City, USA) was used to de-beard the dried seed. Air-screen cleaning was performed using a laboratory sized clipper-cleaner (Blount Agri- Industrial, Indiana, USA). The sieve selections were 3.97 to 4.76mm diameter round hole perforated metal top sieves and 1.32 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.

## ***2.7 - Seed Yield Assessment***

Seed yields and 100 seed weights were determined on a dry weight basis by the low constant temperature method (ISTA, 1993). Samples for drying were drawn using the method of repeated halving (ISTA, 1993) and counted on an electronic seed counter (Baumann Saatzuchtbedarf, Waldenburg, Germany).

## ***2.8 - Seed Germination Assessment***

Seed germination tests were conducted in accordance with the International Rules for Seed Testing (ISTA, 1993). 100 seed samples for germination were counted using the electronic seed counter. Prior to counting, the bulk seed samples were thoroughly mixed to ensure a representative sub-sample for germination. Seeds were germinated in petri dishes lined with 2 layers of Advantec No. 2 filter paper (Toyo Roshi Kaisha, Japan) moistened with deionised water. The petri dishes were placed in clear, airtight plastic containers and held at 20°C (+/- 1°C) in a controlled temperature incubator (Contherm Scientific, Wellington, New Zealand) set for a 12 hour light:12 hour dark cycle. The petri dishes were checked every second day for moisture levels, with deionised water added as required. Depending on the experimental requirements, normal seedlings were counted and removed on a daily basis or at days 7 and 14. All tests were terminated at day 14. Seeds that failed to germinate were classified as either dead or fresh ungerminated based on a squash test (ISTA, 1993). Normal and abnormal seedlings were classified according to ISTA guidelines for seedling assessment (ISTA, 1979).

## ***2.9 - Seed Embryo Assessment***

Carrot seed embryos were examined using the methodology of Gray and Steckel (1983). Representative sub-samples of 40 seeds were soaked in formalin acetic alcohol (FAA) (50% ethanol; 6.5% formalin and 2.5% glacial acetic acid). Four hours later, after the seed tissue had softened, the seeds were rinsed in deionised water and cut to remove the caruncle and a small amount of pericarp. The embryos were extruded by pressing the back of the seed with the flat side of a scalpel blade and examined under a dissecting microscope.

## ***2.10 – Statistical Analysis***

Generally, experiments were conducted either as completely randomised or randomised complete block designs. ANOVA analysis of the data was undertaken using SAS statistical software version 8 (SAS Institute Inc, Cary, USA, 1999). Significant differences between treatment means were assessed using Fisher's LSD (least significant difference) (Steele and Torrie, 1981). Regressions were undertaken using PROC GLM and PROC REG functions in SAS. Analysis of paired data was undertaken using the PROC MEANS function within SAS. Further details of experimental designs, treatment of data and statistical analysis relevant to individual experiments are given in the experimental chapters.

## Chapter 3

### Preliminary Studies of Yield and Germination of Carrot Seed in Australia and New Zealand

*The preliminary work documented in this chapter was undertaken to investigate the causes of low germination of carrot seed produced in Australia compared to New Zealand. The work was based around industry variety trials at production sites in Cambridge, Tasmania, Mount Gambier, South Australia and near Winchmore, New Zealand.*

*Although the random arrangement of a large number of parent lines within the trials precluded a detailed examination of yield limitation in specific hybrids, yield data was collected to provide an indication of male sterile lines that should be used in future studies and some insight into the factors affecting yield.*

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#### **3.1 – Literature Review**

This section provides an overview of literature pertaining to seed germination and yield issues in carrot seed production. It was undertaken to support the preliminary studies. More detailed reviews of specific seed yield and quality issues facing the Australian carrot seed industry are presented in the following chapters.

## Poor Germination of Carrot Seed

Periodic occurrences of poor seed germination in carrot and other members of the Apiaceae have been documented in both Europe and the United States (Borthwick, 1931a; Flemion, 1949; Dean *et al.*, 1989; Simon, 2000). Three reasons for poor germination of carrot seed are generally reported. These are 1) the occurrence of seeds without an embryo; 2) seeds containing a rudimentary embryo; and 3) high levels of seedling abnormality (Flemion, 1949; Robinson, 1954; Dean *et al.*, 1989). Empty seeds were a significant contributor to poor germination of carrot seed lots in one study (Dean *et al.*, 1989), but do not appear to have been mentioned in other studies.

## *Embryoless Carrot Seeds*

It has been reported that the endosperm and other structures of embryoless seeds are present and appear to develop normally (Flemion, 1949). Harrington (unpublished data cited in Robinson, 1954) calculated that the embryo accounts for only 1% of the weight of a carrot seed. Because embryoless seeds have the same shape, size and surface texture as viable carrot seeds and practically the same weight, it is impossible to mechanically separate the two. Extensive studies of the cause of embryoless seeds in the Apiaceae were made by Flemion and reviewed by Robinson (1954). Feeding of nymphal and adult tarnished plant bugs (*Lygus* bugs) *Lygus oblineatus* Say and closely related species (Hemiptera: Miridae) was shown to be responsible for the occurrence of embryoless seeds in 15 genera, including carrot. Embryolessness was reported to be a common condition within seed of the Apiaceae collected from areas in Europe, the United States and Africa where *L. oblineatus* or closely related species were found. Insects of the genus *Lygus* are not known to occur in Australia (CSIRO, 1991) but numerous other seed feeding insects within the Hemiptera are present. There have been no published studies of insect visitation to carrot seed crops in Australia but industry personnel have observed visitation of insects within the genus *Nysius* (Hemiptera: Lygaeidae), which includes a number of seed feeding insects. In the past, pesticide applications to control these insects, based on the assumption that

they may cause damage to carrot seed, appear to have been largely unsuccessful in improving seed germination (Dalrymple, M. *pers. com*<sup>1</sup>).

A low frequency occurrence of embryoless seeds (less than 1%) when plants were grown in insect free cages by Flemion (1949) suggests that some factor other than insect feeding may also cause embryoless seeds. Experiments investigating breeding and variety differences, soil type and nutrition, plant spacing, umbel and flower position, seed yield and seed size suggest that none of these factors are directly involved (Robinson, 1954). Several studies have reported a decrease in percentage germination arising from inadequate levels of pollination (Hawthorn *et al.*, 1955; Rodet *et al.*, 1992), but the reasons for this have not been examined. In some plant species, including members of the Apiaceae, embryoless seeds are occasionally produced by single fertilisation (Jurica, 1922).

A correlation between the incidence of embryoless seed in wild carrot populations and higher summertime temperatures at a range of locations across eastern North America was reported by Lacey (1984). Carrot embryos initially develop slowly after fertilisation (Gray *et al.*, 1984) and may therefore be particularly susceptible to high temperature or other environmental stresses early in their development. The temperature effects that Lacey (1984) observed might, however, be indirect rather than causal. For example, temperature is also likely to impact on population build up or feeding activity of *Lygus* bugs. Higher than average temperatures around the time of seed fertilisation did not affect germination in carrot in one study (Borthwick, 1931 cited in Robinson, 1954), although the temperatures involved were not specified. Several studies have reported that an accumulation of moderate water stress from the start of the reproductive period improved carrot seed germination (MacGillivray, 1949 cited in Steiner *et al.*, 1990; Hawthorn, 1952; Steiner *et al.*, 1990).

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<sup>1</sup>Mr Max Dalrymple, Production Manager, South Pacific Seeds, South Australia

### ***Rudimentary Embryos and Seedling Abnormalities***

Seeds of normal appearance apart from a small, poorly developed (rudimentary) embryo that failed to germinate were first reported by Borthwick (1931a). Such seeds are known to contribute to the periodic low viability of carrot seed lots produced in the United States (Dean *et al.*, 1989) and Europe (Gray and Gent, 1988). Seeds with rudimentary embryos might be capable of germination after prolonged exposure to a moist substrate (Borthwick, 1931a) and were cited as a form of morphological seed dormancy that could be overcome with seed priming treatments by Baskin and Baskin (1998).

Seedling abnormalities have been reported as a significant cause of low germination in carrot (Dean *et al.*, 1989). The International Seed Testing Association (ISTA) lists 2 types of carrot seedling growth abnormality in their guidelines for seedling evaluation (ISTA, 1979). These are seedlings with a short, thick hypocotyl and missing primary root, and seedlings with a twisted or looped hypocotyl. Seeds that germinate late in the test and fail to develop sufficiently to be determined to be normal are also classified as abnormal in industry germination tests.

The developmental processes leading to seed with rudimentary embryos and seedling abnormalities are not well understood. There are many published reports of the effects of agronomic treatments on carrot seed yield and germination but unfortunately, few categorise the types of failed germinant. Flemion and Hendrickson (1932 cited in Robinson 1954) found no relationship between embryolessness caused by *Lygus* bug and the occurrence of rudimentary embryos. Studies of plant spacing (Harrington, 1951; Gray, 1981) indicate that effects of plant density on seed germination are generally small, although a small reduction in the variability of embryo sizes from seed of higher density plantings has been observed (Gray and Steckel, 1983).

It seems plausible that rudimentary embryos and some seedling abnormalities may result from seed that is immature at harvest. This theory is supported by the indeterminate flowering habit of the plant, the late development of the embryo during seed maturation and the once over harvesting strategy employed in commercial carrot

seed production. In onions, immaturity at harvest increased the incidence of seedlings with root abnormalities (Spurr *et al.*, 2001), but even late harvests contained a significant proportion of abnormal seedlings. The germination percentage of seed from higher order umbels generally decreases in carrot when all umbel orders are harvested at the same time (Borthwick, 1931a; Gray, 1979; Jacobsohn and Globerson, 1980). Gray (1979) reported positive correlations between germination percentage and mean embryo size where differences in germination between umbel orders existed. Delaying harvest time has been reported to reduce differences in germination percentage between umbel orders in some studies (Gray and Steckel, 1982) but not others (Gray, 1979; Sandin, 1980). Sandin (1980) and Hawthorn (1961) found that even with a late harvest, carrot seed germination remained low (less than 80%). Unequivocal evidence for or against a relationship between immaturity at harvest and the occurrence of rudimentary embryos or seedling abnormality has not been presented for carrot.

### **Low Hybrid Seed Yields**

Low yielding hybrid carrot seed crops are a problem of varying magnitude in most carrot seed producing areas of the world (Erickson and Peterson, 1978; Evans *et al.*, 1995; Simon, 2000). Average hybrid seed crops typically yield 200 to 700 kg/ha (Rubatzky *et al.*, 1999; Simon, 2000), approximately one-third to one-half of open pollinated yields. Part of the area of hybrid crops (usually between 20 and 40%), planted with the male fertile line does not produce hybrid seed, but after this is accounted for, yields of hybrid seed crops are often considerably lower than open pollinated crops. Although it would be desirable to improve the yield of hybrid seed crops in general, of particular concern is the periodic occurrence of crops that produce very low yields (<100 kg/ha).

The effect of agronomic practices on carrot seed yield has been the focus of many studies. Planting density (Harrington, 1951; Gray, 1981; Gray and Steckel, 1983; Gray *et al.*, 1983; Noland *et al.*, 1988; Oliva *et al.*, 1988), nutrition (Hawthorn, 1952), irrigation (Steiner *et al.*, 1990) and timing of harvest (Hawthorn *et al.*, 1961; Gray, 1979; Sandin, 1980; Gray and Steckel, 1983; Tucker and Gray, 1986) have all been



examined and optima determined for each with respect to seed yield. Given that current Australian production practices (Fulton, 1999) are consistent with the findings of these studies, it is unlikely that any of these variables are common causes of low yields in Australia hybrid carrot seed crops.

Most studies on the causes of low yield in hybrid carrot seed crops focus on processes associated with pollination. Asynchronous flowering of the two parent lines (Erickson and Peterson, 1979), inadequate pollinator levels (Hawthorn *et al.*, 1955; Free, 1965; Delaplane and Mayer, 2000) and low rates of pollinator movement between the parent lines (Erickson *et al.*, 1979; Galuszka and Tegrek, 1987; Rodet and Torre Grossa, 1991) have all been suggested as causes of low seed yield. Although a correlation between insect visitation rate and seed yield between different male sterile lines has been reported (Erickson *et al.*, 1979), there appear to have been no studies that give an unequivocal demonstration of pollination limited yields of hybrid carrot seed.

In many plant species seed yield is limited by the capacity of the mother plant to reproduce rather than processes during pollination (Stephenson, 1981; Fenner, 1985). Mother plant resource limitation of seed yield may result in a restriction on the number of fertile flowers or ovules that are produced, the percentage of successful fertilisations, or more commonly through seed abortion or reduced seed size (Fenner, 1985). Carrot hybrids with F1 male sterile plants often produce greater seed yields than hybrids with inbred male sterile plants (Erickson *et al.*, 1979), which is consistent with, but not evidence of, resource limitation of seed yield.

## **Conclusion**

Three main reasons for poor germination of carrot seed were identified from the literature: the occurrence of embryoless seeds; seeds with rudimentary embryos; and abnormal seedlings. In overseas production locations, feeding damage of *Lygus* bugs appears to cause most embryoless seed. Although this species is not present in Australia, other insects visiting carrot seed crops may cause similar damage.

The reason for the occurrence of seeds with rudimentary embryos and abnormal seedlings in carrots has not been fully determined but it has been hypothesised that rudimentary embryos are immature. If this were correct, rudimentary embryos would be expected to periodically occur in Australian carrot seed as they do in carrot seed produced elsewhere, particularly in cooler seasons with prolonged flowering.

In production areas outside Australia, the causes of low germination of open pollinated and hybrid seed crops and low, variable yields in hybrid seed crops appear to be separate issues. Whilst many studies have examined the effect of cultural factors such as plant spacing, nutrition and irrigation on carrot seed yield, it seems unlikely that these factors are directly responsible for the periodic occurrence of uneconomically low yields. Most studies examining the occurrence of low yields in hybrid carrots have focussed on pollination as a yield-limiting factor. Whilst the published data generally support the hypothesis that hybrid carrot seed yields are limited by some aspect of pollination, clear evidence of this has not been presented. Other possibilities, for example that the availability of assimilates may determine carrot hybrid seed yields, cannot be discounted.

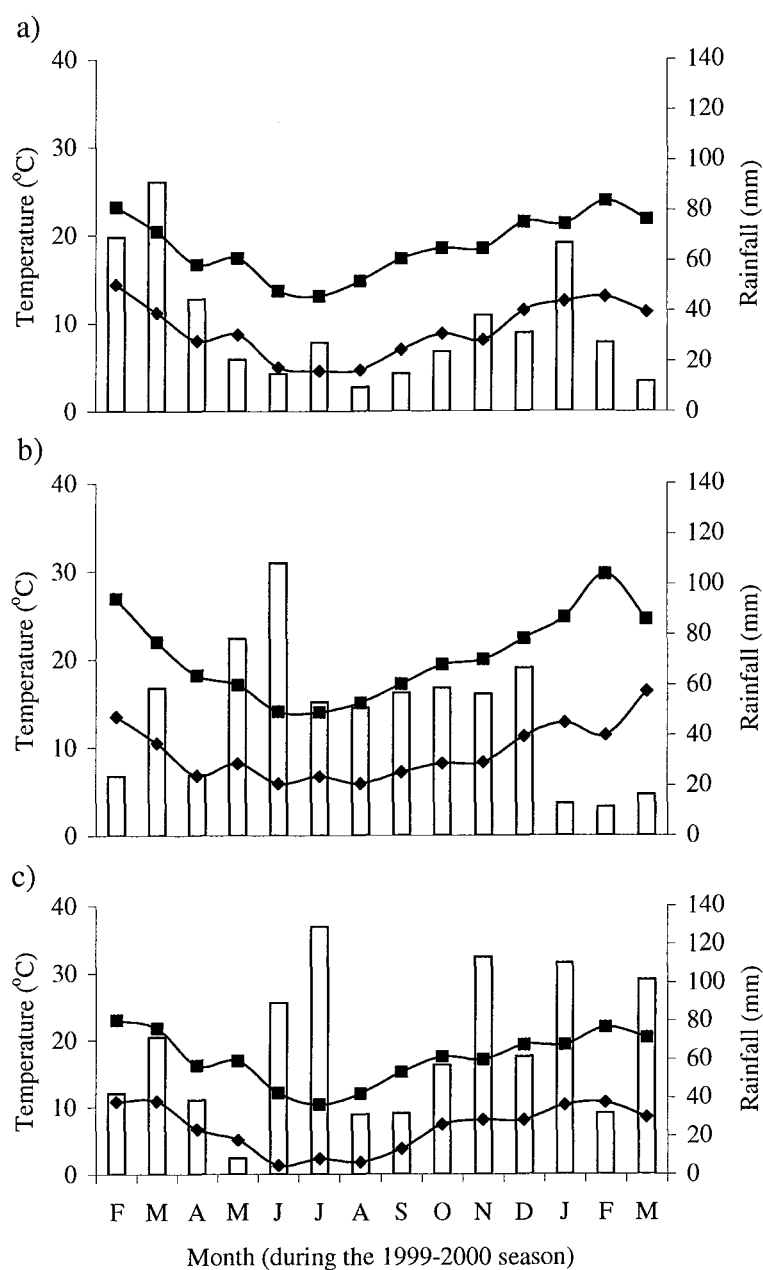
## **3.2 - Materials and Methods**

### **Trial Sites**

The carrot seed parent lines studied in this work were grown in variety trials established by South Pacific Seeds at Cambridge, Tasmania; Mount Gambier, South Australia; and near Winchmore, New Zealand. Climatic conditions during the trials are shown for each location in Figure 3.1. Long-term data for each site are given in Figure 2.2 (Chapter 2). Conditions were near normal for Cambridge and Mount Gambier during the trials, except for a slightly lower than average rainfall from winter onwards at Cambridge. At Winchmore, rainfall was markedly higher and temperatures slightly lower than average during the period between flowering and harvest of the trials (December to March).

### **Cultural Practices and Trial Design**

Soil fertility tests were conducted at each site prior to sowing. 300 to 500 kg/ha of 9:14:17 (NPK) was applied at each site on the basis of the soil test results and industry recommendations for carrot seed crops (Fulton, 1999). The trials were sown at all locations on the 22<sup>nd</sup> of February 1999 using the methods and spacings detailed in Chapter 2. Twenty nine male sterile inbred and F1 hybrid, 25 male fertile and 3 open pollinated lines of Nantes, Amsterdam, Berlicumer and Flakkee root types were included in each trial. Details of each line are provided in Table 3.1. A randomised complete block design was used in which there were 3 replicate blocks consisting of 57, 5m long rows. In each block, each male sterile line appeared in random order in a single row, running the length of the block. Due to the range of male fertile lines and the fact that some experimental male sterile lines did not have a specific partner, male fertile lines were arranged in random order throughout the blocks so that all male sterile lines were adjacent to a pollen source. At each site a standard set of cultural practices was used. These are detailed in Table 2.1, Chapter 2.



**Figure 3.1** – Mean daily minimum (◆) and maximum (■) temperature and total monthly rainfall (□) data from; a) Hobart Airport (site no. 94004), b) Mount Gambier Airport (site no. 026021) and c) Winchmore Research Station during the variety trials. Sources Bureau of Meteorology Australia and Winchmore Research Station, New Zealand.

**Table 3.1** – Varieties included in industry trials at Cambridge, Mount Gambier and Winchmore. Note: 1) embryo examinations were conducted on seed harvested from the 12 lines indicated by \* next to the variety reference code; 2) seed set at Cambridge was determined for lines indicated by a + next to the variety reference code. <sup>1</sup>CMS type not recorded.

Type	Sex	Breeding	Line reference	CMS type	Breeder
Nantes	Male sterile	Inbred	ON44-43A*+	White petaloid	Seminis
			ON44-143A	White petaloid	Seminis
			ON44-158A*	White petaloid	Seminis
			ON44-179A	White petaloid	Seminis
			ON44-193A	White petaloid	Seminis
			ON44-198A+	Pale green petaloid	Seminis
			ON44-227A	Pale green petaloid	Seminis
			ON44-5002A*+	Pale green petaloid	Seminis
			ON44-5015A	White petaloid	Seminis
			ON44-88A	White petaloid	Seminis
			ON70-1260A*+	White petaloid	Seminis
			WO6003	Brown anther	Rijk Zwaan
			WO6014*+	Green/white petaloid	Rijk Zwaan
	Male sterile	F1 hybrid	No.10	Brown anther	Vilmorin
			No.16*+	Green/white petaloid	Vilmorin
			No.22*+	Brown anther	Vilmorin
			No.27*	Brown anther	Vilmorin
	Male fertile	Inbred	ON44-92AxON44-91B	White petaloid	Seminis
			ON44-79C	NA	Seminis
			ON44-145C	NA	Seminis
			ON44-166C	NA	Seminis
			ON44-218B	NA	Seminis
			ON44-5014C	NA	Seminis
			WO8012	NA	Rijk Zwaan
			WO8024	NA	Rijk Zwaan
	Open pollinated		WO158*+	NA	Rijk Zwaan
Amsterdam	Male sterile	Inbred	WO6002	Brown anther	Rijk Zwaan
			NZ1	<sup>1</sup>	Nickerson Zwaan
	Male fertile		NZ2	NA	Nickerson Zwaan
	Open pollinated		WO336*	NA	Rijk Zwaan
Flakkee	Male sterile	Inbred	WO6008	Green petaloid	Rijk Zwaan
			WO6019*	Green petaloid	Rijk Zwaan
	Open pollinated		WO127*+	NA	Rijk Zwaan
Berlicum	Male sterile	Inbred	OB44-155A	Pale green petaloid	Seminis
			WO6006	Brown anther/petaloid	Rijk Zwaan
			WO6010	Green/white petaloid	Rijk Zwaan
	Male fertile		OB44-160C	NA	Seminis

## **Seed Harvests**

All of the male sterile and open pollinated lines included in the variety trials were harvested. To account for the difference in time of seed maturation between lines, weekly harvests were performed from the 9<sup>th</sup> of February to the 15<sup>th</sup> of March 2000. All replicates of a particular line were harvested at the same time. At each location 3 randomly selected plants per replicate were harvested, giving 9 plants in total. At Cambridge 4 randomly selected umbellets were removed from the primary and fourth secondary umbels of all harvested plants of 10 representative hybrid (Nantes) and open pollinated lines (see Table 3.1 for details of the lines sampled). These samples were visually assessed to determine the percentage of flowers setting seeds. The umbels of each plant were removed by hand and threshed using the modified garden mulcher described in Chapter 2 (Section 2.6). The seed of all umbel orders and plants in each replicate was pooled to give 3 replicate seed samples per line. Seed cleaning and storage procedures were as described in Chapter 2 (Section 2.6).

## **Seed Yield and Quality Assessment**

Seed yield data were obtained for each line by the low constant temperature method described in Chapter 2 (Section 2.7). Three replicates of 100 seeds were germinated for each line (1 from each block) according to the method in Chapter 2 (Section 2.8). Final counts of normal seedlings and failed germinants were made at day 14. Failed germinants were classified as dead seeds, fresh ungerminated seeds or abnormal seedlings in accordance with ISTA guidelines (ISTA, 1979; 1993). The caruncles of fresh ungerminated seeds of 12 lines from each location (see Table 3.2) were removed and the embryos extruded with a scalpel blade and examined at 20x magnification under a dissecting microscope. Seeds that failed to produce an embryo using this technique were dissected longitudinally and examined for the presence of embryo tissue. Additional germination and embryo data were collected from commercial and experimental seed lots of Nantes, Amsterdam, Flakkee and Kuroda types grown in South Australia and Tasmania in the 1998-99 and 2000-01 seasons.

## **Statistical Analysis**

Seed yield and percentage germination data were analysed for each location using ANOVA analysis for randomised complete block designs. Significantly different means were separated at the 5% value by Fisher's LSDs. Regression analysis was used to examine the relationships between the occurrence of embryoless seeds in seed lots and percentage germination and seed yield.

### **3.3 - Results**

#### **Seed Yield**

The seed yields of male sterile and open pollinated parent lines from Cambridge, Mount Gambier and Winchmore are shown in Table 3.2. Across all lines, there was a general trend of higher seed yields at Cambridge compared to Mount Gambier or Winchmore, with overall mean seed yields of 10.4, 6.2 and 7.7g/plant at these locations respectively.

There were large differences between the yields of some, but not all, lines at each location ( $P < 0.001$ ) and between locations, ranging from 0.6g/plant for WO6002 at Mount Gambier to 23.4g for ON44-92AxON44-91B at Cambridge (Table 3.2). Within the Nantes varieties, F1 hybrid lines had, on average, higher yields than the inbred lines at each location (for example, 11.4g compared to 8.6g at Cambridge) but, with the exceptions of ON44-92AxON44-91B and No. 27 at Winchmore, the yields of F1 hybrid lines were not significantly higher than the better yielding inbred lines. Similarly, with the exceptions of WO127 at Cambridge and WO336 at Mount Gambier, the yields of open pollinated lines at each location were not significantly higher than many of the inbred or F1 hybrid parent lines.

In both F1 and inbred lines there was a wide range of seed yields at each location. At Cambridge this was due to both variation in plant size and the percentage of flowers on plants that set seeds (Table 3.3). Within the male sterile lines sampled for seed set at Cambridge, between 32% (WO6014) and 64% (No. 22) of flowers with ovaries set seed. In comparison seed set in the two open pollinated lines grown at Cambridge, WO158 and WO127 was 85% and 86% respectively.



**Table 3.2** – Seed yield data (on a dry weight basis) for variety trials at Cambridge, Mount Gambier and Winchmore. Standard errors are given in *italics* (n=3). Differences between lines within each location are statistically significant (P<0.001). LSD (P<0.05): Cambridge, 5.95; Mount Gambier, 2.50; Winchmore, 2.60. \*Data not collected. Lines ranked on mean yield over all sites within root type.

Root type	Sex	Breeding	Line	Mean dry seed yield/plant (g)						Line Mean
				Cambridge		Mt. Gambier		Winchmore		
Nantes	Male sterile	Inbred	ON44-193A	10.1	3.4	17.5	1.8	9.2	2.3	12.2
			ON44-198A	18.1	4.5	5.3	0.8	12.3	1.5	11.9
			ON44-227A	17.5	4.0	6.3	1.0	9.2	3.5	11.0
			ON44-5002A	11.2	5.2	5.7	0.6	9.5	0.4	8.8
			ON44-143A	7.3	1.6	4.0	0.7	9.0	3.3	6.8
			WO6003	6.0	0.9	6.5	0.8	7.6	2.3	6.7
			ON44-5015A	9.9	0.7	4.6	0.6	5.5	1.2	6.7
			ON44-88A	8.9	2.2	4.6	0.5	5.9	2.2	6.5
			ON44-43A	7.3	2.9	3.4	0.4	5.8	0.9	5.5
			ON44-158A	3.9	1.5	4.4	0.4	7.6	2.1	5.3
			ON70-1260A	4.8	2.0	3.5	0.4	7.0	1.3	5.1
			WO6014	3.8	3.3	2.8	0.4	5.3	0.6	4.0
			ON44-179A	3.2	0.6	3.7	0.6	3.2	1.0	3.4
			Mean	8.6	0.2	5.6	0.7	7.5	0.2	
Nantes	Male sterile	F1 Hybrid	ON44-92AxON44-91B	23.4	3.5	7.1	1.2	16.6	2.0	15.7
			27	6.7	1.8	15.9	0.7	17.3	3.9	13.3
			10	10.1	1.5	18.4	2.0	6.4	0.5	11.6
			22	9.8	3.3	8.1	1.2	11.7	2.4	9.9
			16	7.0	1.0	3.1	0.9	7.2	0.5	5.8
			Mean	11.4	1.0	10.5	3.6	11.9	6.6	
Nantes	Open pollinated	na	WO158	13.2	2.2	4.8	0.5	4.7	1.7	7.6
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Berlicumer	Male sterile	Inbred	WO6006	11.9	7.8	4.5	0.5	4.7	0.7	7.0
			OB44-155A	10.0	1.1	2.8	0.4	7.0	1.9	6.6
			WO6010	8.6	2.9	3.8	0.5	4.7	0.9	5.7
			Mean	10.1	0.5	3.7	0.5	5.5	0.7	
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Flakkee	Male sterile		WO6008	9.3	1.5	7	0.7	5.8	0.7	7.4
			WO6019	10.3	1.2	0.8	0.0	4.6	2.3	5.2
	Open pollinated	NA	WO127	19.7	1.4	7.9	2.7	6.0	0.2	11.2
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Amsterdam	Male sterile		NZ1	7.5	0.3	1.2	0.2	6.7	1.1	5.1
			WO6002	10.8	0.8	0.6	0.0	3.7	0.7	5.0
Amsterdam	Open pollinated	NA	WO336	*		7.8	2.1	3.4	0.8	5.6

**Table 3.3** – Average seed set of the primary and 4<sup>th</sup> secondary umbels (expressed as the percentage of flowers with ovaries setting seed) in male sterile and open pollinated lines from the Cambridge variety trial. The result for each line was calculated from pooled samples taken from 9 plants.

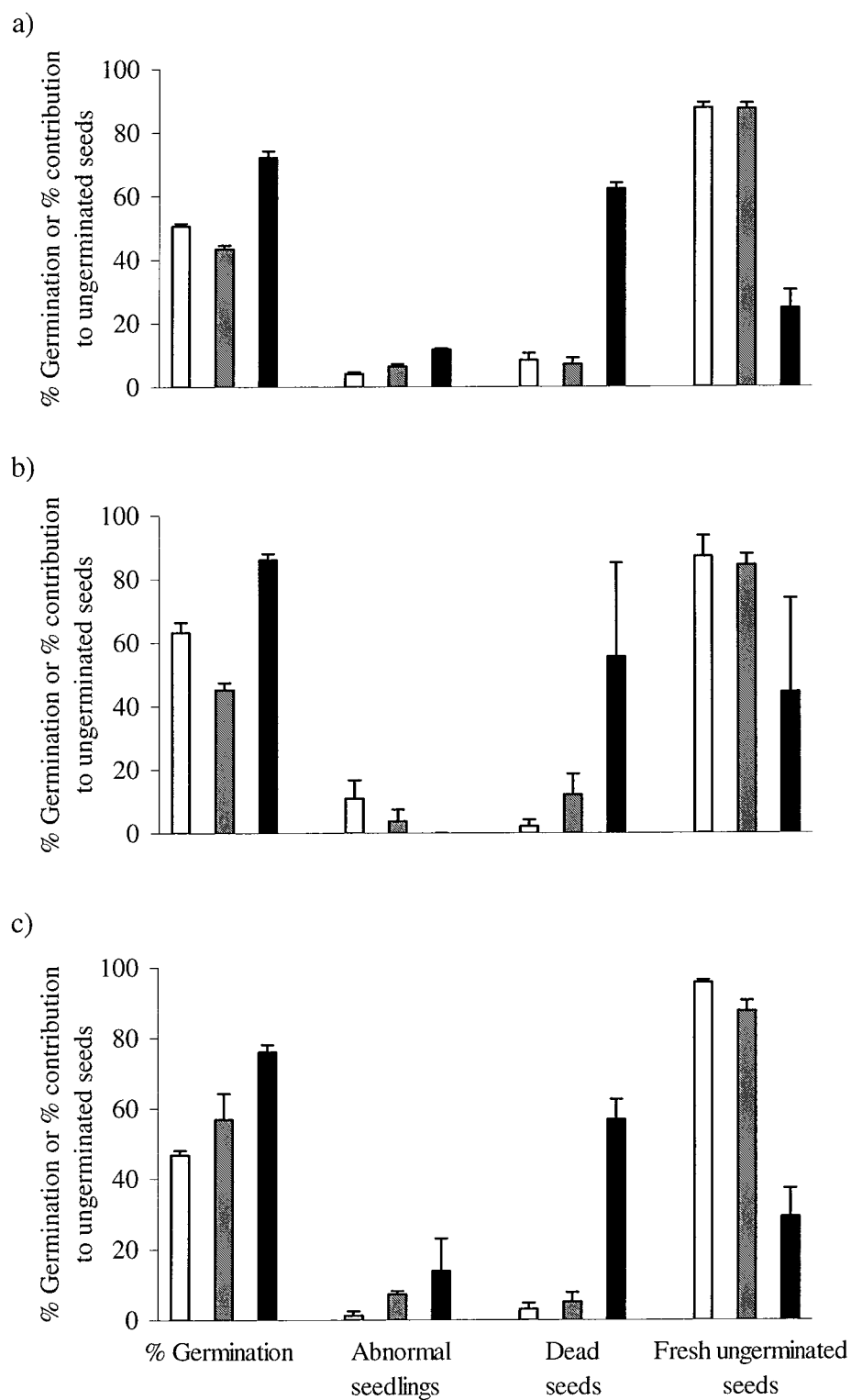
Line reference	Breeding	CMS type	% seed set
WO6014	inbred	Green/white petaloid	32.0
ON44-43A	inbred	White petaloid	32.2
ON70-1260A	inbred	White petaloid	35.5
No. 27	F1	Brown anther	43.7
No.16	F1	Green/white petaloid	57.3
ON44-198A	inbred	Pale green petaloid	63.9
ON44-5002A	inbred	Pale green petaloid	63.9
No.22	F1	Brown anther	64.0
WO158	OP	NA	85.0
WO127	OP	NA	86.0

## Germination

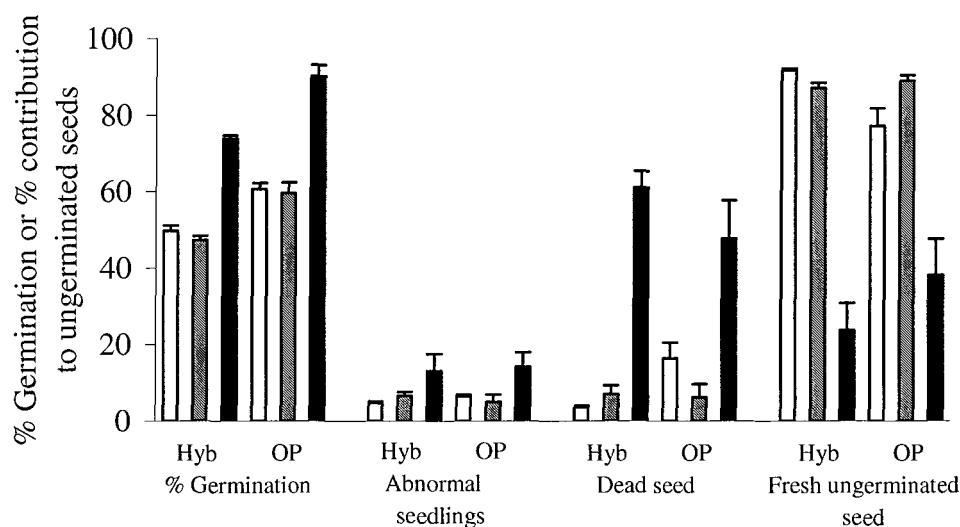
There were significant ( $P < 0.001$ ) differences between the germination percentages of seed of some lines at each location (Table 3.4), with ranges of 24 to 73% at Cambridge, 28 to 74% at Mount Gambier and 38 to 96% at Winchmore. There was little consistency between any of the locations in terms of high or low germinating seed lines. Averaged across all lines, germination was considerably higher for seed from Winchmore (75%), compared to either Cambridge (52%) or Mount Gambier (48%). The pattern of higher germination percentage at Winchmore compared to Cambridge or Mount Gambier occurred across all carrot types (Nantes, Flakkee and Amsterdam) and both hybrid and open pollinated lines (Figures 3.2 and 3.3). Differences of less than 14% in average germination percentage across carrot types were observed at each location, but the best-performed root type varied from Amsterdam at Cambridge (63.2%) and Winchmore (86%) to Flakkee at Mount Gambier (56.9%). The average germination percentage of open pollinated lines was 11 to 16% higher than hybrid seed parent lines at all locations (Figure 3.3).

**Table 3.4** – Day 14 germination test results for seed of male sterile and open pollinated parent lines from the variety trials at Cambridge, Mount Gambier and Winchmore. Standard errors are given in italics (n=3). Differences between individual lines at each location are statistically significant (P<0.001). LSD (P<0.05): Cambridge, 13.3; Mount Gambier, 12.0; Winchmore, 15.8.

Parent Type	Sex	Breeding	Parent line	% Normal seedlings day 14 +/- (s.e)					
				Cambridge		Mt. Gambier		Winchmore	
Nantes	Male sterile	Inbred	ON44-88A	72.2	2.9	51.5	2.8	85.4	1.9
			ON44-227A	68.9	1.4	32.3	3.2	88.6	1.9
			ON44-143A	63.9	0.5	67.9	4.6	48.9	6.6
			ON44-5015A	60.8	4.6	34.4	1.0	39.1	6.5
			ON44-198A	55.2	1.2	58.7	1.2	55.1	9.8
			ON44-5002A	50.6	3.3	51.1	1.3	94.0	1.6
			ON44-179A	49.7	7.8	30.3	5.0	57.9	6.8
			ON70-1260A	49.0	1.9	46.0	3.6	85.3	1.7
			ON44-158A	43.4	5.9	38.1	2.9	37.5	10.6
			ON44-43A	39.2	8.9	34.6	1.4	74.5	4.7
			WO6003	30.9	1.4	27.5	1.3	66.8	2.4
			ON44-193A	28.7	1.4	42.1	5.0	71.0	5.4
			WO6014	24.7	2.0	54.9	5.2	86.9	2.4
	Male sterile	F1Hybrid	27	66.1	4.5	49.5	2.0	84.2	4.7
			22	66.1	2.6	46.2	2.4	75.8	11.2
			10	53.4	4.0	71.0	11.5	89.7	3.8
			16	42.2	2.9	57.4	1.3	66.4	11.1
			ON44-92AxON44-91B	36.3	2.1	27.7	0.1	77.9	2.8
	Open pollinated	NA	WO158	59.3	9.3	49.3	1.6	84.8	9.7
Flakkee	Male sterile		WO127	49.2	3.0	73.6	0.3	90.4	0.5
			WO6008	45.9	4.1	47.4	2.3	81.8	2.8
	Open pollinated	NA	WO6019	44.9	5.0	49.6	3.0	55.8	4.0
Amsterdam	Male sterile		WO6002	78.4	3.4	43.8	1.2	91.5	3.5
			NZ1	37.9	5.3	36.0	2.0	71.0	1.2
	Open pollinated	NA	WO336	73.3	4.0	55.9	3.7	95.6	1.4
Berlicumer	Male sterile	Inbred	OB44-155A	72.6	4.2	62.4	10.8	58.0	11.9
			WO6010	61.1	0.9	41.6	3.4	88.4	4.9
			WO6006	23.7	1.4	61.1	1.2	95.5	1.6



**Figure 3.2** – Mean % normal seedlings at day 14 and % contribution of abnormal seedlings, dead seed and fresh ungerminated seed to failed germinants in germination tests of seed of all Nantes (a), Amsterdam (b) and Flakkee (c) lines at Cambridge (□) Mount Gambier (▨) and Winchmore (■). The number of lines included from each location was: Nantes, 19; Amsterdam, 3; and Flakkee, 3.

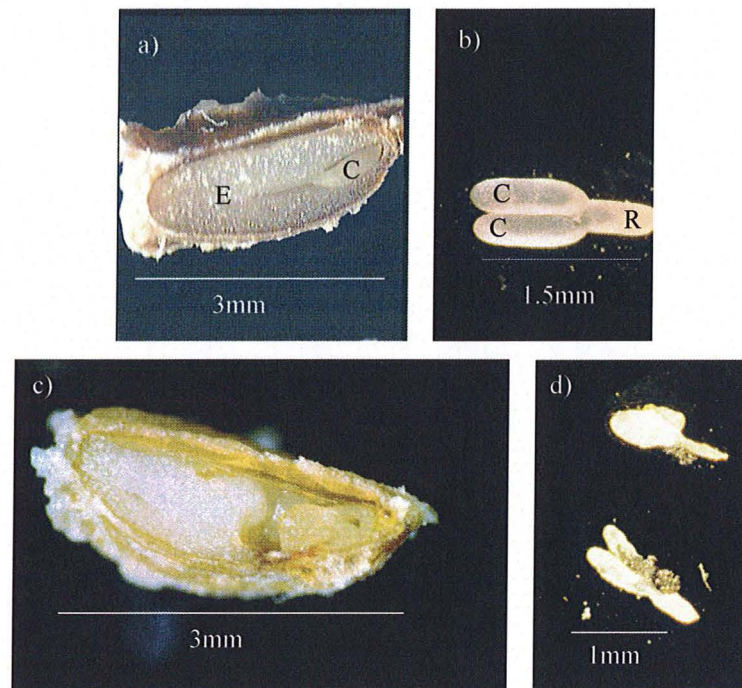


**Figure 3.3** - Mean % normal seedlings at day 14 and % contribution of abnormal seedlings, dead seed and fresh ungerminated seed to failed germinants in germination tests of seed of all open pollinated and hybrid lines at Cambridge (□) Mount Gambier (▨) and Winchmore (■). The number of lines included from each location was: open pollinated, 3; and hybrid, 25.

The reasons for failed germinants were relatively consistent across carrot types and male sterile and open pollinated lines within each location but differed markedly between locations (Figures 3.2 and 3.3). For seed produced at Winchmore, 56 to 74% of germinants that failed to produce normal seedlings were dead, due to a visible fungal infection, or were classified as abnormal seedlings. Most abnormal seedlings were late germinating and failed to develop sufficiently in the duration of the test or had formed stunted primary roots. A smaller proportion, 26 to 44%, was classified as fresh ungerminated. Greater than 84% of all failed germinants from Cambridge and Mount Gambier were fresh ungerminated seeds, with small quantities of abnormal seedlings and dead seed.

Nearly all (98%) of fresh ungerminated seed from Cambridge and Mount Gambier did not contain an embryo or had an embryo that appeared to have been damaged during

or after development (Figure 3.4). The proportions of each varied, but were typically 35 to 45% embryoless and 55 to 65% damaged embryos at both locations.



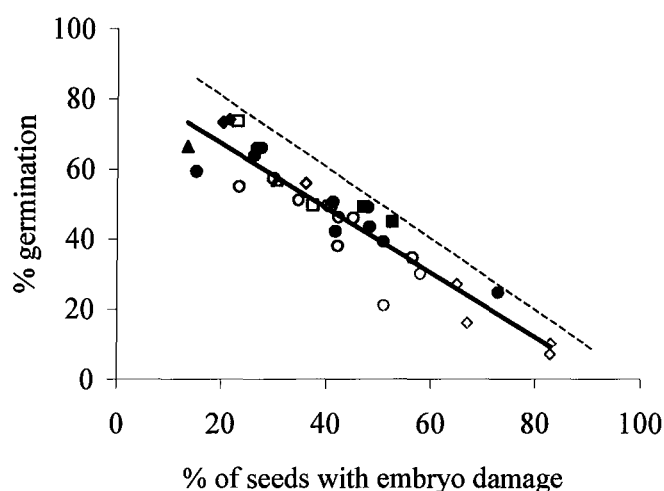
**Figure 3.4** - Embryolessness and embryo damage in carrot seed produced in south-eastern Australia. a) Longitudinal section of mature, healthy carrot seed showing endosperm tissue (E) and embryo embedded in a space in the endosperm at the distal end of the seed. A single cotyledon (C) of the embryo is clearly visible. b) A healthy mature carrot embryo showing 2 cotyledons (C) and the radicle (R). c) Embryoless carrot seed showing damage to the endosperm tissue surrounding the area normally occupied by the embryo. Although not visible in this image, the pocket in the endosperm in which the embryo lies was often visible in dissected embryoless seeds. d) Embryos showing extensive damage to the radicles and cotyledons.

Despite their condition, embryoless and embryo damaged seeds appeared to have otherwise developed normally apart from an area of visible damage or necrosis in the endosperm tissue normally surrounding the embryo (Figure 3.4). There was no visible evidence of damage to the seedcoat. In most embryoless seeds, a pocket had formed in the endosperm where the embryo would otherwise be located. In contrast to healthy carrot embryos that are smooth with a defined shape and firm, white tissue, damaged embryos were irregularly shaped due to a loss of tissue and featured areas of necrotic

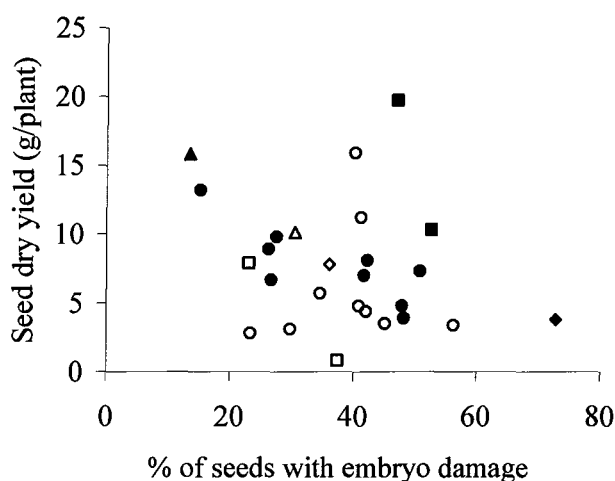
or transparent tissue (Fig 3.4). The damage did not appear to be focussed on any specific area of the embryo, occurring equally on the cotyledons and radicle and occasionally over the entire embryo. Apart from the damaged areas, embryos often appeared otherwise normal, with many of equivalent size to normal healthy embryos (approximately 1.5mm from radicle tip to cotyledon tip).

The remaining 2% of fresh ungerminated seeds from Cambridge and Mount Gambier were observed to have very small embryos (<0.2mm), or were normal in appearance. 72% of fresh ungerminated seeds from Winchmore had rudimentary embryos, or were normal in appearance. The remaining fresh ungerminated seeds were embryoless (24%) or had embryo damage (4%) similar to that observed for seed from the Australian sites.

The relationship between the occurrence of embryo damage (including embryolessness) in seed lines and their germination percentage and yield was examined for three seasons of trials and commercial crops in Tasmania and South Australia. A significant linear relationship ( $P < 0.001$ ,  $r^2 = 0.88$ ) was observed between the occurrence of embryo damage and percentage germination across three European carrot types, Amsterdam, Flakkee and Nantes and the Asian type Kuroda in the three seasons in both states (Figure 3.5). No relationship was observed between the occurrence of embryo damage and yield for carrot seed grown in Australia (Figure 3.6).



**Figure 3.5** – The relationship between the occurrence of embryo damage (embryoless seeds and seeds with embryo damage) and percentage germination for seed of Nantes (●) Amsterdam (◆) Flakkee (■) and Kuroda (▲) parent types grown in 1998-99 (blue), 1999-2000 (black) and 2000-01 (red) at Cambridge (filled shapes) and various locations in South Australia (open shapes). 1999-00 data were collected from experiments reported in this chapter. Other data refer to germination results from commercial production and research reported in the later chapters. The solid line shows the linear regression  $y = -0.9207x + 85.706$  ( $P < 0.001$ ;  $r^2 = 0.88$ ) 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.



**Figure 3.6** - The relationship between the occurrence of embryo damage (embryoless seeds and seeds with embryo damage) and dry yield of seed for Nantes (●) Amsterdam (◆) Flakkee (■) and Kuroda (▲) parent types grown in 1999-2000 at Cambridge (filled shapes) and various sites in South Australia (open shapes).



### **3.4 - Discussion**

The mean seed germination percentage, calculated across all lines, was higher at Winchmore compared to either of the Australian sites. The wetter than average summer to autumn period in Winchmore during the trials was likely to have caused the relatively large proportion of failed germinants with fungal infections in seed lines from this site. It is probable that in typical seasons across all sites the germination difference between Winchmore and the Australian sites would have been even more apparent.

At the Australian trial sites, most failed germinants were embryoless or had embryos that appeared to have been damaged. It is probable that the embryoless seed and embryo damage had common causes, given the extent of variation in embryo damage, the common target in each instance (the embryo) and the similarity of surrounding endosperm damage described for both. In contrast to seed from the Australian sites, embryoless seeds occurred infrequently in seed lines from Winchmore.

The results from variety trials, coupled with data collected from commercial crops produced in 3 seasons at different sites in south-eastern Australia provide clear evidence that embryoless seeds and embryo damage are the primary reasons for low germination of Australian carrot seed. The low frequency of embryoless seed and embryo damage in seed lots from Winchmore compared to the Australian sites indicates that the major cause or causes in Australia were either absent, or had much less impact under the conditions at Winchmore during the variety trials. In Australia, the occurrence of seeds without embryos or with embryo damage was independent of seed yield. This is consistent with the consensus drawn from the literature on overseas carrot seed production (Section 3.2).

The occurrence of embryoless seeds within the Apiaceae in other countries has been well documented (Flemion, 1949; Robinson, 1954). In the United States, Europe and Africa embryoless seed in the Apiaceae has been attributed to feeding damage caused by the tarnished plant bug (or *Lygus* bug) *Lygus obliateus* Say and several closely related species. (Flemion, 1949; Robinson, 1954). *Lygus* bugs do not occur in

Australia but other Hemipteran seed feeding insects occur in the areas of Australia in which carrot seed is grown (Tillyard, 1926; Carver *et al.*, 1991). Although there has been no reported examination of the insect fauna visiting carrot seed crops in south-eastern Australia, seed feeding members of the genus *Nysius* have been reported in carrot seed crops by industry personnel and were observed in the carrot seed parent line trials at Cambridge and Mount Gambier.

Alternative mechanisms proposed for the formation of embryoless seed in the Apiaceae such as single fertilisation (Jurica, 1922) do not appear to be responsible for the majority of embryoless seeds observed in this work. During development, the embryo is surrounded by endosperm. Some of this tissue appears to be broken down to support embryo growth, thereby forming a pocket in which the mature embryo rests (Borthwick, 1931b). In many embryoless seeds observed in this work, a pocket had formed in the endosperm where the embryo would normally lie, suggesting that embryo development had commenced. The observation that single fertilisation occurs with a relatively low frequency in the Apiaceae (Jurica, 1922) is also inconsistent with the high frequency of embryoless seed observed in Australian seed lines.

Embryo abortion does not explain the embryoless seed and embryo damage that was observed. Firstly, the damage often occurred on otherwise fully developed embryos with no consistent location on the embryo where it occurred. Furthermore, there is a general lack of references to abortion of carrot embryos, or reductions in germination percentage when treatments are applied that should alter resource partitioning during flowering and seed development (Gray, 1981; Gray *et al.*, 1986; Steiner *et al.*, 1990). Negative correlations between temperature and seed quality in surveys of production regions in North America have been observed (Lacey, 1986; Dean *et al.*, 1989). These studies do not specify if the reduction in seed quality was due to an increased frequency of embryoless seed and, if it was, it is unclear if the effect was causal. Neither single fertilisation, nor abortion of the embryo accounts for the damage to the endosperm surrounding the embryo that was observed in this work.

Other than seedling decay, the major limitations of germination at Winchmore were the incidences of seeds with rudimentary embryos and abnormal seedlings. Although documented in the literature as significant causes of low germination from overseas

production locations (Robinson, 1954; Dean *et al.*, 1989), abnormal seedlings and seeds with rudimentary embryos were relatively infrequent in germination tests of Australian carrot seed. Temperature conditions at Winchmore during flowering and seed maturation were slightly lower than average and cooler than conditions at the Australian sites. Industry personnel managing the variety trial at Winchmore reported a longer than normal flowering period. Such observations are consistent with the hypotheses proposed in the literature review (Section 3.2) that either condition could be associated with immaturity of the embryo at harvest.

The variety trials studied in this work were not specifically designed to examine seed yields but several observations relevant to crop yield were made. Many of the yields of male sterile seed parents were high compared to the yields achieved in commercial crops, suggesting that there is potential for higher commercial seed yields. For example, in commercial production centred on Mount Gambier, yields of Nantes male sterile lines typically range from less than 1 to 7 grams per plant (Fulton, 1999) compared to 2.8 to 17.5g at Mount Gambier in these trials. The consistent trend of higher yields at Cambridge compared to either Mount Gambier or Winchmore suggests that yield improvement could be achieved through choice of production environment, but the key environmental differences promoting higher yields are unclear.

Examination of seed set at Cambridge showed that, despite the generally higher than normal yields, a relatively low percentage of flowers with ovaries set seeds on inbred and F1 male sterile lines compared to open pollinated lines. Seed yields of hybrid varieties may therefore be limited in part by processes during pollination or early seed development. It has been suggested that inadequate pollination may limit the yield of hybrid carrot seed crops (Erickson and Peterson, 1978; Erickson and Peterson, 1979; Erickson *et al.*, 1979; Galuszka and Tegrek, 1987; Galuszka, 1989; Rodet and Torre Grossa, 1991) but conclusive data have not been presented. Although Erickson (1979) and Galuszka *et al.* (1989) have reported different levels of pollinator attraction between CMS types, there was little evidence of an overriding effect of CMS type on seed yield within the lines studied in this work.

## Chapter 4

### **Feeding Damage and Management of *Nysius vinitor* Bergroth (Hemiptera: Lygaeidae) in Relation to Carrot Seed Yield and Quality**

*The work reported in Chapter 3 established that the low germination percentage of Australian carrot seed was primarily due to high levels of embryoless seeds and seeds with damaged embryos. The only documented cause of embryoless seeds in the Apiaceae is the feeding of the tarnished plant bug (Lygus oblineatus Say) and several other closely related species. These insects are endemic to the United States, Europe and Africa (Flemion, 1949) but are not found in Australia. It is possible that other insects found in Australia cause the same damage in carrot seed crops. Insects of the Hemipteran genus Nysius are often reported in carrot seed crops by field officers and were observed in the Tasmanian variety trial (Chapter 3). Applications of pesticide to carrot seed crops following observation of Nysius appear to have been largely ineffective in increasing the percentage of seed lines achieving export germination standard. This could indicate that Nysius is not the cause of low germination, or that the control strategy that was used was ineffective.*

*The research that is reported in this chapter examined the visitation of seed feeding insects to carrot seed crops in South Australia and Tasmania. The hypothesis that Nysius vinitor Bergroth (Hemiptera: Lygaeidae) caused loss of germination in carrot seed was tested. Subsequent experiments examined some important aspects of the biology and management of N. vinitor in Australian carrot seed crops. The research is supported by a literature review on the distribution, biology, behaviour and management of Nysius.*

## **4.1 - Literature Review**

### ***Nysius* (Dallas) in Australia and New Zealand**

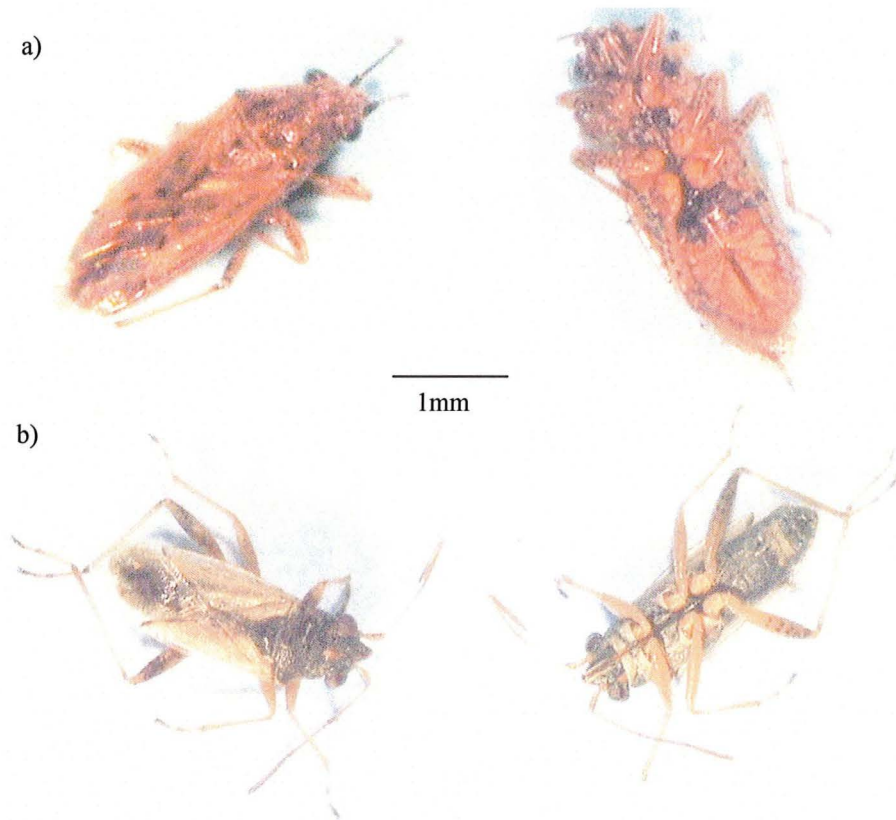
*Nysius* (Dallas) is a large, cosmopolitan genus containing over 100 species. It is classified within the Hemiptera as follows: sub-order Heteroptera; superfamily Lygaeoidea; family Lygaeidae; subfamily Orsillinae (Carver *et al.*, 1991). It includes important agricultural pests from South Africa, the United States, the Mediterranean and the Asia Pacific region ( Martin Kessing and Ronald, 1993; Hill, 1994; DuPlessis, 2002).

Three endemic species of *Nysius* are reported from Australia, *N. cleavlandensis* Evans, *N. vinitor* Bergroth and *N. turneri* Evans (Woodward, 1964). These species are commonly known as the grey cluster bug, the Rutherglen bug and the Invermay bug respectively. No other species of *Nysius* are known to be present in Australia.

*N. vinitor* occurs in all Australian states (Evans, 1943) and causes economic damage to a wide variety of cultivated crops including fruit (French, 1918 cited in Attia, 1982; Emmett *et al.*, 1992), vegetables (Prescott, 1940 cited in Attia, 1982; Dillard *et al.*, 1993) and oilseed crops, notably sunflower and canola (Broadley and Rossiter, 1982; Broadley *et al.*, 1986). It is considered the most important agricultural pest of the three *Nysius* species present in Australia (Broadley and Rossiter, 1982).

Descriptions of *N. vinitor* have been given by a number of authors including McDonald (1977) and Carver *et al.* (1991). The adult female Rutherglen bug (Figure 4.1a) measures about 3-5mm in length. It is narrow bodied and of a general greyish brown colouration, with darker brown markings. The eyes are black and prominent; the antennae are four segmented; and two simple eyes (ocelli) are located on the top of the head. Two pairs of wings are present and these are folded back over the body when the insect is at rest. The sucking labium is long, and is held horizontally beneath the body when in repose. The adult male (Figure 4.1b) is

noticeably smaller than the female and somewhat darker in colour. The eggs, which are approximately 1mm in length, are white when first laid, but become reddish-brown before hatching. The wingless nymphal Rutherglen bugs are red-brown in colouration.



**Figure 4.1** – Adult Rutherglen bugs (*Nysius vinitor*): a) adult female dorsal (left) and ventral (right) views and b) male dorsal (left) and ventral (right) views.

*N. cleavlandensis* and *N. turneri* are very similar in appearance to *N. vinitor*, but can be distinguished by the presence of long, bristly hairs in addition to pubescent setae, which are particularly obvious on the pronotum (Malipatil, pers. comm.<sup>1</sup>). *N. vinitor* is sparsely covered in pubescent hair but not bristles. Recent studies by Malipatil (unpublished) suggest that *N. turneri* is a synonym of *N. cleavlandensis*, which is in turn a synonym of a south-west Pacific species *N. caledoniae*. Although it is likely that *N. turneri* and *N. cleavlandensis* will be reclassified as *N. caledoniae* in the future, the current classifications are used in this review.

*N. cleavlandensis* and *N. turneri* have been reported to attack summer crops in New South Wales, Queensland and Tasmania, but often as minor species in infestations consisting primarily of *N. vinitor* (Woodward, 1964; Broadley and Rossiter, 1982). *N. vinitor* predominates in the southern states of mainland Australia and in the drier parts of the interior whilst *N. cleavlandensis* is most apparent in coastal areas at subtropical latitudes (Woodward, 1964).

None of the species of *Nysius* found in Australia have been reported from New Zealand but three endemic species occur, *N. convexus* (Ushinger), *N. huttoni* (White), and *N. liliputanus* (Tillyard, 1926; Eyles and Ashlock, 1969). Of the three species, only *N. huttoni*, commonly known as the New Zealand wheat bug, has been identified as an agricultural pest (Eyles and Ashlock, 1969; Every *et al.*, 1992). *N. huttoni* attacks wheat grains at the milk stage of development, which results in the condition known as sticky dough when baking with the flour of damaged wheat (Every *et al.*, 1992). It also feeds on cultivated cruciferous seedlings, with the resulting puncture marks around the stem causing cankerous growth of the tissue or collapse of the plant (Gurr, 1952; 1957 cited in Eyles and Ashlock, 1969). Despite apparent similarities to *N. vinitor* and its wide distribution throughout New Zealand (Eyles and Ashlock, 1969), *N. huttoni* is infrequently observed in carrot seed crops (Ferguson, pers. comm.<sup>2</sup>).

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## **The Biology of Australian *Nysius***

Studies of the biology of *Nysius* in Australia have mainly focussed on *N. vinitor*, with some comparative study of *N. cleavlandensis*. Apart from Evans' (1943) description of the species, there appear to be no studies of *N. turneri* in the literature.

### ***Life Cycle***

Under laboratory conditions (25-30°C) *N. vinitor* and *N. cleavlandensis* have similar life cycles (Malipatil, 1979; Attia, 1982). Both species pass through a teneral period of 1.5 days, with the females commencing oviposition approximately 3 to 4 days after the final moult (Attia, 1982). In the field the eggs are deposited in compact groups of 2 to 7 in the soil, between the glumes of grass spikelets, or in the flower heads of composite plants (Evans, 1943). In excess of 400 eggs may be laid by a single female in her lifetime (Swincer, 1977). These hatch 6 to 8 days after oviposition (Malipatil, 1979; Attia, 1982). Following emergence, there are 5 nymphal moults before becoming an adult (Evans, 1943). The nymphal period ranges between 21 and 29 days (Malipatil, 1979). Adult males and females typically live for 55 to 60 days (Kehat and Wyndham, 1972b). For both species, the rates of development through all stages and overall longevity are largely dependent on temperature and other environmental variables including humidity and food quality. During summer in New South Wales the life cycle from egg to egg is approximately 4 weeks (Anon, undated). Depending on the environment at a given location, between 3 and 8 generations of *N. vinitor* may occur in the field in a year (Nichols, 1932; Kehat and Wyndham, 1972b; Attia, 1982). Whilst in some warm climates, populations may breed year round (Malipatil, 1979), over-wintering usually occurs in the egg form (French, 1918 cited in Attia, 1982) or as 5<sup>th</sup> instar nymphs or adults sheltering under soil and dead vegetation (Evans, 1943; Attia, 1982).



## *Ecophysiology*

The effects of temperature and relative humidity on the life history of *N. vinitor* and *N. cleavlandensis* have been studied (Kehat and Wyndham, 1972b; Kehat and Wyndham, 1974; Attia, 1982). Inverse relationships between temperature and adult longevity, generation time and developmental rate in the range 22 to 35°C under laboratory conditions were observed (Kehat and Wyndham, 1972a). Over this temperature range, decreases in median adult female longevity and generation times were from 90 days to 9 days and 80 days to 19 days respectively. A threshold temperature for development of around 15°C was calculated. This relatively high threshold is consistent with the reported variation in the number of generations per year at different locations. In many areas of south-eastern Australia it is likely that development is largely confined to the warmer months while, during winter, its rate would be very slow.

Whilst data obtained under laboratory conditions are rarely directly applicable to field populations, the results are likely to represent the range for field development. Predictions based on ambient temperatures and the day degree estimates derived from the Kehat and Wyndham (1972b) study overestimated generation times for *N. vinitor* observed under field conditions in Victoria (McDonald and Smith, 1988). Similarly, reports of up to 5 generations annually in Tasmania (Nichols, 1932) and 7 to 8 generations in a year under conditions approximating a field environment in New South Wales (Attia, 1982) suggest more rapid generation times than predicted by the model. Such discrepancies may be explained in part by behavioural adaptations of *N. vinitor* aimed at maintaining optimum body temperatures. When desirable *N. vinitor* appears able to achieve a rapid increase in body temperature during the day through basking (McDonald and Smith, 1988). At night it may maintain higher than ambient temperatures by moving to warmer micro-niches (Wilmer, 1982). *N. vinitor*, like many poikilotherms, also displays a capacity for increased developmental rates under fluctuating temperatures compared to constant temperatures (Kehat and Wyndham, 1972b).

Fecundity and egg survival of *N. vinitor* appear optimal at temperatures between 25 and 30°C (Kehat and Wyndham, 1972b). In excess of 50% mortality was observed in eggs held at 18°C for 18 days, with 100% mortality recorded in eggs held at 12°C. Mortality amongst young nymphs was also dramatically increased by temperatures below 15°C (Kehat and Wyndham, 1974). This suggests that if *Nysius* over-winters in south-eastern Australia, it would most likely be as adults or nymphs in the last stages of development. The longevity of adults at low temperature and under starvation conditions suggests they could probably survive through a relatively mild southern Australian winter period (Kehat and Wyndham, 1974; Attia, 1982). Of several studies that examined the life cycle of *N. vinitor* in the field in south-eastern Australia over a number of years, only McDonald and Smith (1988) reported an over-wintering population in a single, unseasonably warm year.

The estimated capacity for increase ( $r_c$ ) of *N. vinitor* populations increased linearly from 0.43 to 1.69 over the temperature range 22 to 35°C (Kehat and Wyndham, 1972a). This suggests an adaptation of *N. vinitor* to warm habitats. Compared to  $r_c$  values published by Andrewartha and Birch (1954), the upper values for *N. vinitor* are comparatively high for insects, indicating that under favourable conditions, *N. vinitor* populations are able to increase rapidly. Since mean monthly temperatures in south-eastern Australia, even in summer, are generally below 25°C, this potential for increase is unlikely to be fully realised in average seasons.

Although the temperature response of *N. vinitor* has received most attention, similar longevity, generation intervals and fecundity were observed in populations of *N. vinitor* and *N. cleavlandensis* cultured over a year at ambient temperatures in the range 14.7 to 28°C (Attia, 1982). Marked differences in adaptation to humidity between *N. vinitor* and *N. cleavlandensis* have been observed (Attia, 1982). For increasing relative humidities from 45 to 90% the fecundity of individual females of *N. vinitor* halved, when measured over the lifespan of the insect. Over the same range of increasing humidities, fecundity of individual females of *N. cleavlandensis* increased by more than 25%. In both instances the differences were due to the number of eggs laid rather than the percentage that hatched. This difference in

adaptation of *N. vinitor* and *N. cleavlandensis* to humidity is generally consistent with the reported distribution of both insects throughout Australia.

### ***Food and Water Requirements***

The majority of species, both ground and plant living, in the family Lygaeidae, subsist on seeds (Sweet, 1960). The species of *Nysius* present in Australia appear to conform to this generality. A wide range of plants, notably those within the families Asteraceae, Poaceae and Brassicaceae and various others including wireweed (*Polygonum aviculare*; Polygonaceae) (McDonald and Farrow, 1988) and purslane (*Portulaca oleracea*; Portulacaceae) (Elshafie, 1976) are colonised. Most of these hosts are common weed species of Australian pastures and waste grasslands (Donald, 1970). A range of summer crops including sunflower (Broadley *et al.*, 1986), safflower (Attia, 1974 cited in McDonald and Farrow, 1988) and grapes, vegetables and stonefruit (Dillard *et al.*, 1993; McDougall, 1998) are also utilised as temporary hosts.

In most instances, seeds appear to be an essential dietary item for nymphal development and egg production in *N. vinitor* and *N. cleavlandensis* (Kehat and Wyndham, 1972a; Attia, 1974; Chinajariyawong *et al.*, 1989). In the field, nymphs of *N. vinitor* and *N. cleavlandensis* were not detected on sunflowers until seed development had commenced (Broadley and Rossiter, 1982). Diets consisting of the leaves and stems of various weeds and crop hosts visited by *N. vinitor* prevented nymphal development and egg production (Kehat and Wyndham, 1972a; Chinajariyawong *et al.*, 1989). The requirement for a seed based diet was overcome in nymphs and adults of *N. vinitor* that were fed flowers of the composite weeds dandelion (*Taraxacum officinale*) and capeweed (*Arctotheca calendula*) (Kehat and Wyndham, 1972a). Nymphs have also been reared on vegetative growth of beans, although adults fed the same diet did not produce eggs (Attia, 1974).

Differences in the nutritive value of seeds of different plants have been observed for *N. vinitor*. Nymphs fed the seeds of 24 out of 34 species of plants tested developed normally to adulthood within 19 days with low mortality (Kehat and

Wyndham, 1972a). The remaining seed types including 3 from the Apiaceae were less suitable, causing prolonged development, high mortality levels and stunted adults (carrot and celery) or total mortality (parsley). There appears to have been no examination of the suitability of seeds from the Apiaceae for the adult stages of *Nysius* but differences in the types of seeds suitable for nymphs and reproductive females have been reported (Kehat and Wyndham, 1972a; Attia, 1974). Continuity of food supply appears important for optimal levels of egg production in adult females (Kehat and Wyndham, 1972a; Chinajariyawong *et al.*, 1989). Given that the longevity of starved females was equal to that of egg laying females provided with food (Chinajariyawong *et al.*, 1989), most of the food consumed by reproductive females appears to be utilised in egg production.

Access to water is critical to the survival of *N. vinitor* and *N. cleavlandensis* (Kehat and Wyndham, 1972a; Chinajariyawong *et al.*, 1989). Water deprivation results in an almost immediate increase in mortality of adults and nymphs and arrested egg production due to the inability of either species to store water. Because both species are predominantly seed feeders, the sources of nutrients and water may be different. If low water content seeds are the main food supply, water may be obtained from other plant tissues, dew, ground moisture or free water. As most leaf, stem and fruit tissue appears nutritionally inadequate for development and reproduction, it is possible that the heavy infestation of non-seed bearing crops may be to satisfy the need for water.

### **Migration and Dispersal of Australian *Nysius***

Insect migration usually occurs by flight. In general the migrants either travel short distances under their own power within the surface boundary layer (dispersal flights), or climb rapidly to altitudes of several hundred metres to be carried on low-level jet streams that establish at night above the temperature inversion. These strong, stable winds enable migration over several hundred kilometres in a single night's flight, with longer migrations often taking place over a succession of nights (Pedgley and Reynolds, 1995). For all species that migrate above the surface boundary layer, variations in wind velocity and direction impose a strong stochastic

effect on the direction and distance of migratory flight, and hence the destination (Gatehouse and Zhang, 1995).

Long distance migration is an important behavioural adaptation for *N. vinitor*, enabling it to maximise survival and reproductive success in the face of the large temporal and spatial variations in the flowering periods of its major host plants, which are patchily distributed throughout subtropical and temperate Australia. *N. vinitor* was one of the most abundant species in samples of insects migrating in the upper air (100-300m altitude) throughout the year at Trangie in New South Wales (McDonald and Farrow, 1988). Since long distance migrants probably descend at random, without specific targets (McDonald and Farrow, 1988), dispersal flights are likely to be involved in location of suitable hosts after migration. In addition, localised or 'trivial' movements of juvenile and adult *N. vinitor* within and between weeds and neighbouring crops are used to achieve gradual population shifts (Ramesh, 1984 cited in McDonald and Farrow, 1988).

In contrast to *N. vinitor*, *N. cleavlandensis* has not been observed to undertake long distance migratory flights (McDonald and Farrow, 1988). This may be explained by unsuitable climatic conditions for the insect in southern Australia (Woodward, 1964; Attia, 1982) or a lack of suitable airflows from subtropical regions at the time migrations could occur (McDonald and Farrow, 1988). Flights of *N. cleavlandensis* detected throughout the year in Queensland were not phototactic (Woodward, 1964). This suggests that migratory behaviour is not a characteristic of the species, with dispersal in its northerly habitat apparently achieved through dispersal flights and localised displacement.

### ***Seasonal Migration Patterns Within Australia***

Although laboratory studies suggest it is feasible (Kehat and Wyndham, 1974; Attia, 1982), over-wintering populations of *N. vinitor* in south-eastern Australia have rarely been observed. *N. vinitor* is known to over-winter and breed on weedy hosts distributed in parts of New South Wales and Queensland with higher minimum temperatures (Malipatil, 1979). Similarly, over-wintering may occur in

warmer areas of South Australia and in the Northern Territory (McDonald, 1988). Little is known of non-crop host plants in these areas, but during autumn and winter purslane (*Portulaca oleracea*) and various native daisies appear to be important hosts (McDonald, 1988).

In September and October large numbers of *N. vinitor* migrate from subtropical Australia to pastures in south-eastern Australia and sunflower crops in Queensland (Kehat and Wyndham, 1973a; Broadley and Rossiter, 1982; McDonald and Farrow, 1988). During the springtime migrations over Trangie in New South Wales, densities of around 60 000 insects/ha were estimated to be airborne at any point in time (McDonald and Farrow, 1988). A succession of flights of this magnitude would readily explain the reports of rapid colonisation of weeds and crops during spring and summer in most seasons in south-eastern Australia (Kehat and Wyndham, 1973a; McDonald and Smith, 1988).

The density and timing of springtime migrations to southern Australia appear to be a function of host availability and distribution during the winter and spring period (McDonald, 1988) and the occurrence of synoptic airflows suitable for transporting the insects (McDonald and Smith, 1988). The level of winter and springtime rainfall is believed to be an important determinant of the numbers of migrants and hence the infestation pressure experienced in agricultural crops, because of its limiting effect on the growth of the host plant species (Swaine and Ironside, 1983). It is unclear if this effect is due to a reduction in the production of migrants, or a reduction in the quality of food supplies, which may prevent individuals from accumulating sufficient reserves to undertake migratory flights. Localised flight activity of *N. vinitor* was reported to be less affected by drought conditions than migratory flight activity (McDonald and Farrow, 1988).

Capeweed (*Arctotheca calendula*) and other weeds of the Asteraceae, which are a major component of spring time pastures of southern Australia (Donald, 1970) serve as important host plants for the immigrants to south-eastern Australia (Kehat and Wyndham, 1973a). Eggs are laid in the flowers of the hosts, producing one large generation (McDonald and Smith, 1988). After a period of hot weather in mid November to early December, capeweed rapidly dries off, an occurrence that

coincides with the dispersal or migration of the new generation of adults. The main destination of adults, which depart *en masse* from dried off weeds in November and December is unclear, but is likely to be early sunflower crops and a range of horticultural crops (McDonald, 1988). Other than these, the only potential hosts seem to be late flowering *A. calendula* in mountainous and coastal areas of southern Australia (McDonald and Smith, 1988). The source of insects known to invade summer crops after December has not been established but may be late maturing spring weeds or earlier crops further to the north (McDonald, 1988). The summer generation ends with a movement of adults to autumn weeds such as purslane and wireweed (McDonald and Smith, 1988). Whilst these may provide some opportunity for breeding, autumn populations decline to very low levels at the start of winter (Kehat and Wyndham, 1973a). Year round presence and breeding of *N. vinitor* in south-east Queensland has been observed (Malipatil, 1979) but it is unclear if the subtropical winter population consists mainly of individuals that persist in the area through the summer or immigrants that return from southern Australia in autumn.

Throughout the seasonal cycle, host plant preferences appear to be strongly influenced by region. Aggregations of insects on *A. calendula* and *P. aviculare* have been reported from both South Australia (Kehat and Wyndham, 1973b) and Victoria (McDonald and Smith, 1988); but in New South Wales, where these weeds are also prolific, *Portulaca oleracea* was considered the major host (Elshafie, 1976). Late flowering sunflowers (March to May) failed to attract *N. vinitor* (Broadley and Rossiter, 1982), despite the fact that breeding continues in late summer and autumn on other preferred hosts such as *Polygonum aviculare* (McDonald and Smith, 1988). Summer crops, including sunflower, may provide a temporary niche habitat until *P. aviculare* or other preferred hosts commence flowering in February.

There has been no study of the migration and dispersal of *Nysius* in Tasmania. Given that the winter climate in the south-east of mainland Australia appears unsuitable for the breeding or survival of *N. vinitor*, annual reinvasion from the mainland is probably an important factor in its population dynamics in Tasmania. The flight distance involved is within the range of migratory flights of *N. vinitor*

calculated by McDonald (1988). Similar migrations into Tasmania are known to occur for other insect species including the noctuid moths *Persectania ewingii*, *Heliothis punctiger* and *Agrotis munda* (Drake *et al.*, 1981) and the bush fly *Musca vetustissima* (Hughes, 1970 cited in Drake *et al.*, 1981). The seasonal distribution of *N. turneri* in Tasmania has not been studied.

### ***Triggers of Migratory Behaviour***

Although several environmental conditions conducive to large-scale migratory flights of *N. vinitor* have been identified, the causal factors remain unclear, which makes it difficult to accurately predict the timing of these flights. It is often suggested in the literature that migration in *N. vinitor* is a facultative response to lack of water (for example Kehat and Wyndham, 1973a; Swincer, 1977; McDonald and Farrow, 1988). The requirement of the species for almost continuous access to water for survival and reproduction, and observations that it appears to always migrate when host plants desiccate (Kehat and Wyndham, 1972a) add weight to this argument. Because the majority of migrants are newly emerged adults (Kehat and Wyndham, 1973a; McDonald and Farrow, 1988) it is difficult to establish if they are obligate migrants or, if migration is a facultative response to lack of water. *N. vinitor* has also been observed to migrate when host plants are green and succulent and there seems to be no shortage of food (Kehat and Wyndham, 1973a). Seasonal migration of *N. vinitor* has been reported to follow the source population curve, with a continual production of migrants and succession of flights of departing individuals (Kehat and Wyndham, 1973b). Similarly, migratory flights were observed over Trangie in New South Wales during all months of the year except July and August when temperatures were too low to permit flight (McDonald and Farrow, 1988). These observations cannot be explained by the hypothesis that lack of water triggers migration but suggest that migratory behaviour may be an obligate response, or is promoted by other environmental cues.

Migratory flights of *N. vinitor* occur during day or night under a range of synoptic conditions (Kehat and Wyndham, 1973a; McDonald and Farrow, 1988) resulting in



the displacement of individuals in various directions and distances according to the conditions at the time of flight. The major migratory flights of *N. vinitor* into south-eastern Australia occurred at night after a dusk take-off when a warm evening followed a hot day (Kehat and Wyndham, 1973a). Major flights were associated with periods of disturbed weather and unstable northerly airflows ahead of a cold front (McDonald, 1988). The reasons for the increased levels of flight both within the surface boundary layer and in the upper air under prefrontal conditions are not well understood, although this phenomenon is widespread amongst migratory insects (Pedgley and Reynolds, 1995). Under such conditions, nighttime temperatures are typically above average, particularly at the top of the inversion layer where migrants are known to concentrate (Farrow, 1986). During pre-frontal conditions there is also an increased probability of rain occurring, the dew point is generally elevated and electrical activity is increased (McDonald and Farrow, 1988). It is unclear if any of these factors exert an effect on the flight activity of *N. vinitor*.

The timing of mass flights may also be regulated by the relatively short period of egg production for each generation. Under laboratory conditions at 25 and 30°C over 70 and 80% of eggs respectively were laid within 14 days (Kehat and Wyndham, 1972b). Under field conditions most nymphs achieve maturity over a restricted period (McDonald and Smith, 1988). Similar synchronisation of development and mass migration has been reported for other Heteroptera including *Dysdercus* spp. (Sweeney, 1960 cited in Kehat and Wyndham, 1972b) and the sunn pest *Eurygaster intergriceps* (Brown, 1965).

### **Crop Damage**

*N. vinitor* and, to a lesser extent *N. cleavlandensis*, are considered important pests in a range of Australian agricultural and horticultural crops. Nymphal and adult insects are known to suck sap from the new shoots of grapevines (Fletcher, 1997) and potatoes (Keller *et al.*, 1997), and seedlings of canola and cereals (Miles and McDonald, 1999; Mangano, 2001) amongst others. On a hot day, when the

transpiration rate is high, or under other conditions of moisture stress, this behaviour results in wilting and ultimately abortion of the tissue or death of the plant (Evans, 1943). In stonefruit and citrus crops *N. vinitor* feeds on the fruit, which causes pitting and exudation of a gum like substance, resulting in unsaleable fruit (Fletcher, 1997).

Amongst seed crops, damage of *N. vinitor* and *N. cleavlandensis* has been reported from oilseeds including sunflower, safflower and canola (McDonald, 1977; Swincer, 1977; Broadley and Rossiter, 1982; Broadley *et al.*, 1986). Feeding damage in sunflower crops has received most attention. Three stages of damage have been described (Swincer, 1977). Prior to flowering adult bugs congregate on the upper stem beneath the developing bud. Their feeding disrupts the vascular tissue and restricts sap flow to the bud, which wilts and then aborts, resulting in a significant reduction in yield potential. During flowering, the bugs feed by penetrating the flowers, causing them to abort. After flowering, the bug punctures the seeds and sucks their contents. This reduces seed weight, oil yield and seed germination percentage. 95% of visible damage to sunflower seeds was reported to occur on the cotyledons (Broadley *et al.*, 1986) but this may reflect the architecture of the sunflower head and accessibility of the tightly packed seeds rather than a preference for a particular area of the seed.

In northern NSW, extremely high populations of Rutherglen bug (up to 500 adults and 1500 nymphs per sunflower head) significantly reduced grain yield, oil content and seed germination (Forester and Saini, 1982 cited in Broadley *et al.*, 1986). Plants suffering from moisture stress experienced higher levels of damage than did irrigated plants, indicating that soil moisture level was a significant factor in damage development. Reductions in germination percentage and yield of up to 20% and 500 kg/ha respectively in unsprayed plots of non-water stressed sunflowers compared to plots sprayed from budding to harvest were observed by Broadley *et al.* (1986).

## Management Options

Control of *N. vinitor* and *N. cleavlandensis* in field crops is based on the use of pesticides and, where possible, fallowing or spraying of areas of weedy alternative hosts. A native parasite of *Nysius* has been identified (Attia, 1973), but has not been developed as a control option for use in agriculture. Little research has addressed the issue of threshold levels of crop infestation at which controls should be implemented. Recommendations for several crops have been made on the basis of practical experience. These include 10 adults or 20 nymphs per plant for canola oil crops in South Australia (Fulton, pers. comm.<sup>1</sup>) and, 10 individuals per plant at budding or 25 per plant before the end of flowering in sunflowers (Broadley *et al.*, 1986; Anon, 2003). If *Nysius* did damage carrot seed germination such recommendations would be of little value to producers because of the difference between the crops, and because recommendations for oilseed crops aim to preserve oil yields rather than to prevent loss of germination.

In the United States, sweep netting of carrot seed fields at 4 to 7 day intervals from flowering to harvest is recommended for monitoring *Lygus* bug population levels (Krivholavek, 1999). With this monitoring technique, thresholds for pesticide application have been set (by trial and error) at 0.2 adults and 0.05 nymphs per 180-degree sweep of a 0.5m diameter net mounted on a 1.2m handle (Krivholavek, 1999). Industry attempts to monitor *Nysius* populations have also been hampered by the inconspicuous nature of the insects on carrot seed plants, which makes a visual assessment of population levels difficult. The sweep netting methodology used for *Lygus* has also proven ineffective due to the tendency of the insects to burrow into the umbels or hide in the leaf axils when approached (Dalrymple, pers. comm.<sup>2</sup>). Although portable vacuum samplers have been used to measure *Nysius* populations in research (Broadley and Rossiter, 1982; McDonald, 1986b), practical alternatives for commercial monitoring do not appear to have been examined in the literature.

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## Conclusion

*Nysius vinitor* and, to a lesser extent, *N. cleavlandensis* are considered important pests in Australian agriculture. The success of *N. vinitor* in particular in Australia is linked to the flexibility of its behaviour and habitat use. Its mobility and polyphagous feeding habit combined with the capacity for rapid multiplication under favourable conditions enable it to fully exploit niche habitats provided throughout the season by a succession of annual spring and autumn weeds and summer crops.

Whilst it remains unclear if the damage observed in Australian carrot seed is caused by an insect, the nature of the damage and the fact that *Lygus* bugs are the only known cause of embryoless seed in members of the Apiaceae make this a logical starting point. Based on this information several lines of argument can be advanced which justify an examination of the role of *Nysius*, in particular *N. vinitor*, in the production of non-viable carrot seed in Australia.

Unidentified species of *Nysius* are known to visit carrot seed crops. The literature on the distribution of the species suggests that these would most likely be *N. vinitor* on the Australian mainland and *N. vinitor* or *N. turneri* in Tasmania. Summertime crops are known to provide an important food source in the succession of *Nysius* between weedy springtime and autumn hosts. Given the large number of insects that breed up on springtime hosts, the relative shortage of summertime hosts and the later maturation of carrot seed compared to other major summer hosts such as canola, it seems probable that *Nysius* would infest carrot seed crops in large numbers in some seasons.

The symptoms of embryoless and embryo damaged carrot seeds (Chapter 3) are consistent with the type of damage caused by certain seed feeding Hemiptera. Australian species of *Nysius* subsist on the seeds of a range of plants. In the case of sunflower seed, the feeding damage of *N. vinitor* is mainly confined to the embryo in the same way that it is in carrot seed. Although the seeds of carrots appear a less suitable food source than some other seeds for nymphal development of *N. vinitor*, they did not completely prevent development. Thus carrot seed may be used as a

food source if the seeds of more favoured species are unavailable. The value of carrot seed as a food source for adults has not been examined.

The large proportion of embryoless and embryo damaged seed in Australian seed lots indicates that a causal insect would need to infest in large numbers or consist of individuals capable of damaging a large number of seeds. In the oilseed crops in which it has been studied, *N. vinitor* is known to infest in large numbers. Furthermore, relatively low-level infestations appear to cause economic damage to both yields and seed quality.

If Australian species of *Nysius* were causal agents of seeds without embryos and seeds with embryo damage, it would provide a plausible explanation for the very low levels of seeds with these problems in New Zealand seedlots compared to those from Australia. None of the Australian species of *Nysius* are present in New Zealand, and the species that are present have not been noticed in carrot seed crops (Ferguson, pers. comm.<sup>1</sup>).

Collectively, the arguments outlined above provide a strong foundation for the hypothesis that the genus *Nysius*, specifically *N. vinitor* reduces the germinability of carrot seed produced in Australia.

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## 4.2 - Materials and Methods

The work reported in this chapter consists of the series of field experiments outlined in Table 4.1 below.

**Table 4.1** – A summary of experiments reported in Chapter 4

Experiment	Seasons	Location	Purpose
Survey of seed feeding insects in carrot seed crops	1999 - 00 2000 - 01	Cambridge, Tasmania Naracoorte, South Australia	Identification of seed feeding insects visiting carrot seed crops during seed development
Exposure of carrot plants to <i>N. vinitor</i> during mid seed development	1999 - 00	Cambridge, Tasmania	To establish if <i>N. vinitor</i> can damage carrot seed quality
Seasonal prevalence of <i>N. vinitor</i> in carrot seed crops	2000 - 02	Cambridge, Tasmania Richmond, Tasmania Tea Tree, Tasmania Millicent, South Australia Naracoorte, South Australia	To define the time period over which carrot seed crops are infested by <i>N. vinitor</i> . To establish the stages of <i>N. vinitor</i> that infest carrot seed crops. To examine the relationship between populations of <i>N. vinitor</i> in carrot seed crops and surrounding areas
The effect of timing and level of adult <i>N. vinitor</i> infestation of carrot crops	2000 - 01 2001 - 02	Cambridge, Tasmania Naracoorte, South Australia	To establish timing and rate of damage to seed yield and quality by <i>N. vinitor</i> within the timeframe for infestation defined by the seasonal prevalence studies
Monitoring of <i>N. vinitor</i> populations in carrot seed crops	2000 - 02	Cambridge, Tasmania Richmond, Tasmania Millicent, South Australia Naracoorte, South Australia	To evaluate the use of sticky traps for monitoring of <i>N. vinitor</i> in carrot seed crops. To provide guidelines for the placement of sticky traps in carrot seed crops

### Survey of Seed Feeding Insect in Carrot Seed Crops

Surveys were undertaken in Tasmania in 1999-2000 and South Australia in 2000-01 to identify the species of *Nysius* visiting reproductive carrot plants and to ascertain if other seed feeding insects visited. In South Australia the trial site was located in a commercial Nantes type hybrid seed crop located at Naracoorte. The

survey site at Cambridge was a plot of the Nantes hybrid, Vilmorin No. 22, grown in trial grounds at the University of Tasmania Farm. At each location a 15m long block consisting of 2 male fertile and 6 male sterile rows, buffered on all sides by a minimum of 5m of plants was sampled. Insecticides were not applied to the sample or buffer areas during the period from bolting to seed maturation.

Two sampling techniques were used: vacuum sampling of 30 randomly selected male sterile carrot plants; and placement of yellow 200mm x 100mm sticky traps (Agrisense, Pontypridd, UK) at 10cm above canopy height on each side of the survey plots for 1 week. Vacuum sampling and sticky trap placement occurred between 2 and 4pm 0, 30 and 60 days after peak bloom at Cambridge; and 7, 28 and 50 days after peak bloom at Naracoorte. The insects captured with each technique were examined under a dissecting microscope. Those capable of causing seed damage were identified using taxonomic keys (Carver *et al.*, 1991) and descriptions provided by Malipatil (pers. comm.<sup>1</sup>), and counted.

At maturity a sample of seed was collected by hand from 20 randomly selected plants at the two locations. Representative sub-samples of 100 seeds from each location were dissected and examined using the method outlined in Chapter 2 (Section 2.7) for the occurrence of embryoless seeds and seeds with embryo damaged from the surveyed plants.

### **Seed Yield and Quality Effects from Exposure of Carrot Plants to *N. vinitor* During Mid Seed Development**

Parent lines of the Nantes hybrid Vilmorin No. 22 grown at the University of Tasmania Farm trial grounds at Cambridge were used. One week prior to anthesis, 16 plants of the male sterile line were caged individually with single plants of the male fertile line from an adjacent row. The cages were constructed with four, 2100mm steel posts supporting white shade cloth tents with a 30% shade rating. At ground level the cages were firmly sealed with a layer of soil to prevent insects

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<sup>1</sup> Dr Mallik Malipatil, Systematic Entomology Unit, Agriculture Victoria, Knoxfield, Australia

entering or leaving. Immediately after caging, each plant was sprayed with the pyrethroid insecticide Dominex<sup>®</sup> (a.i. alpha-cypermethrin) to kill any insects present. A single nucleus hive of honeybees was placed in each cage at the start of flowering to effect pollination. The honeybees and male fertile plants were removed after the first three orders of male sterile umbels had flowered (approximately 4 weeks). One week after pollination was completed, adult Rutherglen bugs (*Nysius vinitor*) were collected from nearby carrot plants and added to 8 of the cages at a density of 40 per plant, with the remaining 8 cages serving as controls in which all insects were excluded. A completely randomised design was used. Due to the difficulty of sorting live Rutherglen bugs by gender, the populations in each cage were of mixed sex (60:40 M:F), being representative of the populations naturally occurring on adjacent carrot plants. After 20 days exposure, the Rutherglen bugs were killed with a second application of Dominex<sup>®</sup>. All cages were left in place until harvest, 35 days after removal of the Rutherglen bugs. During this period regular inspections were made to ensure no insects had entered or hatched within the cages. For each plant, all umbels bearing seed were pooled and then threshed with a modified plant mulcher, cleaned and stored for subsequent seed yield, 100 seed dry weight and germination data collection. All seed handling and data collection methods were as outlined in Chapter 2 (Sections 2.6 to 2.8). The presence of embryoless seeds or embryo damage was checked for 40 seeds from each plant using the method for embryo excision detailed in Chapter 2 (Section 2.9). The lengths of intact embryos from each treatment were recorded using a video camera mounted on a dissecting microscope. The video image was projected to a colour monitor where it was measured and corrected for magnification.

### **Seasonal Prevalence of *N. vinitor* in Carrot Seed Crops**

Surveys of the occurrence of *N. vinitor* were made in 7 carrot seed fields near Cambridge, Richmond and Tea Tree in the Coal River Valley (Tasmania) and Naracoorte and Millicent (South Australia) in the 2000-01 and 2001-02 seasons (Table 4.2). Within each crop, sampling was undertaken in a 20m long section of 10 beds of plants, consisting of 4 beds of male sterile plants (12 rows) on either side



of 2 beds of male fertile plants. The sampling area plus an additional 5m buffer around all sides was not sprayed with insecticides after bolting. Although the location of the survey sites varied to facilitate commercial spraying operations, they were generally positioned 5m from the end of the rows in the second spray bay from one side of the crop. Temperature data for each site during the survey periods were obtained from temperature loggers placed in Stevenson's Screens located at canopy height within the survey areas.

Each survey site was divided into 120, 2m long, plots of male sterile plants for sampling, with the plots numbered individually. Sampling started at South Australian sites at the end of flowering (2000-01) and at the late stage of bud development (2-3 weeks prior to flowering) (2001-02). In Tasmania sampling commenced when *N. vinitor* was first detected in weekly scouting of the carrot field and surrounding weedy areas. Weekly scouting commenced in July each year. At each site 30 randomly selected plots were sampled for *N. vinitor* at weekly intervals until harvest. Each plant's umbels and stems were vacuumed with a modified leaf blower (Makita, California, USA) to collect any insects present. Preliminary trials indicated that this method collected greater than 95% of *N. vinitor* present on the carrot plants. The vacuum samples were transferred to plastic phials and stored at -20°C prior to identification and counting. For every third plant sampled, the primary umbels were collected for determination of seed moisture content using the low constant temperature method (Chapter 2, Section 2.7).

**Table 4.2** –Commercial crops and experimental plots of carrot seed production used in *N. vinitor* seasonal prevalence and sticky trap monitoring studies. Note: x denotes inclusion of a crop/plot in the specified study.

Season	Location	Line	Seasonal prevalence	Sticky trap evaluation
2000-01	Cambridge	No. 22 Nantes hybrid (experimental)	x	x
	Millicent	Kuroda open pollinated (commercial)	x	
	Naracoorte	W0535 Nantes hybrid (commercial)	x	
2001-02	Richmond	ON44-88A x ON131C Nantes hybrid (commercial)	x	x
	Tea Tree	WO030 hybrid (commercial)	x	
	Millicent	Kuroda open pollinated (commercial)	x	x
	Naracoorte	Nantes hybrid (commercial)	x	x

In 2001-02, sampling was undertaken to determine if *N. vinitor* were present on the soil surface in carrot seed crops. A 0.16m<sup>2</sup> square quadrat was placed at the base of every third plant sampled. The area within the quadrat was vacuumed and the samples bagged and stored at –20°C for subsequent examination.

Observations of *N. vinitor* prevalence in weedy areas adjacent to the survey site were made at Cambridge during 2000–01. In 2001–02, 2 to 3 sites containing alternative host species within 1km of each survey site were identified (Table 4.3), divided into 100 plots and sampled at weekly intervals. Sampling commenced at the time at which *N. vinitor* were first detected in weekly scoutings of the carrot fields and weedy areas. At each sampling time a 0.16m<sup>2</sup> area of 20 randomly selected plots was vacuum sampled as described above, with the samples bagged and stored at –20°C for subsequent examination.

In the laboratory, individuals of *N. vinitor* were sorted out of each sample and classified as nymphs, male adults or female adults and counted.

**Table 4.3** – Site descriptions for examination of *N. vinitor* populations in weedy areas near carrot seed crops. \*Observation only

Season	Location	Site	Major species composition	
			Common name	Taxonomic classification
2000-01	Cambridge - University Farm*	1	Capeweed	<i>Arctotheca calendula</i>
		2	Wireweed and Brassica weeds	<i>Polygonum aviculare</i> <i>Raphanus raphanistrum</i> <i>Sinapsis arvensis</i>
2001-02	Richmond - Strathayr	1	Capeweed	<i>Arctotheca calendula</i>
		2	Wireweed	<i>Polygonum aviculare</i>
2001-02	Tea Tree - Gunn	1	Mixed cultivated and weedy grasses	<i>Lolium sp.</i> , <i>Dactylis glomerata</i> and <i>Hordeum leporinum</i>
		2	As for site 1	
2001-02	Millicent	1	Capeweed	<i>Arctotheca calendula</i>
		2	Canola stubble	<i>Brassica napus</i>
2001-02	Naracoorte	1	Capeweed	<i>Arctotheca calendula</i>
		2	Capeweed	<i>Arctotheca calendula</i>
		3	Canola stubble	<i>Brassica napus</i>

### **The Relationship of Timing and Level of Adult *N. vinitor* Infestation to the Yield and Quality of the Seed Produced**

Caging experiments were conducted at the University Farm at Cambridge in 2000-01 and 2001-02 and Naracoorte in 2001-02 to examine the effect of timing and level of *N. vinitor* infestation on seed yield and quality. Due to an incident involving pesticide drift from an adjacent site and extensive wind damage to the tents used at Cambridge in 2001-02, data were collected from this location in 2000-01 only. Climatic data for Cambridge and Naracoorte during the experimental periods are presented with long-term averages in Table 4.4. The experiments were conducted on four, 20m long adjacent blocks of a 'seed to seed' carrot crop. Each block consisted of a single row of male fertile and male sterile plants 0.8m apart on a 1.2m wide bed. Irrigation was provided by dripper tape laid at the soil surface. In

mid November, approximately 5 weeks prior to flowering, a 20m x 10m isolation tent of white shade cloth with a 30% shade rating was erected over each experiment to prevent insects moving into the experimental plots. At the onset of flowering the four blocks within each experiment were divided lengthwise into 20 plots each, giving a total of 80 plots. Each plot was thinned to leave 3 healthy, uniformly mature male fertile and male sterile plants and sprayed with Dominex<sup>®</sup> to kill any insects present. At peak bloom, 2 large hives of honeybees were placed in each tent for pollination. Both the beehives and the male fertile plants were removed from the experiment 21 days later.

**Table 4.4** – Climatic data from the nearest weather stations to the Cambridge and Naracoorte trial sites, Hobart airport, site no. 094008, and Naracoorte, site no. 026023 during the caging trials (plain text). Both weather stations were less than 10km from the trial sites. Long-term averages of 43 years data (Hobart Airport) and 133 years data (Naracoorte) for the same months are given in italics. Source Australian Bureau of Meteorology climate records.

Month	Mean daily maximum temperature		Mean daily minimum temperature		Rainfall (mm)	
Hobart Airport						
December	21.8	20.5	11.1	10.6	35.4	56.2
January	24.0	22.3	13.1	11.9	10.4	40.5
February	25.4	22.3	13.7	12.0	3.2	36.9
-----						
Naracoorte						
December	23.7	25.4	7.5	10.8	27.4	30.4
January	27.1	28.1	9.3	12.2	10.6	23.3
February	27.3	28.8	8.8	12.6	3.2	19.9

Treatments consisting of 5 levels of adult Rutherglen bug infestation (0, 5, 10, 20 and 40 adults per plant) of 10 days duration were applied at 4 stages of seed development. The treatments were arranged in a randomised complete block design. Twenty of the small cages described earlier were used to enclose the insects within the plots. These were erected and removed immediately before and after each treatment. The net shading effect of the cages and the isolation tent was approximately 51%. Air temperatures outside the isolation tent and within the small

cages (Table 4.5) were logged at canopy height during each treatment with a Hobo-temp<sup>®</sup> (Onset Computer Corporation, Massachusetts, USA) temperature logger mounted in a Stevenson screen. Treatments were applied at 12, 29, 47 and 65 days after full bloom at Cambridge and 21, 32, 50 and 64 days after full bloom at Naracoorte. The dates treatments were applied and the age range of seed exposed at the start of each treatment are shown for both locations in Table 4.6. To enable pollination to proceed for 21 days in plots used in the first set of treatments at Cambridge, a single nucleus hive of honeybees was placed in each cage for the duration of the treatment. Rutherglen bug stocking levels were adjusted in these treatments to correct for the presence of 4 plants (2 male fertile and 2 male sterile) within the cages.

**Table 4.5** – A comparison of climatic conditions within the cages enclosing plants (plain text) with those in the field outside the isolation tent (*italics*) during the *N. vinitor* infestation treatments. Temperatures from both positions were measured at canopy height. Temperatures outside the isolation cage were recorded amongst plots of male sterile plants of the carrot hybrid used in the trial. Due to the use of shade cloth for caging, treatments were exposed to rainfall. Total precipitation data during the infestation treatments were sourced from the Australian Bureau of Meteorology weather stations at the Hobart Airport and Naracoorte.

Time of treatment (Days after full bloom)	Commencement date	Mean daily maximum temperature °C		Mean daily minimum temperature °C		Total rainfall during treatment (mm)
Cambridge						
12 – 21	27/12/00	30.8	28.0	7.1	8.0	13.2
29 – 38	13/1/01	34.8	30.2	12.1	12.6	1.0
47 – 56	31/1/01	32.6	32.2	11.7	11.9	0.0
65 – 60	18/2/01	32.8	30.8	11.8	13.6	0.8
Naracoorte						
21 – 30	20/12/01	23.0	21.1	9.6	7.7	3.0
32 – 41	31/12/01	25.2	25.0	10.2	8.5	7.2
50 – 59	18/1/02	26.9	26.2	10.7	8.9	3.4
64 – 73	1/2/02	23.2	22.2	10.2	8.4	1.0

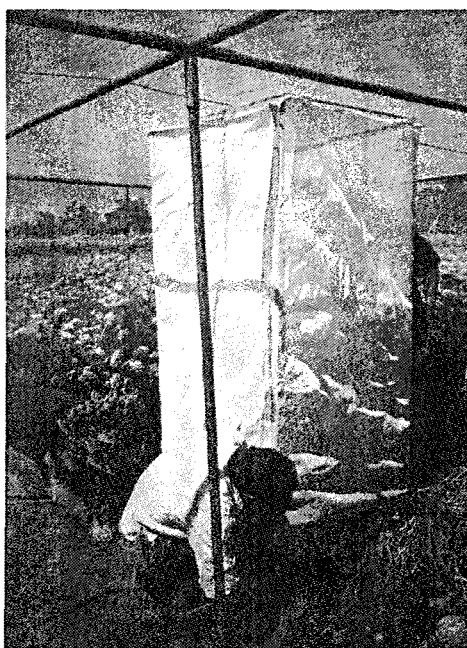
The cages were stocked with *N. vinitor* collected from nearby carrot plants 1 day prior to each treatment period. Representative samples of the insects used for each

infestation period were killed and sexed to determine the proportion of males and females in each treatment (Table 4.6). In each case, except the third stocking at Naracoorte, the ratio of male to female insects was approximately 60:40. Two of the three male sterile plants in each plot were infested. The remaining plants were removed and pooled for determination of mean moisture contents (all umbel orders) at the 4 times of exposure, using the low constant temperature method (Chapter 2, Section 2.7).

**Table 4.6** – The timing of *N. vinitor* exposure treatments at Cambridge and Naracoorte and the percentage of male and female *N. vinitor* in bulk samples used to stock the cages at these times.

Time of treatment (Days after full bloom)	Commencement date	Age range of seed at start of treatment (days)	<i>N. vinitor</i> sex proportions	
			% Male	% Female
Cambridge				
12 – 21	27-Dec-00	-9 to 12	61.5	38.5
29 – 38	13-Jan-01	8 to 29	60.0	40.0
47 – 56	31-Jan-01	26 to 47	60.0	40.0
65 – 74	18-Feb-01	44 to 65	57.1	42.9
Naracoorte				
21 – 30	20-Dec-01	1 to 21	62.0	38.0
32 – 41	31-Dec-01	12 to 32	59.0	41.0
50 – 59	18-Jan-02	30 to 50	44.0	56.0
64 – 73	1-Feb-02	44 to 64	60.0	40.0

All trial plots were harvested when the primary and secondary umbels had turned brown. The seed of the two plants in each plot was pooled and threshed, cleaned and stored as described in Chapter 2 (Section 2.6). Seed yields, 100 seed dry weights and the presence of embryos or embryo damage were determined for each plot using the methods outlined in Chapter 2 (Sections 2.7 to 2.9).



**Figure 4.2** –Erecting cages for stocking with *N. vinitor* in a plot of male sterile carrot plants at Naracoorte. Within the timing and level of infestation experiments all plots were contained in a commercial isolation tent (20m x 10m). The ceiling and a support for this tent are visible in the foreground.

### **Monitoring of *N. vinitor* Populations in Carrot Seed Crops**

The potential for monitoring of *N. vinitor* levels in carrot seed crops with sticky traps was examined at the survey sites for *N. vinitor* seasonal occurrence at Cambridge in 2000-01 and Richmond, Millicent and Naracoorte in 2001-02 (Table 4.2). The survey areas at each site were lengthened by 12m to accommodate 4 plots. Each plot consisted of a 6m long section of 4 rows of male sterile plants. The

4 plots were evenly spaced throughout the survey areas. Commercially available yellow Agrisense<sup>®</sup> (Agrisense, Pontypridd, UK) 200 x 100mm double-sided sticky traps were used. A single trap was attached to a steel picket 10cm above canopy height in the centre of each plot. The trap catches were counted 3 times a week, with the traps removed and replaced at weekly intervals coinciding with the survey (vacuum) sampling. Counts of *N. vinitor* in the vacuum samples taken at the end of each week of trapping were compared with the numbers caught on the sticky traps during the preceding week.

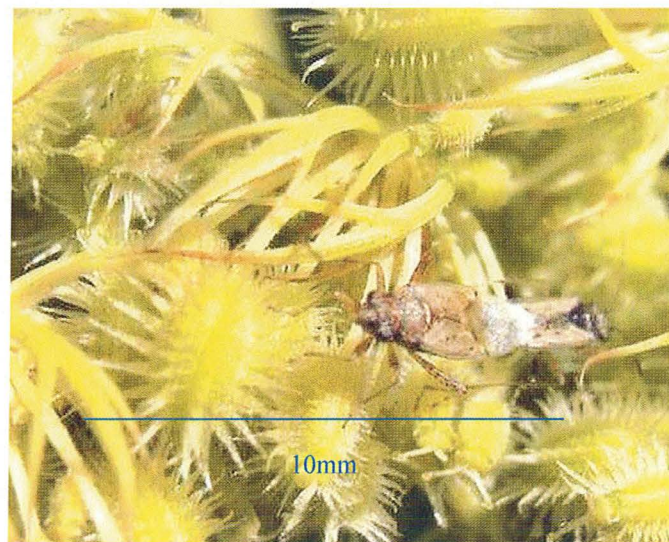
The distribution of *N. vinitor* in carrot fields was examined at 2 commercial sites at Millicent and Naracoorte in 2001-02. Each crop was divided equally into 9 rectangles in a 3 x 3 grid, with an Agrisense<sup>®</sup> sticky trap positioned at 10cm above canopy height in the corner of each rectangle within the grid. This gave 16 traps spaced evenly throughout the crop. Traps located on the periphery of the crop were positioned so that they were 5m in from the edge. On 3 separate occasions throughout the period from flowering to harvest the traps were set and then removed for counting 7 days later. The data collected was analysed using the regression functions of SAS V8 (SAS Institute Incorporated, Cary, USA) and plotted using the contour plot function within Sigmaplot V5 (SPSS, Chicago, USA) to produce maps of the distribution of *N. vinitor* within the two fields over the three weeks of sampling.



### 4.3 – Results

#### Survey of Seed Feeding Insects in Carrot Seed Crops.

Several insects that were known seed feeders or thought to be capable of seed feeding were found in carrot seed plots in Cambridge and Naracoorte (Table 4.7). At both sites, sticky trap monitoring accounted for more insect species than vacuum sampling. Only adult Rutherglen bugs, *Nysius vinitor*, occurred in considerable numbers in vacuum samples and on sticky traps at both locations. Two unidentified species of Mirids were caught on sticky traps at Cambridge, but none were recorded in samples vacuumed from the carrot plants. Carrot seed samples collected from the survey sites at Cambridge and Naracoorte had 17 and 12% seeds with missing or damaged embryos respectively.



**Figure 4.3** – Female (foreground) and male (background) *N. vinitor* on a maturing carrot umbellet.

**Table 4.7** – Insects considered to have the potential to cause carrot seed damage collected by vacuum and sticky trap sampling of carrot trials at Cambridge in 1999-2000 and Naracoorte in 2000-2001. Vacuum samples were collected between 2 and 4pm on each day. Note Miridae 1, 2 and 3 denote unidentified species of Mirid.

Survey method	Time (days after anthesis)	Identification Family	Genus / species	Count / sticky trap or plant
Cambridge Sticky trap	0	Lygaeidae	<i>Nysius vinitor</i>	51
		Miridae	<i>Nezera veridula</i>	7
		Miridae 1		32
		Miridae 2		39
	30	Coreidae	<i>Amorbus sp.</i>	2
		Lygaeidae	<i>Nysius vinitor</i>	22
		Miridae	<i>Nezera veridula</i>	7
		Miridae 1		5
		Miridae 2		2
	60	Lygaeidae	<i>Nysius vinitor</i>	34
		Miridae	<i>Nezera veridula</i>	6
		Miridae 1		9
		Miridae 2		144
Vacuum	0	Lygaeidae	<i>Nysius vinitor</i>	0.2
	30	Lygaeidae	<i>Nysius vinitor</i>	0.5
	60	Lygaeidae	<i>Nysius vinitor</i>	4.3
<hr/>				
Naracoorte Sticky trap	7	Lygaeidae	<i>Nysius vinitor</i>	28
	28	Coreidae		2
		Miridae	<i>Nezera veridula</i>	2
		Lygaeidae	<i>Nysius vinitor</i>	33
	50	Miridae 3		2
		Lygaeidae	<i>Nysius vinitor</i>	47
Vacuum	7	Lygaeidae	<i>Nysius vinitor</i>	0.6
	28	Lygaeidae	<i>Nysius vinitor</i>	2.0
	50	Lygaeidae	<i>Nysius vinitor</i>	2.1

### Seed Yield and Quality Effects of Post Fertilisation Exposure to *N. vinitor*

Infestation of male sterile parent plants for 20 days with 40 adult *N. vinitor* from one week after the completion of pollination of the first three umbel orders significantly ( $P<0.001$ ) reduced seed quality but did not affect seed yield or 100 seed weight (Table 4.8). Seed from plants exposed to *N. vinitor* had a germination percentage of 49.2% normal seedlings compared to 88.4% for control plants from which all insects were excluded. The difference in germination was mainly due to a reduction in the percentage of seeds with viable embryos from 97.2% in control plants to 54.8% in plants exposed to *N. vinitor* (Table 4.8). Seeds without viable embryos were either embryoless or had damaged embryos. In both instances the symptoms were identical to those described in Chapter 3 (See Figure 3.4). That is; apparently normal development apart from the absence of an embryo and damage to an area of surrounding endosperm, or necrosis of embryo tissue not confined to any particular area of the embryo. There was no difference in the size of undamaged embryos from seed exposed to *N. vinitor* compared to seed from which all insects were excluded.

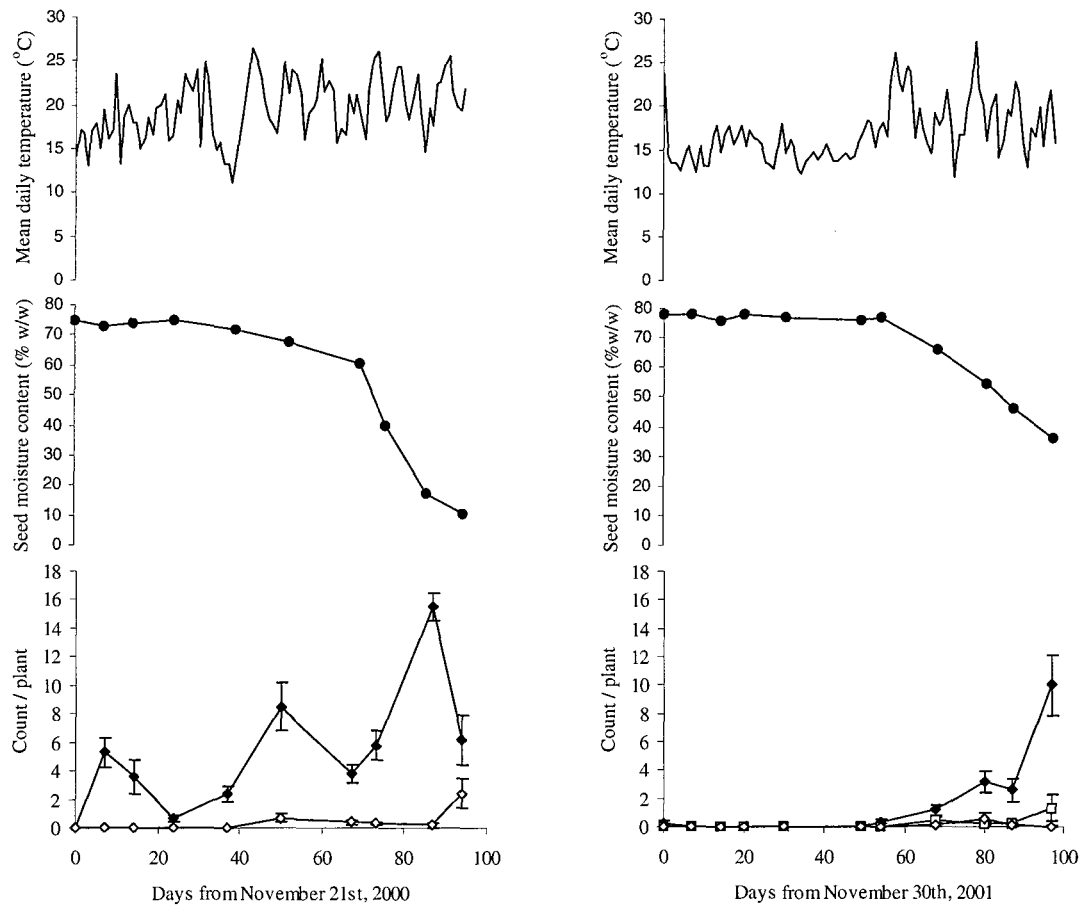
**Table 4.8** –The effect of 20 days exposure to *N. vinitor* (40 adults/plant) from one week after flowering on seed yield and quality in male sterile plants of the hybrid No. 22 (n=8).

	Treatment		LSD ( $P<0.05$ )
	All insects excluded	Exposure to <i>N.</i> <i>vinitor</i>	
Mean dry seed yield / plant (g)	20.9	18.6	NS
Seed dry weight (mg)	1.8	2.2	NS
% Normal seedlings (day 14)	88.4	49.2	4.5
% Seeds with intact embryos	97.2	54.8	2.8
Mean embryo length (mm)	1.74	1.76	NS

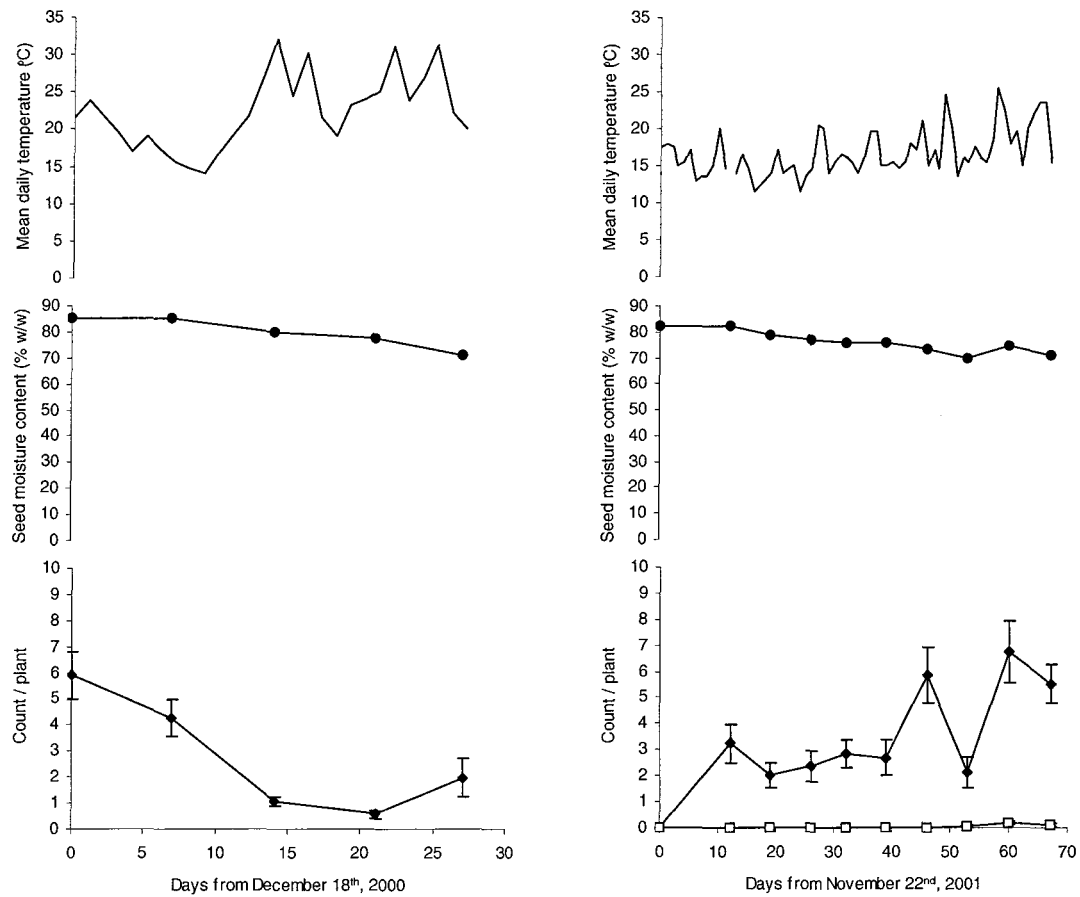
### Seasonal Prevalence of *N. vinitor* in Carrot Seed Crops

In 2000-01 and 2001-02 at production sites near Richmond, Millicent and Naracoorte, *N. vinitor* was shown to infest carrot plants from the late stages of flower bud development (2 to 3 weeks prior to flowering) until seed harvest, which occurred at primary umbel seed moisture contents as low as 10% (Figures 4.4 – 4.6). In 2001-02 the first infestation of the carrot seed crops at all 3 locations was detected, with each occurring in the period between the 30<sup>th</sup> of November and the 6<sup>th</sup> of December. Over time, infestation levels across all sites varied from 0 to a maximum of 16 insects per plant, recorded at Cambridge in 2000-01. In 2001-02 the two Tasmanian carrot seed crops under survey differed markedly in infestation levels. At Richmond, a late season infestation of up to 10 adults per plant was observed (Figure 4.6). At Tea Tree no *N. vinitor* were observed at any time during the reproductive phase of the crop despite the two sites being separated by only 6km. Embryoless seeds were found in seed harvested from all survey sites except for Tea Tree.

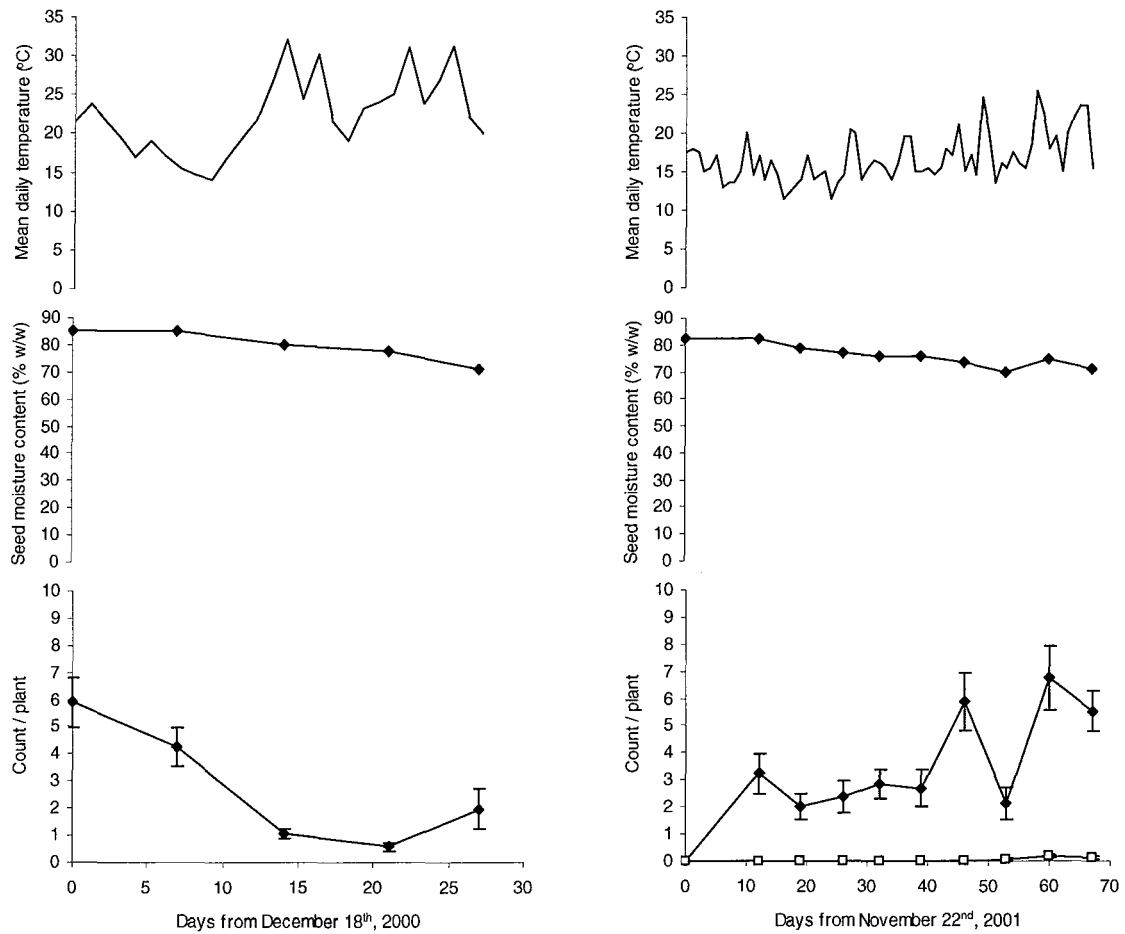
Across all sites and both seasons up to 3 distinct peaks in the levels of infestation were observed at intervals of 20 to 45 days during the period from pre-flowering to harvest (up to 97 days). No other members of the *Nysius* genus were observed in the infestations, which consisted largely of adult insects of both sexes. The ratios of male to female adults in populations on the carrot plants were examined for all sites in 2001-02 and were consistently around 60:40 at each location for the entire monitoring period. Nymphs were only observed on carrot plants in small numbers at the later stages of seed maturation, or not at all (Figures 4.4 - 4.6). Compared to the levels of *N. vinitor* on the plants, in general very few were observed in samples taken from the soil surface at the base of the carrot plants (Figures 4.4 – 4.6). Within the plants, most insects were collected from umbels containing seed, but other were present on flowering umbels, umbels at the late stage of bud development and within the leaf axils.



**Figure 4.4** – Prevalence of *N. vinitor* adults (◆) and nymphs (◇) on male sterile carrot seed plants at Cambridge in 2000-01 (left) and Richmond in 2001-02 (right) during the period from the late bud stage (pre-flowering) to harvest. Data are not shown for the site at Tea Tree in 2001-02 as no *N. vinitor* were observed over the entire survey period. In 2001-02 samples were collected from the soil surface at the base of the plants, with total counts of *N. vinitor* adults and nymphs per soil unit surface area occupied by a single plant ( $0.08\text{m}^2$ ) depicted in the figure for 2001-02 (□). Also shown for both seasons are the mean daily air temperatures (canopy height) at the survey sites and changes in moisture content of seed from the primary umbel (●) over the survey periods. 2000-01 data were collected from trial plots of the Nantes hybrid No. 22. 2001-02 data were collected from a commercial crop of the Nantes hybrid WO535. Error bars depict standard errors. (N=30 for plant samples; 10 for soil surface samples).



**Figure 4.5** – Prevalence of *N. vinitor* adults (♦) and nymphs (□) on male sterile carrot seed plants in commercial crops near Millicent in 2000-01 (left) and 2001-02 (right) during the periods from the end of flowering to harvest (2000-01) and late bud stage (pre-flowering) to harvest (2001-02). In 2001-02 samples were collected from the soil surface at the base of the plants, but no *N. vinitor* were found in these. Also shown for both seasons are the mean daily air temperatures (canopy height) at the survey sites and changes in moisture content of seed from the primary umbel (●) over the survey periods. Error bars depict standard errors. (N=30 for plant samples; 10 for soil surface samples).

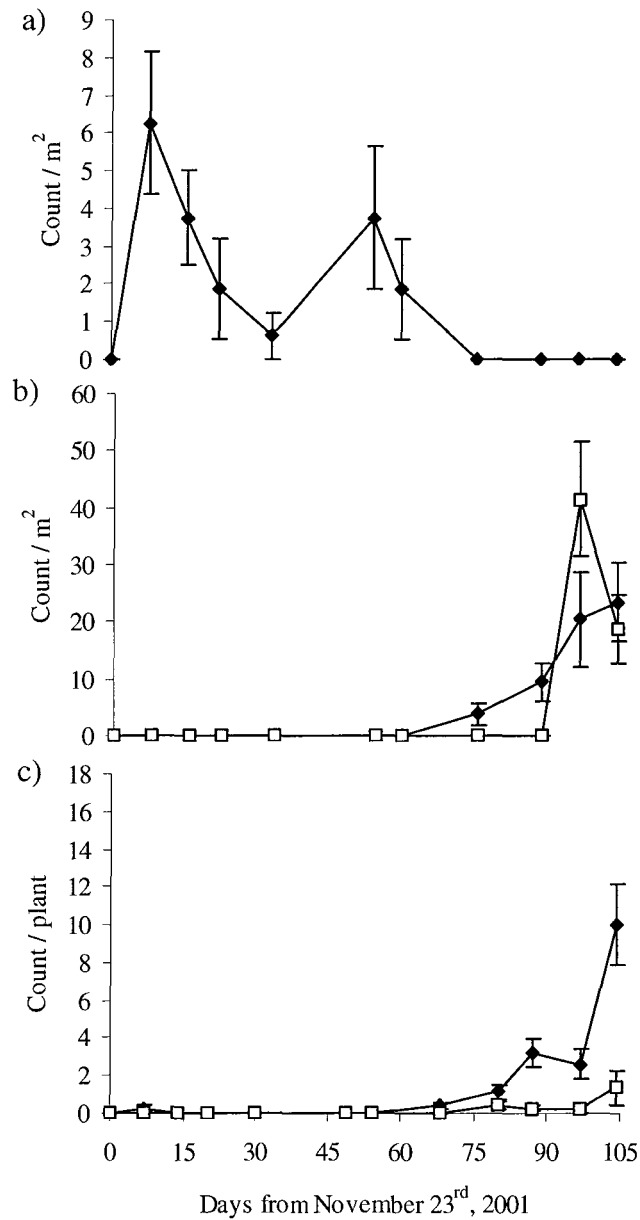


**Figure 4.6** – Prevalence of *N. vinitor* adults (♦) on male sterile carrot seed plants in commercial crops near Naracoorte in 2000-01 (left) and 2001-02 (right) during the periods from the end of flowering to harvest (2000-01) and late bud stage (pre-flowering) to harvest (2001-02). No nymphs of *N. vinitor* were observed in these surveys. In 2001-02 samples were collected from the soil surface at the base of the plants. Total counts of *N. vinitor* adults per unit surface area occupied by a single plant ( $0.08\text{m}^2$ ) are depicted in the figures for 2001-02 (□). Also shown for both seasons are the mean daily air temperatures (canopy height) at the survey sites and changes in moisture content of seed from the primary umbel (●) over the survey periods. Error bars depict standard errors. (N=30 for plant samples; 10 for soil surface samples).

In 2000-01 a small population of *N. vinitor* was detected under flowering capeweed at Cambridge on the 28<sup>th</sup> of November, the same time the carrot plants were first infested. This population persisted until the 10<sup>th</sup> of January, when the capeweed had dried off. A large population of nymphs was subsequently observed in weedy borders consisting of wireweed (*P. aviculare*) and Brassica weeds. Adults were observed in this population on the 16<sup>th</sup> of February, which coincided with the timing of the largest infestation of carrot plants that season (Figure 4.4).

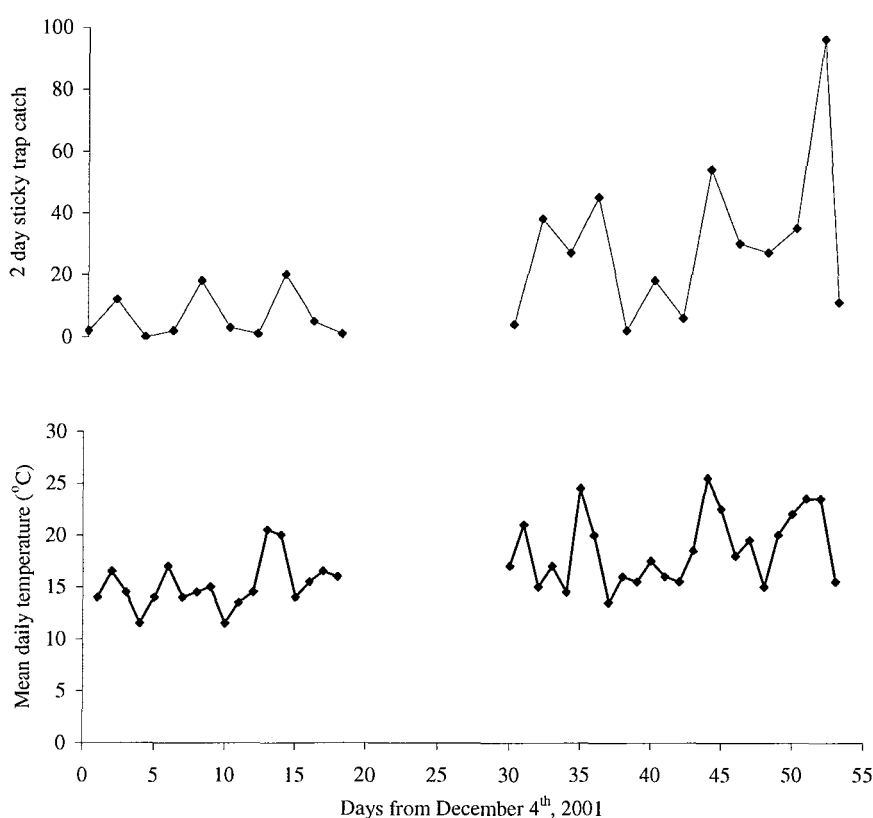
In 2001-02, very few *N. vinitor* were collected from weedy areas surrounding the sites at Naracoorte and Millicent and none were collected at Tea Tree, (data not shown). At Richmond, Rutherglen bugs were first detected in 2001-02 under capeweed plants on the 30<sup>th</sup> of November (Figure 4.7a). This population persisted through the flowering and seeding of the capeweed and ultimately dispersed when the plants dried off in mid January. The population initially consisted mostly of females (80%), but this gradually shifted to an equal proportion of males and females in the last two weeks that the population was present. No nymphs were observed under capeweed plants during the period that adults were present, or in following samples, which continued until the end of February. The initial detection of *N. vinitor* under capeweed occurred at the same time that small numbers were detected on carrot plants at the late stage of bud development (primary umbels) (Figure 4.7c). The decline of the population under capeweed in mid to late January coincided with a gradual infestation of seeding wireweed and carrot plants by *N. vinitor* adults (first detected on the 23<sup>rd</sup> of January). Initially the population under the wireweed consisted mostly of females (75%) but by mid February the male to female ratio had altered to around 50:50, at which it remained until the end of the survey period on the 25<sup>th</sup> of February. Nymphs were observed under wireweed 14 days after the adults were first detected (Figure 4.7b). The maturation of this population into adults coincided with the marked late season increase in the numbers of adult *N. vinitor* observed on the carrot seed plants.



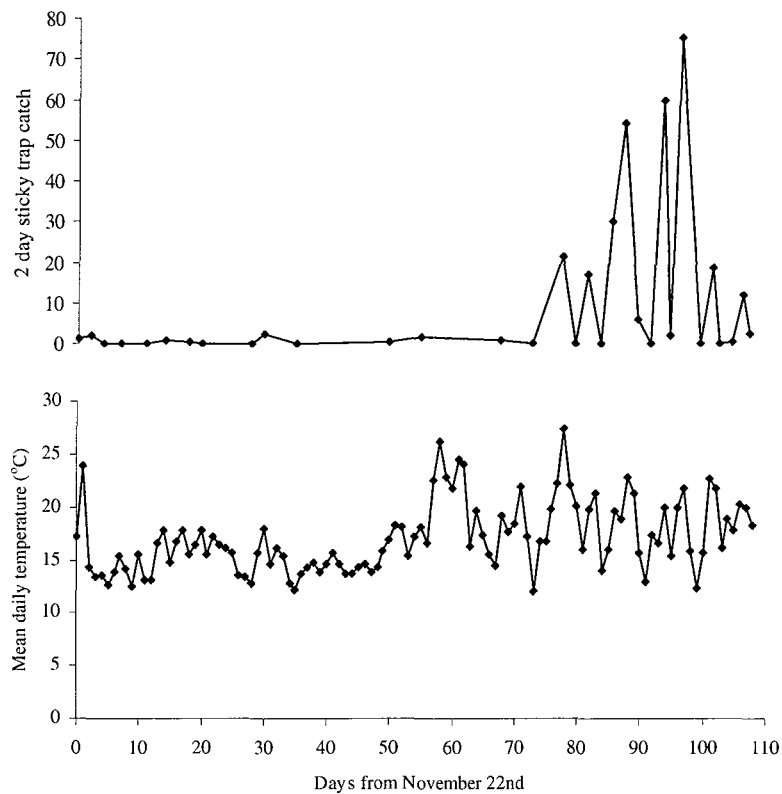


**Figure 4.7** – Occurrence of *N. vinitor* adults (◆) and nymphs (□) under a) capeweed (*Arctotheca calendula*) and b) wireweed (*Polygonum aviculare*) in weedy borders surrounding a commercial carrot seed crop near Richmond in 2001–02. Infestation patterns on male sterile carrot plants within the crop are shown in (c). Error bars indicate standard errors (n=30).

Data collected from the catches of *N. vinitor* on sticky traps at 2-day intervals throughout the period from flowering to harvest at the Richmond and Naracoorte survey sites in 2001-02 showed a relationship between flight activity of adult *N. vinitor* within the plots and air temperature (Figures 4.8 and 4.9). At both sites, peak flight activity coincided with periods when the mean daily air temperatures were at or above 16.5°C. Mean daily air temperatures of less than 15°C markedly decreased this activity. The data collected from weekly vacuum sampling of plants (Figures 4.4 and 4.6) show that the periods of peak flight activity detected by sticky trapping were in part due to the migration of *N. vinitor* into the carrot seed plots.



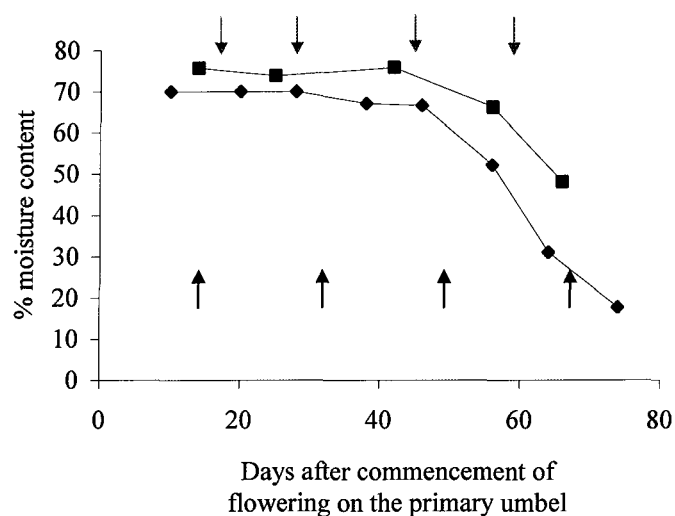
**Figure 4.8** – Relationship between flight activity of *N. vinitor* (top) and mean daily air temperature at canopy height (bottom) during the period from December 4<sup>th</sup>, 2001 to January 28<sup>th</sup>, 2002 in a commercial carrot seed crop at Naracoorte. Flight activity was based on the 2-day catches of 4 yellow Agrisense<sup>®</sup> sticky traps positioned within the trial plots used to survey seasonal prevalence of *N. vinitor*.



**Figure 4.9** – Relationship between flight activity of *N. vinitor* (top) and mean daily air temperature at canopy height (bottom) during the period from November 29<sup>th</sup>, 2001 to February 14<sup>th</sup>, 2002 in a commercial carrot seed crop at Richmond. Flight activity was based on the 2-day catches of 4 yellow Agrisense® sticky traps positioned within the trial plots used to survey seasonal prevalence of *N. vinitor*.

### **Seed Yield and Quality with Respect to the Level and Timing of Infestation of Adult *N. vinitor***

The changes in mean seed moisture content in caging trials at Cambridge in 2000 and Naracoorte in 2001 are shown in Figure 4.10. Mean seed moisture contents remained above 65% for the first 46 days after full bloom at Cambridge and 56 days after full bloom at Naracoorte before dropping sharply at both locations. At the start of the 4 periods of infestation, the seed moisture contents at Cambridge and Naracoorte were 70.0, 70.1, 66.6 and 31% and 77.5, 73.8, 75.9 and 66.2% respectively.

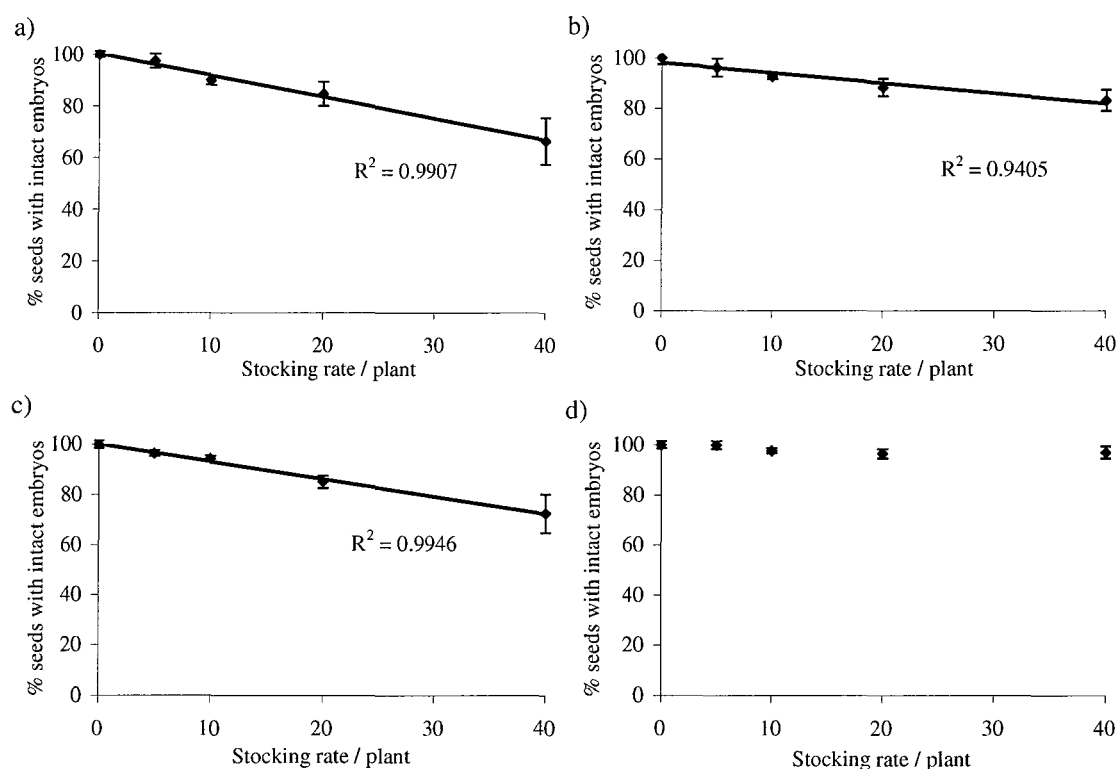


**Figure 4.10** – The change in moisture content of seed pooled from all umbel orders with time during the caging trials at Cambridge in 2000-01 (◆) and Naracoorte in 2001-02 (■). The start of each period of infestation is indicated by the black (Cambridge) and red (Naracoorte) arrows.

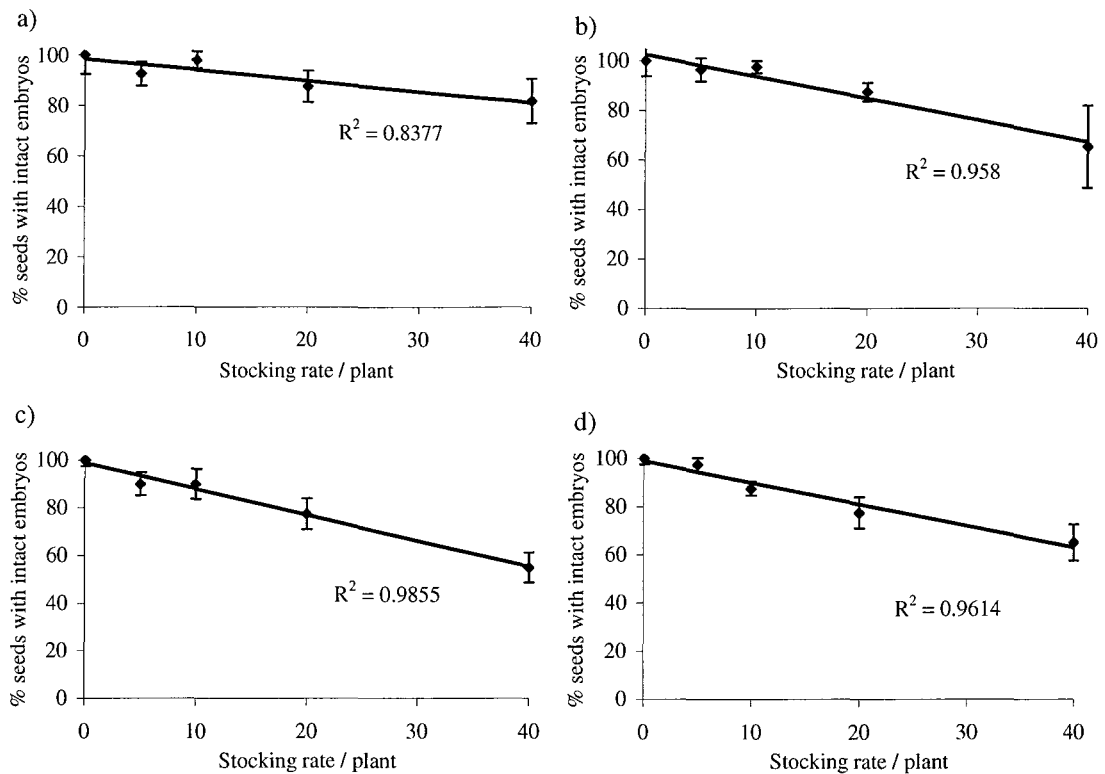
The dry seed yields in the caging experiments varied considerably between treatments, with ranges of 4.8 to 6.4g per plant at Cambridge and 2.8 to 3.8g per plant at Naracoorte recorded. There were no effects of timing or level of adult *N. vinitor* infestation on seed yield. Similarly, seed size was unaffected by the treatments, ranging from 2.8 to 3.5mg at Cambridge and 2.3 to 2.8mg at Naracoorte.

At Naracoorte, embryo damage occurred at all of the stages of seed development where *N. vinitor* were introduced (Figure 4.12). In contrast to the first three times of exposure at Cambridge, the final set of treatments (imposed at lower seed moisture content than the others or the Naracoorte treatments) did not produce any embryo damage (Figure 4.11). At both locations at all stages of seed development in which embryo damage was observed there were significant negative linear relationships

( $P < 0.05$  to  $P < 0.001$ ) between the level of infestation and the percentage of exposed seeds with healthy embryos (Table 4.9).



**Figure 4.11** – The relationship between the percentage of seeds with intact embryos and adult *N. vinitor* infestation at four stages of seed development a) 12, b) 29, c) 47 and d) 65 days after full bloom at Cambridge in 2000–01. The bars indicate standard errors,  $n=4$ .



**Figure 4.12** – The relationship between the percentage of seeds with intact embryos and adult *N. vinitor* infestation at four stages of seed development a) 21, b) 32, c) 50 and d) 64 days after full bloom at Naracoorte in 2001–02. The bars indicate standard errors,  $n=4$ .

At both locations significant ( $P<0.01$ ) differences were observed between the slopes of the lines fitted to the data from each infestation time (Table 4.9). Averaged over all stocking rates, the extent of damage for the first three sets of treatments at Cambridge and all treatments at Naracoorte ranged from 1.0 to 2.0 and 0.2 to 0.8 seeds per insect per day respectively. These values equated to reductions in potential seed lot viability (as indicated by the presence of a healthy embryo) of between 0.04 and 0.08% and 0.04 and 0.11% /insect/plant/day at Cambridge and Naracoorte respectively.

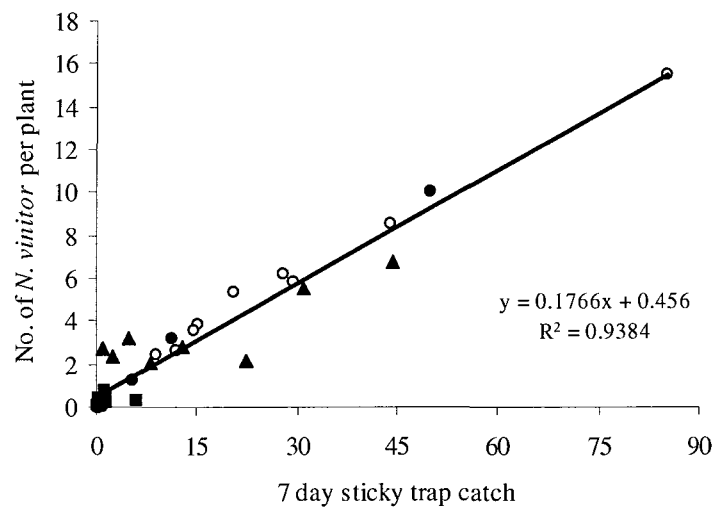
**Table 4.9** - Summary of the relationship between adult *N. vinitor* and the occurrence of embryoless and embryo damaged seeds at 4 stages of seed development in caging trials at Cambridge in 2000–01 and Naracoorte in 2001–02. The damage relationships indicate the percentage of seeds with intact embryos, y, after 10 days exposure to a population of x adult *N. vinitor* per plant. Standard errors are given in italics, (n=4).

Location	Time of exposure (DAF)	Damage relationship	Significance of relationship	Daily damage rate/ adult insect / plant	
				No. of damaged seeds	% decrease in seeds with intact embryos
Cambridge	12	$y = 100.36 - 0.84x$	$P < 0.001$	1.40 0.32	0.08 0.02
	29	$y = 98.11 - 0.40x$	$P < 0.001$	1.04 0.14	0.04 0.01
	47	$y = 100.12 - 0.70x$	$P < 0.001$	1.97 0.24	0.07 0.01
	65	No relationship			
Naracoorte	21	$y = 98.43 - 0.43x$	$P < 0.05$	0.20 0.05	0.04 0.01
	32	$y = 102.68 - 0.89x$	$P < 0.001$	0.46 0.12	0.09 0.01
	50	$y = 98.81 - 1.09x$	$P < 0.001$	0.79 0.17	0.11 0.02
	64	$y = 99.00 - 0.90x$	$P < 0.001$	0.74 0.13	0.09 0.02

### Monitoring of *N. vinitor* in Carrot Seed Crops

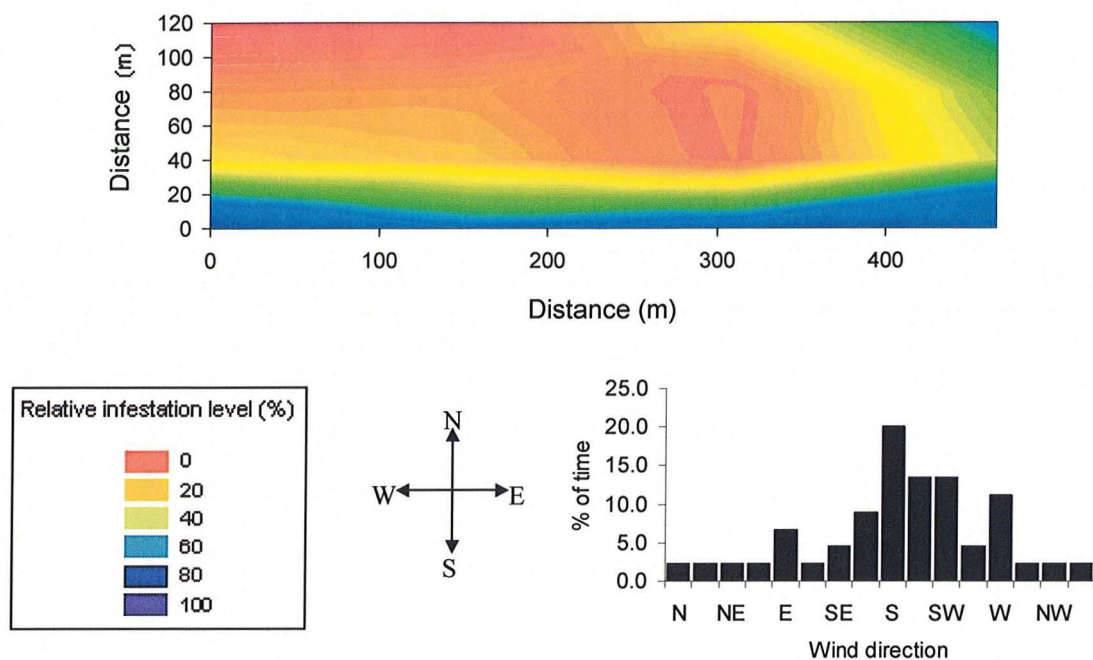
There was a highly significant ( $P < 0.0001$ ) linear relationship between the number of adult *N. vinitor* collected from carrot plants and the catches from sticky traps in the survey areas for the preceding 7 days across all locations in both 2000–01 and 2001–02 (Figure 4.13). The relationship was described by the equation  $y = 0.1766x + 0.456$  ( $r^2 = 0.94$ ), where y = the number of insects per plant and x = the total sticky trap catch for the preceding 7 days. In a commercial carrot seed crop in Naracoorte in 2001–02, the distribution of *N. vinitor* throughout the fields was non-random ( $P < 0.0001$ ). *N. vinitor* densities were highest on the southern edge of the field that faced the prevailing winds during the trapping period and decreased with distance from this edge (Figure 4.14). The distribution was described by the polynomial equation  $z = 18.9850 - 0.9885x - 0.1378y + 0.01750x^2 - 0.0007y^2 - 0.0015xy$  ( $r^2 = 0.92$ ), where z = the mean 7 day trap catch at a position x metres north and y metres east of the south-western corner of the field. The experiment was repeated in Millicent in the same season. Although there was a trend of greatest population densities around the periphery of the crop (Figure 4.15) facing the prevailing wind,

the pattern was not significant, partly because of the extremely low incidence of *N. vinitor* at this site (Figure 4.5).

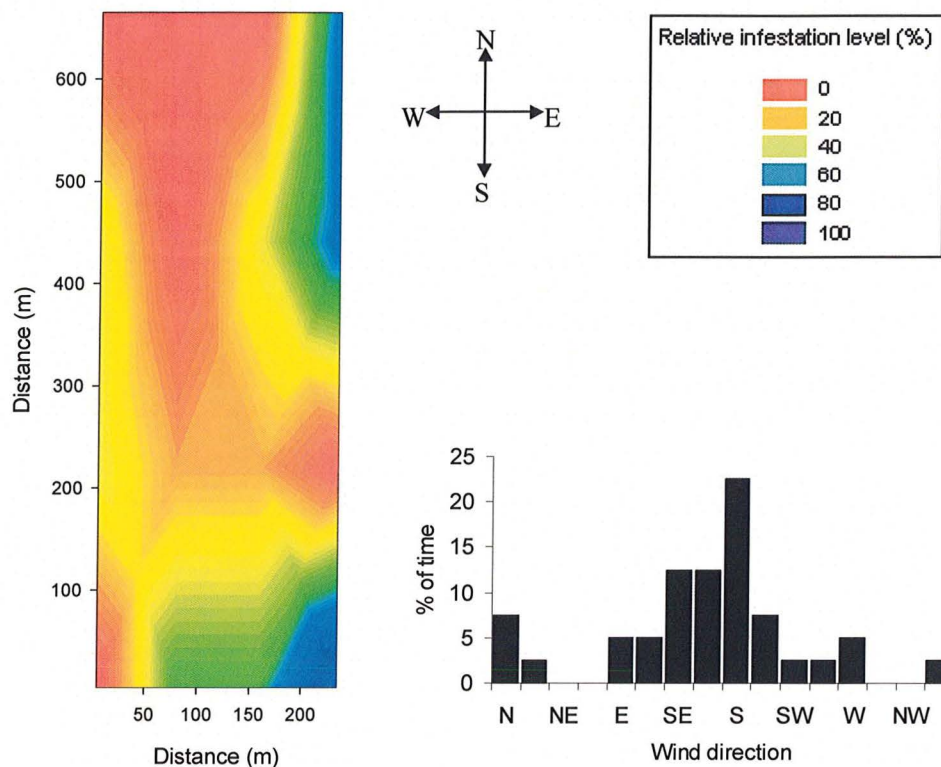


**Figure 4.13** – The relationship between 7 day sticky trap catches and the numbers on male sterile plants on the 7<sup>th</sup> day for adult *N. vinitor* in hybrid carrot seed crops in Millicent (2001-02) (■), Naracoorte (2001-02) (▲) and Cambridge (2000-01, 2001-02) (○, ●). Per plant numbers are based on a standard planting density of 12 plants/m<sup>2</sup>. Each data point is based on the mean catch of 4 sticky traps and 30 plant samples.





**Figure 4.14** –The infestation pattern of *N. vinitor* in a commercial carrot seed field at Naracoorte in the 2001–02 season. Data were collected over 3 weeks in the period between the 20<sup>th</sup> of December, 2001 and the 21<sup>st</sup> of January, 2002, when *N. vinitor* activity within the crop was highest. The colour scale indicates the average relative infestation levels throughout the field (as a percentage of maximum infestation level) over the three weeks of sampling. The orientation of the field is indicated by the compass points, with the position coordinates taken from the south-western corner of the crop. The frequency distribution of wind direction at the field site during the sampling periods is indicated in the bar graph. These data were extracted from the daily records of 9am and 3pm wind direction at the nearest Weather Station at Naracoorte, site no.026023.



**Figure 4.15** – The infestation pattern of *N. vinitor* in a commercial carrot seed field at Millicent in the 2001–02 season. Data were collected in 3 weeks in the period between the 15<sup>th</sup> of December, 2001 and the 18<sup>th</sup> of February, 2002, when *N. vinitor* activity within the crop was highest. The colour scale indicates the average relative infestation levels throughout the field (as a percentage of maximum infestation level) over the three weeks of sampling. The orientation of the field is indicated by the compass points, with the position coordinates taken from the south-western corner of the crop. The frequency distribution of wind direction at the field site during the sampling periods is indicated in the bar graph. These data were extracted from the daily records of 9am and 3pm wind direction at the nearest weather station, Mt. Gambier Aerodrome, site no. 026021.

## 4.4 - Discussion

The data presented in this chapter demonstrate that adult Rutherglen bugs (*Nysius vinitor*) are a major cause of low germination of Australian carrot seed. Feeding of *N. vinitor* caused carrot seeds that were embryoless or that had damaged embryos. These forms of damage were identified as the main reasons for failed germination in open pollinated and hybrid carrot seed lines grown in South Australia and Tasmania in 3 seasons (See Chapter 3, Figure 3.5). Exposure to *N. vinitor* at all stages of seed development from peak bloom to harvest maturity had no effect on the incidence of abnormal seedlings or seed yields. Over the same range of maturities there were no effects of *N. vinitor* on mean seed weight or the size of embryos that remained intact in seed samples from exposed plants. The lack of effect on seed yields and seed weight were presumably due to the isolation of damage to the embryo and a small amount of surrounding endosperm. The embryo has been estimated to occupy less than 3% of the volume of a mature carrot seed (Gray, 1984).

Other insect species that could potentially damage the germination of carrot seed were observed in initial surveys of carrot seed plots at Cambridge and Naracoorte but *N. vinitor* was the only species collected directly from seeding carrot plants at both sites. Neither of the closely related species *N. cleavlandensis* (mainland Australia) or *N. turneri* (Tasmania) were observed in carrot seed fields or adjacent weedy areas in this study. *N. vinitor* was found in all carrot seed crops that were surveyed over three seasons in South Australia and Tasmania except one at Tea Tree, which produced the only seed lot free of embryoless seeds and embryo damage.

*N. vinitor* is not found in New Zealand (Tillyard, 1926), which provides an explanation for the low incidence of embryoless seed and embryo damage in carrot seed from Winchmore compared to south-eastern Australia (see Chapter 3). However, a small percentage of carrot seeds without embryos were found in New Zealand seed lines (Chapter 3). This suggests that another insect may cause relatively low levels of damage to carrot seed embryos in New Zealand, or

alternatively, there is another factor causing a low-level incidence of seeds without embryos. Incomplete fertilisation has previously been reported as a cause of low levels of embryoless seed in the Apiaceae (Jurica, 1922).

Effective management of *N. vinitor* in carrot seed crops requires information on seasonal occurrence, the time period over which seed damage occurs, the insect density – damage rate relationship and techniques for monitoring. No previous studies of these aspects have been published.

Despite regular scouting of carrot seed crops and adjacent areas of weedy hosts near Cambridge and Richmond in the seasons of 2000–01 and 2001–02 *N. vinitor* was not found until adults were detected under capeweed, and in carrot seed crops from late November to early December. This pattern is consistent with the current understanding of temperature requirements for over-wintering of *N. vinitor* (Kehat and Wyndham, 1972b; Kehat and Wyndham, 1974), which indicate that the Tasmanian winter is generally too cold for survival of any stage of the insect. Immigrants from central Australia are reported to be a common source of adults initiating spring and summer generations in Victoria (McDonald and Smith, 1988). In 2001, the first infestation of carrot seed crops at both sites in South Australia and Tasmania appear to have occurred synchronously around the first week of December. This would be consistent with a coordinated movement of *N. vinitor* into south-eastern Australia. In the few days preceding the detection of *N. vinitor* in 2000–01 and 2001–02, a northerly weather pattern extended from mainland Australia to Tasmania. Such conditions would be necessary for migration of the insect into Tasmania from mainland Australia.

Adults of *N. vinitor* were found in carrot seed fields from the late stages of flower bud development (2–3 weeks prior to flowering) until harvest, up to 97 days later. The large majority of insects were found on the umbels or in the leaf axils of carrot plants, with few found at ground level. During the time that *N. vinitor* were present in carrot seed crops there were up to 3 distinct peaks in immigration separated by 20 to 45 days. This suggests up to 3 generations of immigrants. Compared to reports for sunflowers (Broadley and Rossiter, 1982) and anecdotal evidence for carrot seed crops, the infestations observed over the three years of the study (up to

16 insects per plant) were relatively low. Wide variation in *N. vinitor* population size in sunflowers between seasons has been noted (Broadley and Rossiter, 1982). Such variation is presumed to be a function of the availability and quality of host plants and temperature conditions during winter and spring (Swaine and Ironside, 1983; McDonald, 1988).

In contrast to the adults, nymphs of *N. vinitor* were infrequently observed on carrot seed plants. Their occurrence was restricted to the later stages of seed development, when other hosts were unavailable, and was usually at low levels compared to the numbers of adults. Reduced rates of development and increased mortality in nymphs fed carrot seed have been observed (Kehat 1972a). When available, the nymphs may infest other more favourable hosts in preference to seeding carrot plants and consequently play a minor role in damaging carrot seed.

Observations in Tasmania in 2000–01 suggested that populations of *N. vinitor* in adjacent weedy areas consisting predominantly of flowering capeweed (*Arctotheca calendula*) and wireweed (*Polygonum aviculare*) contributed to the infestation of adjacent carrot seed fields. Data collected in 2001–02 showed that the decline in populations of *N. vinitor* under capeweed as it desiccated in mid-summer and the maturation and dispersal of adults from wireweed in early autumn coincided with the main periods of infestation of an adjacent carrot seed field. Thus initial migrants and generations that subsequently develop on weedy hosts in early and late summer may account for the three main stages of infestation observed in carrot seed crops at Cambridge and Richmond. Although a nymphal generation was observed under capeweed in 2000-01, none was recorded in 2001-02. This may have been because mean daily temperatures in the period from invasion of capeweed until the plants dried off were typically around 15°C. Such temperatures are reported to be unfavourable for survival of eggs or nymphs of *N. vinitor* (Kehat and Wyndham, 1972b; Kehat and Wyndham, 1974). From the data collected in 2001-02 it is unclear if the first significant migration of adults into carrot seed crops consisted of the original immigrants to capeweed (and probably other spring weeds), or if it consisted of a new generation that developed in habitats distant to the sites that were studied.

In 2001-02 significant numbers of *N. vinitor* were observed in a carrot seed field at Richmond but none were observed in another crop located at Tea Tree, despite the two crops being separated by just 6km. The latter crop was isolated from significant populations of capeweed, wireweed and other preferred hosts of *N. vinitor* by several kilometres. Localised dispersal from weedy hosts is reported to be an important mechanism of infestation in sunflower crops (Ramesh, 1988 cited in McDonald and Smith, 1988) and may play a similar role in carrot seed crops in the lower Coal River Valley in Tasmania. If confirmed across seasons and locations, this mechanism of infestation would have important implications for the monitoring and management of *N. vinitor* in the Coal River Valley. The origin of migrants into crops at Millicent and Naracoorte in this study was unclear, although it was apparent that they did not originate from the nearby (within 1km) areas of preferred hosts including capeweed and canola during the 2001-02 season.

*N. vinitor* feeding damage occurred in cage trials at flowering and subsequent stages of seed development at mean seed moisture contents (all umbels) above 31%. The lack of damage at seed moisture contents below 31% was most likely due to an inadequate supply of water within the cages or an inability of *N. vinitor* to penetrate the dry seed. *N. vinitor* requires uninterrupted access to water (Kehat and Wyndham, 1972a) but has been successfully reared on diets of mature cracked seeds when provided with water (Kehat and Wyndham, 1972a; Attia, 1974). The commercial seed crops studied in this work were windrowed at seed moisture contents between 20 and 75%. Based on the observations of seasonal occurrence and timing of damage, most commercial crops of carrot seed are likely to be susceptible to *N. vinitor* feeding damage during the period from flowering until windrowing or later.

Close linear relationships were observed between *N. vinitor* population levels and seed damage rates in cage trials. Over a 10-day period in cage trials, individual insects damaged between 1 and 2 seeds/day at Cambridge and 0.2 to 0.8 seeds/day at Naracoorte. This equated to losses of seed viability between 0.04 and 0.11% / insect / plant / day for plants yielding 2.8 to 6.4 grams of seed, which is within the typical yield range of many hybrid seed crops in the field (see Table 3.2, Chapter 3). The difference between damage rates at Cambridge and Naracoorte may reflect

temperature differences between the two locations. Temperature effects on the feeding behaviour of *N. vinitor* have not been examined, but temperatures above 25°C appear optimal for development (Kehat and Wyndham, 1972b). During the caging experiments mean daily maximum temperatures at Naracoorte, were 23.0 to 26.9°C compared to 30.8 to 34.8°C at Cambridge.

In practical terms, relatively small numbers of *N. vinitor* have the capacity to rapidly reduce the germination percentage of carrot seed crops below commercially acceptable standards. Control strategies must be applied in rapid response to infestations to preserve seed viability. Previous industry management of Rutherglen bug failed to improve carrot seed germination, because in these attempts, pesticides were not applied until insect numbers accumulated to at least 6 per plant by visual estimation (Dalrymple, pers comm<sup>1</sup>).

To effectively manage *N. vinitor* in carrot seed crops, the capacity for accurate forecasting of infestation events or detection of them soon after they occur appears necessary. Peaks in flight activity of *N. vinitor* in carrot seed crops, which generally coincided with immigration into the crop, were observed when mean daily temperatures were above 16.5°C. Whilst this relationship may facilitate the forecasting of some infestation events, or periods when infestation is not expected, temperatures generally exceed the threshold for flight during the majority of the summer period in south-eastern Australia.

Given the current understanding of triggers of migration for *N. vinitor*, monitoring of population levels within carrot seed crops or other nearby hosts is likely to be more useful than attempts to forecast infestation events. Visual assessment of population levels and sweep netting of crops, as recommended for monitoring of *Lygus* in carrot seed fields (Krivholavek, 1999) are reported to be ineffective for monitoring of *Nysius* (Dalrymple pers. comm<sup>2</sup>). Sticky trap monitoring may provide a potentially cost and time effective method of tracking *N. vinitor* levels within carrot seed crops without the problems of the previous two methods. In this

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study, sticky trap monitoring detected infestation events and accurately estimated the population of adult *N. vinitor* on carrot seed plants in trial plots within commercial fields. Studies at two sites suggested that *N. vinitor* might have a non-random distribution in carrot seed fields, with the highest population densities located on the windward edges of the crop. Placement of sticky traps around the outer edges of carrot seed fields is therefore likely to be satisfactory for detection of *N. vinitor* infestation events, but further study of the distribution of *N. vinitor* in carrot seed fields is required to determine the optimum placement for monitoring of population levels.

In summary, the research reported in this chapter confirms that *Nysius vinitor* is a major cause of low germination of Australian carrot seed. This loss of germination is due to the removal of embryos, or damage to the embryos resulting from the feeding of the insect. Given the levels of infestation of carrot seed crops that were observed at some sites, and the rate at which individuals can damage carrot seeds, *N. vinitor* has the ability to cause considerable loss of germination in carrot seed crops in a relatively short time. The fact that carrot seed crops are susceptible to damage for an extended period of up to 80 days from flowering until windrowing means that effective management of *N. vinitor* is critical for achieving successful carrot seed production in Australia. An important component of the management of *N. vinitor* in carrot seed crops is the ability to detect infestations and quantify population levels. Sticky trap monitoring appears to have the potential to achieve both objectives. Whilst placement of sticky traps around the periphery of crops appears satisfactory for detection of infestation events, further research is required to establish guidelines for their use in quantifying population levels.



## Chapter 5

### Techniques for Handling, Storing and Testing the Germination Capacity of Carrot Pollen

*Appropriate handling and viability testing techniques for carrot pollen were required for use in studies of yield limitation in hybrid seed crops (Chapter 6). Techniques for storage of carrot pollen without loss of viability were also considered desirable so that pollen viability could be studied between the main periods of fieldwork. This chapter addresses these issues. It commences with a review of the literature for pollen handling, storage and viability testing techniques that could be used with carrot. As no techniques specifically for use with carrot pollen were found, the experimental component of the chapter tests the suitability of a range of techniques that have been successfully used with other pollen systems. Recommendations for handling, storing and viability testing of carrot pollen are given in the conclusion.*

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#### 5.1 – Literature Review

##### The Pollen Grain

The pollen grain is the male gametophyte of seed plants. In the angiosperms the mature male gametophyte consists of 3 cells; a vegetative cell and two sperm cells (Raven *et al.*, 1992). With dehiscence of the anthers, the pollen grains are transferred to the stigmas in a process called pollination. Pollination is achieved in different plant species with a range of vectors including wind, insects and other animals. Upon germination on the stigmatic surface, the pollen grain produces a pollen tube that

grows down through the style into the ovary, delivering the non-motile sperm to the female gametophyte so that fertilisation may occur.

Unlike most exposed plant cells, pollen grains lack a cuticle; the cells are instead encased in the sporoderm, which consists of two elastic layers (Dafni, 1992). The outer protective layer, the exine, is formed from the sporophytic tapetal layer of the anther (McCormick, 1991). It is made of sporopollenin, which is indigestible to most insects and absorbs ultraviolet radiation (Keith-Lucas, 1999). The exine is often highly sculpted. The resulting patterns are a diagnostic feature for many plant families and, in some instances, individual species (Moore *et al.*, 1991). The inner layer, the intine, is derived from gametophytic gene expression (McCormick, 1991). Proteins from the intine are involved in pollen germination, pollen tube growth (Van den Ende, 1976) and self-incompatibility (Frankel and Gulen, 1977). The regions within the sporoderm from which the pollen tube emerges during germination are called apertures. The apertures may appear as pores or longer furrows on the surface of the sporoderm.

Two forms of pollen are classified on the basis of the number of cells present in the pollen grain at anthesis. Binucleate (bicellular) pollen occurs in gymnosperms and approximately two thirds of angiosperm species (Brewbacker, 1967; Gardner, 1975). At anthesis binucleate pollen consists of a vegetative cell involved in pollen tube growth and metabolism, and a generative cell that undergoes a mitotic division within the pollen tube to produce the two sperm cells (Keeton, 1980). Trinucleate (tricellular) pollen occurs in approximately one third of angiosperm species. The generative cell divides precociously, so that all three cells are present within the pollen grain at anthesis. The sperm cells are sited wholly within the cytoplasm of the vegetative cell, separated not by walls but by their own plasma membrane and that of the host cell (Dafni, 1992).

In a survey of the nuclear condition of mature pollen in nearly 2000 angiosperms belonging to 265 families, most taxa had either binucleate (179 families) or trinucleate pollen (54 families) (Brewbacker, 1967). Only 5 genera and 32 families were reported to have both types, but no single species contained both. Based on the phylogeny of the binucleate and trinucleate families, the binucleate condition appears to be the more

primitive of the two. Evolution of trinucleate species from binucleate ancestors appears to have been an irreversible step (Brewbacker, 1967). Brewbacker (1967) and Gardner (1975) examined the pollen of 22 species of Apiaceae, but none of *Daucus*, and found that all were trinucleate. On the basis of these studies it seems likely that carrot pollen is trinucleate, but this does not appear to have been confirmed.

Trinucleate pollens are characterised by a series of physiological phenomena. They have relatively short lag times to germination after deposition on the stigma, and undergo rapid pollen tube growth compared to binucleate pollen. In one study, pollen tube emergence occurred within 3 minutes of incubation for the trinucleate pollen of *Aster tripolium* but took 75 minutes for binucleate pollen of *Typha latifolia* (Hoekstra, 1979). This difference appears to arise because trinucleate pollen is shed with a fully developed complement of mitochondria, whilst binucleate pollen contains incomplete mitochondria at anthesis, which develop during the lag phase after deposition on the stigma (Hoekstra, 1979). In contrast to binucleate pollen, trinucleate pollen is generally difficult to germinate *in-vitro* (Shivanna and Rangaswamy, 1992). It often requires water restricting conditions (Bar-Shalom and Mattsson, 1977 cited in Hoekstra, 1979) or high concentrations of sucrose and other metabolites (Hoekstra and Bruinsma, 1975b; Mulchay and Mulchay, 1988), but develops relatively short tubes (Hoekstra and Bruinsma, 1975b). The viability of trinucleate pollen is easily lost, even in conditions routinely used for storage of binucleate pollen (Hoekstra, 1979). *In-vivo*, trinucleate pollen germinates mostly on 'dry' stigmas without exudate (Heslop Harrison and Shivanna, 1977 cited in Hoekstra, 1979). In incompatibility reactions, trinucleate pollen is generally rejected on the stigma surface. The genetic basis of the incompatibility system is either sporophytic, or gametophytic, based on a complex S gene system as in the Poaceae (Lundqvist, 1975 cited in Hoekstra, 1979), whereas the incompatibility of the binucleate species is always gametophytically determined (Hoekstra and Bruinsma, 1979).

### **Pollen Viability and Longevity**

Pollen viability is generally considered to indicate the ability of the pollen grain to perform its function of delivering the sperm cells to the embryo sac following

compatible pollination. Assessment of pollen viability on the basis of its function is cumbersome, time consuming and not always feasible (Shivanna *et al.*, 1991). Many short-cut methods that reflect the competence of the pollen to perform its normal function in the pistil have been devised. These have lead to definitions of viability based on the ability of pollen grains to germinate on the stigma, germinate *in-vitro*, pick up certain stains or effect seed set after pollination (Firmage and Dafni, 2001). Where pollen germination is used as an indicator, germination is usually defined as having occurred if the pollen tube length is greater than the diameter of the pollen grain (Shivanna and Rangaswamy, 1992).

Longevity is defined as the period during which pollen remains able to germinate on the appropriate (receptive and compatible) stigma (Dafni, 2000). As the loss of viability in pollen samples is a continuous variable rather than a dichotomous condition (Kearns and Inouye, 1993) descriptions of longevity such as pollen half-life have been adopted. The half-life of a pollen sample is the time taken for its viability to decrease by 50%. Under natural conditions, the half-lives of different species of pollen range from a few hours to in excess of a year (Frankel and Gulan, 1977). Such differences in pollen grain longevity between plant species are due to fundamental differences in their pollen grain biology and are an evolutionary adaptation to the time period required to reach a compatible, receptive stigma after anthesis. The latter is affected by the method of pollination (self vs. cross) and the mode of dispersal (Chichiricco, 2000; Dafni and Firmage, 2000).

### ***Factors Affecting Pollen Viability and Longevity***

A range of factors during pollen development may cause pollen abortion or the production of infertile pollen, referred to as pollen sterility (Shivanna and Johri, 1989 cited in Dafni and Firmage, 2000). These include genetic and nutritional factors and environmental conditions during pollen development (Dafni and Firmage, 2000). Pollen appears to be most sensitive to environmental stress in the period around meiosis (Sato *et al.*, 2000 cited in Aloni *et al.*, 2001). Low and high temperatures (Chang and Struckmeyer, 1976b; Subedi *et al.*, 1998), photoperiod (Yuan *et al.*, 1993; Voyiatzis and Paraskevopoulou-Paroussi, 2002), light intensity (Yoshida and

Tanimoto, 1999), water stress (Rawson and Subedi, 1996; Sheoran and Saini, 1996) and atmospheric carbon dioxide concentration (Aloni *et al.*, 2001) have been demonstrated to influence pollen sterility. The mechanisms by which environmental factors cause pollen sterility or affect longevity are not fully understood, but many authors propose a role for disruption of assimilate supply to the developing grains (for example, Sheoran and Saini, 1996; Goetz *et al.*, 1998; Aloni *et al.*, 2001). In addition to meeting the energetic requirements of pollen, carbohydrate plays an important role as a desiccation protectant (Vasil, 1987).

In many species, the state of the membrane of the vegetative cell appears to be the principal determinant of pollen longevity after anthesis (Heslop Harison and Heslop Harison, 1970; Shivanna and Heslop Harison 1981; Abdul-Baki and Stommel, 1995). Regulation of water loss is therefore a critical factor in maintenance of pollen viability since water is a major determinant of the structural integrity and stability of cellular membranes (Taylor and Helper, 1997). In most cases, as pollen matures the anthers dehydrate, resulting in partially dehydrated pollen with a water content less than 30% of its fresh weight at anthesis. Partially dehydrated pollen is in a quiescent state and as such is more capable of withstanding the stresses during dispersal (Nepi *et al.*, 2001). Pollen of a few species, for example members of the Poaceae (Shivanna and Heslop Harison, 1981), disperses with a relatively high water content (above 30%). Such pollen is classified as partially hydrated (Nepi *et al.*, 2001). Partially hydrated pollen grains germinate more rapidly after contact with the stigma compared to partially dehydrated pollen because less time is required to reorganise the cytoskeleton (Pacini, 2000). Structurally, partially hydrated pollen is not well guarded against water loss during dispersal, and desiccation rapidly reduces germinability (Pacini and Franchi, 1999).

Partial dehydration causes radical changes to the organisation of the plasma membrane of the vegetative cell (Heslop Harrison, 1979) and alters the DNA conformation (Osborne and Boubriak, 1994) and the volume and shape of the pollen grain (Pacini, 2000). Collectively these changes are referred to as harmomegathy (Wodehouse, 1935 cited in Nepi *et al.*, 2001). In its partially dehydrated state, the plasma membrane is ineffective as an osmotic barrier because it does not have a lamellar structure with a continuous lipid bilayer (Heslop Harrison, 1979). Recovery

of the osmotic properties of the plasma membrane, an essential prerequisite to germination, takes place on rehydration, but only if favourable structural relationships have been preserved in the modified membrane during the period of desiccation (Heslop Harrison, 1979). Longevity is thus related to the stability of the system in its partially dehydrated state, with the loss of viability over time reflecting the progressive loss of capacity of the vegetative cell membranes to regain a normal structure on rehydration. Similarly, it has been suggested that the capacity of DNA to regain its integrity during rehydration may also account for differences in pollen longevity (Osborne and Boubriak, 1994).

The structural changes associated with harmomegathy are directly related to water movement into and out of the pollen grain and therefore fluctuate with changes in relative humidity during dispersal and rehydration. This causes mechanical stresses to the sporoderm, plasma membrane and protoplast (Blackmore and Barnes, 1986 cited in Nepi *et al.*, 2001), which also contribute to reduced viability (Bassani *et al.*, 1994 cited in Aronne *et al.*, 2001)

The type of carbohydrates present in pollen grains plays a role in the regulation of susceptibility to desiccation and pollen longevity (Dafni and Firmage, 2000). Generally, long-lived pollens shed in a partly dehydrated state are rich in sucrose and fructose, whilst short-lived pollens released in a partially hydrated state have low levels of these sugars (Chichiricco, 2000). The role of sucrose and fructose in protection against desiccation appears to be in the maintenance of structural integrity of the plasma membrane and other cellular structures during harmomegathy. Specifically, sucrose appears to prevent folding of the dehydrated plasma membrane by binding to its phospholipids in replacement for water lost during desiccation (Spearanza *et al.*, 1997). Polymerisation and depolymerisation of fructans within the pollen grain enables it to regulate osmotic pressure as a buffer against fluctuations in water content and thermal shock (Pacini, 2000). Hydrolysis of starch in the cytoplasm by acid invertase during the later stages of pollen development contributes to the accumulation of sucrose and fructose in pollen grains (Pacini, 2000; Aloni *et al.*, 2001). The presence of high levels of starch in shed pollen is therefore considered an indication of short-lived pollen or incomplete development typical of sterile pollen (Chichiricco, 2000; Dafni and Firmage, 2000).

In addition to the type of carbohydrate present, the level of reserves within the pollen grain may play an important role in pollen longevity. For short-lived trinucleate pollens, it has been suggested that the formation of the sperm cells prior to anthesis deprives the pollen of sufficient reserves for longevity (Frankel and Gulen, 1977). In contrast to binucleate pollen, trinucleate pollen was reliant on exogenous sources for at least some of its metabolites from the start of germination (Mulchay and Mulchay, 1988). The trinucleate pollen of several species of Asteraceae was observed to halve in viability in about 3 hours compared to 5 to 35 hours for binucleate pollens under conditions of 30°C and 97% relative humidity (Hoekstra and Bruinsma, 1975a). This rapid loss of viability was associated with a respiratory rate 2 to 3 times higher than that of binucleate pollen and a high turnover of ATP (Hoekstra and Bruinsma, 1979). In the Asteraceae the link between respiration rate and longevity is supported by observations of increased longevity and restricted respiration rates under low humidity conditions, and a positive relationship between temperature and rates of viability loss (Hoekstra and Bruinsma, 1975a).

### **Pollen Storage**

The ability to maintain pollen viability in storage is an important component of both plant breeding programs and pollination research. Storing pollen for use in breeding programs eliminates different flowering times or geographical isolation as barriers to successful crossings (Johri and Vasil, 1961; Boyle, 2001). Storage of pollen also eliminates the need for continuous growing of male fertile lines for breeding or research, prevents the variability associated with daily collection of pollen samples, and allows experimental studies of pollen throughout the year (Shivanna and Rangaswamy, 1992).

Long-term storage of pollen has been studied for many species but there appears to have been no research in carrot or related species that has examined pollen longevity or storage effects. The preceding section has highlighted that pollen longevity is greatly affected by the environmental conditions to which the pollen is exposed. For pollen shed in a partly dehydrated state, high or variable humidity and high

temperature are most damaging to longevity (Johri and Vasil, 1961; Chang and Struckmeyer, 1975; Shivanna and Rangaswamy, 1992), but the combination of optimal preparation and storage conditions have to be determined for each species of pollen (Frankel and Gulan, 1977). Whilst binucleate pollens generally store well, under the same storage conditions, the longevity of trinucleate pollen is often short (Hoekstra, 1979).

Drying prior to storage extends pollen longevity in many species (Johri and Vasil, 1961) and is critical for species stored below the temperature at which water would freeze in the undried pollen (Boyle, 2001). After drying, pollen of *Solanum tuberosum* (Johri and Vasil, 1961) and *Pinus radiata* can be stored for extended periods at 4-5°C (Siregar and Sweet, 2000), whilst *Brassica* sp. (Brown and Dyer, 1991) *Petunia hybrida* and *Malus* sp. required storage temperatures of -10 and -35°C to maintain them long-term (Frankel and Gulan, 1977). Repeated freezing and thawing are damaging to pollen viability (Shivanna and Rangaswamy, 1992), so if pollen is needed at frequent intervals, it should be stored in multiple sets.

Long-term pollen storage is generally most effective under ultra-low temperatures (Shivanna and Rangaswamy, 1992). Freeze drying and storage in liquid nitrogen (-196°C) have been shown to be the most successful methods of long term preservation of pollen of *Vitis vinifera* (Parfitt and Almeihdi, 1983), *Narcissus* sp. (Bowes, 1990), *Anigozanthos manglesii* (Sukhvibul, 1993) and the tree genera *Quercus*, *Fagus* and *Catanea* (Joergensen, 1990).

After low humidity storage, pollen generally shows some improvement of germination following exposure to a humid atmosphere (Hoekstra and Bruinsma, 1975b; Shivanna, 1981; Sukhvibul and Considine, 1993). This period of rehydration enables the reorganisation of the pollen membranes from their dissociated state in the dehydrated grain, an essential step for them to function normally during germination. Optimal conditions for rehydration vary according to the species involved and the degree of drying prior to storage but varying periods of exposure to 30°C and 97% relative humidity have proven successful for several species (Hoekstra and Bruinsma, 1975b; Sukhvibul and Considine, 1993).



## **Pollen Viability Testing**

Information about the ability of pollen to perform its function when it reaches a compatible, receptive stigma is valuable for both horticultural purposes and research. Viability tests provide a means of estimating the potential of pollen to do this. The importance of measurements of the viability of pollen used in hand pollination experiments for the interpretation of the results was highlighted by Stone *et al.* (1995). Despite this, in their survey of 283 papers reporting hand pollination experiments, pollen viability was assessed infrequently.

A number of pollen viability tests have been developed and performed on pollen of many plant species (Dafni, 1992; Kearns and Inouye, 1993). These can be grouped into 3 main classes: tests of fruit or seed set, pollen culture and histochemical methods (Knox, 1984). Although acetocarmine staining has been used in the past by vegetable breeders as an indicator of pollen viability in carrot (Markiewicz-Ladd, K. pers. comm<sup>1</sup>.), the relationship between the results of this test and the capacity of pollen to germinate have not been published. In fact, the methods that are most appropriate for testing the viability of carrot pollen appear unknown, as no references to viability testing of pollen of carrot or other members of the Apiaceae could be found in the literature.

## ***Seed Set Tests***

Whilst artificial pollination of receptive flowers and assessment of seed set or pollen tube growth (*in-vivo* germination) provides the most authentic test of pollen viability, it has several important limitations. Factors such as uncertainty about the amount of pollen deposited on the stigma (Young and Young, 1992), stigmatic receptivity (Stone *et al.*, 1995), incompatibility reactions between the pollen and the pistil (Heslop Harrison and Heslop Harrison, 1984) and abortion of the developing seed or fruit after

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fertilisation (Stephenson, 1981) can confound the assessment of pollen viability using this method. Waiting for seed maturation is time consuming and the seed counts that are obtained may be more qualitative than quantitative because, in many species, a small number of viable pollen grains may be all that is necessary for full seed set (Stone *et al.*, 1995).

### ***Pollen Culture***

*In-vitro* germination tests circumvent some of the drawbacks of *in-vivo* germination tests. For many species they provide a direct and reliable assessment of pollen germination capacity and correlate with fruit or seed set (Visser, 1955 cited in Dafni and Firmage, 2000). The base of the germinating medium is a sucrose solution, typically of 10-40% concentration (Kearns and Inouye, 1993) into which other essential ions and nutrients are added. The pollen grains are suspended in the germinating medium in hanging drops, on microscope slides or on agar or gelatin media and usually incubated for 1 to 12 hours before an assessment of germination is made (Shivanna and Rangaswamy, 1992). Under natural conditions, the stigma and style provide water, sugar and amino acids for nourishment and the appropriate osmotic conditions for germination and tube growth (Kearns and Inouye, 1993). The germination medium must therefore be formulated for each species to replicate these conditions. Failure to provide optimal conditions can result in a false negative assessment of germination potential in *in-vitro* tests (Dafni and Firmage, 2000). For many binucleate pollen systems, only three elements, sucrose, boric acid and calcium nitrate are required for germination (Shivanna and Rangaswamy, 1992). The addition of magnesium sulfate and potassium nitrate to this basic medium produced a medium that was found to be suitable for the germination of pollen of 86 species (Brewbacker and Kwack, 1963). In addition to the germination medium, the results of *in-vitro* germination are strongly influenced by other factors such as incubation temperature and the concentration of pollen grains in the media (Brewbacker and Kwack, 1963). Whilst binucleate pollen germinates *in-vitro* with relative ease, suitable media for a number of trinucleate pollen species, including the Apiaceae have not been developed. The difficulty in developing media for trinucleate pollen appears to be caused by its reliance on exogenous sources for at least some of its metabolites from the start of

germination, compared to binucleate pollen, which uses its own reserves during the first phase of germination (Mulchay and Mulchay, 1988). An additional disadvantage of *in-vitro* germination is that media that promote optimal germination of fresh pollen may not be suitable for stored pollen (Shivanna and Rangaswamy, 1992).

### ***Histochemical Tests***

Histochemical tests can be divided into three types: the fluorochromatic reaction, enzyme tests and stainability. The histochemical tests have one common characteristic: all are asymmetric in their predictive value. In each case, a positive result establishes the possibility, but not the certainty of effective function, whilst in a properly designed test, a negative should establish the certainty of non-function. The reliability of a histochemical test therefore depends on the closeness of the relationship between the property assessed and loss of germinability in the pollen system under investigation.

The fluorochromatic reaction procedure (FCR test) (Heslop Harison and Heslop Harison, 1970) is principally based on the relationships between pollen viability and the integrity of the plasma membrane of the vegetative cell and the presence of active esterases within the pollen cytoplasm. Pollen samples are suspended in a sucrose solution of appropriate concentration containing the non-polar, non-fluorescent ester of fluorescein, fluorescein diacetate (FDA). FDA readily penetrates biological membranes. Cytoplasmic esterases cleave FDA to release fluorescein, which traverses intact membranes with greater difficulty and so accumulates intracellularly, where it can be detected by fluorescence microscopy. Thus, with the FCR test, viable pollen grains are indicated by their fluorescence under ultraviolet light. The results of the FCR tests are influenced by the concentration of sucrose in the FDA solution (Kearns and Inouye, 1993), through its effect on the osmotic potential of the solution. Optimal sucrose concentrations must be determined for the species of pollen under investigation. These typically range from 2% to 40% (Kearns and Inouye, 1993). A 20% sucrose solution was recommended for a range of species from the Poaceae, Asteraceae and Gesneriaceae (Heslop Harison and Heslop Harison, 1970).

The FCR test is a relatively simple procedure that provides a reliable estimate of pollen germinability and the capacity to effect fertilisation in a range of species (Shivanna *et al.*, 1991; Aronne *et al.*, 2001). A high correlation ( $r=0.86$  to  $1.00$ ) was observed between FCR and *in-vitro* germination of bi- and trinucleate species belonging to the Iridaceae, Cyperaceae, Poaceae, Scrophulariaceae, Plantaginaceae and Caprifolaceae (Shivanna, 1981). La Porta and Roselli (1991, cited in Kearns and Inouye, 1993) also found that the FCR test and *in-vitro* germination were highly correlated ( $r=0.99$ ) and determined that the FCR test was an easier and faster technique. The FCR test accurately predicted *in-vitro* germination of pollen of *Nicotiana tabacum*, *Agave* sp., *Tradescantia virginiana* and *Iris* sp. exposed to heat and high humidity stresses (Shivanna *et al.*, 1991) and *Helleborus niger* and *Narcissus pseudonarcissus* exposed to heat stress and treatment with 10% dimethylsulphoxide, which increases the permeability of the plasma membrane and enhances leakage (Heslop Harrison *et al.*, 1984). In contrast, the FCR test significantly overestimated the *in-vitro* and *in-vivo* germination capacity of heat stressed *Brassica* pollen (Rao *et al.*, 1992).

The FCR test has an element of subjectivity associated with the scoring procedure, due to the continuum of intensity of fluorescence observed in some pollen samples (Kapyla, 1991 cited in Dafni and Firmage, 2000). Lipids are autofluorescent under the UV light used in the FCR test, so even dead, empty pollen grains will show some weak fluorescence (Shivanna, 1981). This problem has, in part, been addressed with the application of computerised image analysis and scoring techniques (Aronne *et al.*, 2001). Since the FCR test measures the potential for germination, rather than germination itself, it may overestimate viability in some cases, particularly if immature pollen is tested (Shivanna, 1981). As a positive result in the FCR test is reliant on a functional plasma membrane, the test can also yield false negative scores if dehydrated pollen is not properly pre-conditioned by rehydration before testing (Shivanna and Rangaswamy, 1992). Unlike *in-vivo* or *in-vitro* germination, where pollen tube growth rates can be measured, the FCR test provides no indication of pollen vigour (Shivanna *et al.*, 1991).

Tests of enzyme activity in pollen include the x-gal test (Trognitz, 1991 cited in Firmage and Dafni, 2001) and tetrazolium tests (MTT and Bakers solution tests)

(Shivanna and Rangaswamy, 1992; Kearns and Inouye, 1993). The x-gal test is based on the hydrolysis of 5-bromo-4-chloro-3-indoyl- $\beta$ -galactosidase to 5-bromo-4-chloro-indigo, a blue dye, by  $\beta$ -galactosidase (Trognitz, 1991 cited in Firmage and Dafni, 2001). Tetrazolium tests of pollen viability are based on the reduction of the tetrazolium salt by dehydrogenase enzymes to produce formazan, which stains the pollen cytoplasm red. A positive stain is taken as an indication of a functioning respiratory system, an essential prerequisite for germination and tube growth. After staining, the pollen grains may vary from pale to deep red, making it difficult to establish a consistent cut-off point for viability (Shivanna and Rangaswamy, 1992).

For many species enzyme tests are a reliable indication of pollen viability (Stone, 1995); for others, they may yield false positive scores for viability when compared with the results from *in-vitro* germination tests (Heslop Harrison *et al.*, 1984; Rodriguez-Riano and Dafni, 2000 cited in Dafni and Firmage, 2000). Therefore the reliability of enzyme tests needs to be established for each study species. Of a range of viability tests suitable for use in the field, MTT and Baker's solution provided the best estimates of *in-vitro* germination of pollen of 14 of 17 species examined by Firmage and Dafni (2001).

Stains specific to pollen components can be used to test for viability. These include aniline blue in lactophenol, which stains callose; acetocarmine, which stains cytoplasm; and phloxin-green, which stains cytoplasm and cellulose (Kearns and Inouye, 1993). The reliability of such stains is reduced in many systems because immature or non-viable pollen often contains enough of the target tissues to cause staining, and viable pollen of some species does not stain well (Kearns and Inouye, 1993). Stains appear to often provide the least reliable means of estimating pollen viability (Heslop Harrison and Heslop Harison, 1984; Shivanna and Rangaswamy, 1991).

## Conclusion

Although appropriate handling, storage and viability testing procedures for pollen should be determined for individual species, there appears to be no published information indicating suitable methods for use with carrot or related species. The information presented in this review provides a useful starting point for the experimental determination of such methods. The nuclear condition of the pollen system of interest is an important factor and should be established before attempts are made to determine appropriate methods for storage and viability testing. Although it seems likely from the study of other members of the Apiaceae that carrot pollen is trinucleate, there appears to be no published confirmation of this. Trinucleate pollens are generally shorter-lived than binucleate pollens and require more extreme storage conditions to maintain longevity. Despite this, the principles of reduced temperature and relative humidity during storage, and the need to rehydrate stored pollen before use apply to most species of both pollen types.

As there have been no published reports of appropriate pollen viability tests for use with carrot, potential tests must be identified and screened for suitability. Acetocarmine staining has previously been used as an indicator of carrot pollen viability, but the information presented in this review suggests that it is unlikely to be the most reliable test. *In-vitro* germination has been successfully used for many species of pollen, but the lack of suitable media for some trinucleate families including the Apiaceae is likely to preclude its use in this study, if the pollen of carrot is in fact trinucleate. In contrast, the FCR test has proven to provide a reliable indication of pollen viability in many bi- and trinucleate pollen systems, and appears a logical inclusion in screening trials. Similarly, the reports of successful application of tetrazolium tests in the viability testing of a range of bi- and tri-nucleate pollen species warrants their inclusion in such trials.

## **5.2 – Materials and Methods**

### **Examination of the Nuclear Number of Carrot Pollen at Anthesis**

The number of sperm nuclei in carrot pollen grains was determined using the acetocarmine staining method described by Brewbacker (1967). Mature anthers of WO8024 were macerated in prepared acetocarmine stain solution (Southern Biological Services, Victoria, Australia) on a microscope slide, covered with a cover slip and allowed to stand for 20 minutes under laboratory conditions. The number of sperm nuclei in individual pollen grains was determined by examination of the prepared slides under a light microscope (Leica Leitz DM RBE, Heerbrugg, Switzerland) at 1000x magnification with oil immersion. Sperm nuclei stained red against a pale pink background of cytoplasm.

### **Evaluation of Pollen Viability Tests for Use with Carrot Pollen**

As preliminary attempts to germinate carrot pollen at a range of temperatures between 15 and 30°C *in-vitro* using Brewbacker and Kwack's medium (Brewbacker and Kwack, 1963) at pHs between 5 and 9 in both hanging drops and on agar were unsuccessful, *in-vitro* germination was excluded from the evaluation of viability tests. Three tests of pollen viability were examined: the fluorochromatic reaction (FCR) test (Heslop Harrison and Heslop Harrison, 1970); the MTT (2,5-diphenyl tetrazolium bromide) test (Firmage and Dafni, 2001) and acetocarmine staining (Kearns and Inouye, 1993). *In-vivo* germination was used as a benchmark against which the other tests were compared. Specific details of each test procedure are provided below. Evaluation of the tests was performed on random samples of pollen drawn from pools of freshly dehiscent pollen of each of the lines No. 22, WO8024 and ON44-88A x ON44-131C. Umbels of each line with mature, undehiscent anthers were collected from the field at 8am and transported to the laboratory. During a 30 minute period the anthers were allowed to dehisce under the ambient conditions and the pollen collected using the technique detailed in Chapter 2 (Section 2.4). The screening procedure was

repeated with fresh pollen collected on 2 days during the flowering period. On each day all tests were prepared for scoring within 15 minutes of collection of the dehiscent pollen.

### ***The FCR Test***

The method of FCR testing was based on the original method of Heslop Harrison and Heslop Harrison (1970). Prior to conducting this test, an optimum sucrose content for the fluorescein diacetate (FDA) test solution was determined. Pooled samples of freshly dehiscent pollen from glasshouse-grown plants of the lines No. 22 and WO8024 were used. 0, 5, 10, 20, 30, 40 and 50% (w:v) solutions of sucrose were made up with deionised water. 1ml of FDA dissolved in acetone (2mg/ml) was added to 10mls of each sucrose solution, resulting in solutions of persistent turbidity. For each line, sub-samples of pollen were drawn at random from the pooled sample and 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 in random order 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 (Figure 5.3). The results were reported as the percentage of FCR positive grains out of the total number counted. The procedure was repeated with freshly collected pollen on 3 subsequent occasions, giving 4 replicates in total.

During the screening of pollen viability tests, the FCR testing methodology was the same as described above, but an FDA solution with 20% sucrose was used.

### ***The MTT Test***

The MTT staining procedure was adapted from Firmage and Dafni (2001). A staining solution of 1% MTT (w:v) in 20% sucrose solution (w:v) was prepared. Four replicate



samples of pollen were brushed onto individual microscope slides, suspended in a drop of the staining solution under a cover slip and incubated in darkness at 30°C for 30 minutes. Following incubation the slides were examined under a light microscope at 100x magnification. Pollen grains staining red were scored as viable whilst unstained pollen grains were scored as non-viable (Figure 5.3). Six fields of view were examined for each replicate.

### ***Acetocarmine Staining***

Four replicate samples of pollen were suspended in a drop of prepared acetocarmine stain solution (Southern Biological Services, Victoria, Australia) on individual microscope slides. After cover slips had been added, the slides were allowed to stand under laboratory conditions for 20 minutes. The slides were subsequently examined under a light microscope at 100x magnification. Pollen grains with cytoplasm stained pink were scored as viable (Figure 5.3). Six fields of view were examined for each replicate.

### ***In-Vivo Germination***

*In-vivo* germination tests were undertaken in a glasshouse, using glasshouse grown male sterile lines. Preliminary trials established that, for the range of male fertile lines used, pollen germinated on the stigmas of the corresponding male sterile line as well as it did on 2 other male sterile lines of different breeding. Thus only the corresponding male sterile line within the hybrid cross was used for *in-vivo* germination testing.

On both days of screening, samples of pollen from the male fertile lines were sparingly hand pollinated onto the receptive flowers within a single umbellet of a secondary umbel from 4 plants of the corresponding male sterile line. The corresponding male sterile lines were No. 22 male sterile (for No. 22 male fertile), WO6014 (for WO8024 male fertile) and ON44-88A (For ON44-131C male fertile). 24 hours after pollination, 6 pollinated flowers were removed from each plant, and the

stylar and stigmatic tissue excised under a dissecting microscope. The excised tissue was mounted on a microscope slide in aniline blue stain (0.05% water-soluble aniline blue in 0.1M K<sub>3</sub>PO<sub>4</sub>). The prepared mounts were observed at 100x magnification using a Leica Leitz DM RBE fluorescence microscope fitted with an HBO 50 Watt mercury vapour lamp, BP 340-380 excitation filter, RKP 400 dichromatic mirror and LP 430 suppression filter (Leica, Heerbrugg, Switzerland). All pollen grains present on the stigmatic surface were clearly visible, whilst the pollen tubes of germinated grains fluoresced brightly (Figure 5.4). Pollen grains located on the stylar tissue were scored for germination on the basis of the presence of a pollen tube. Typically, between 10 and 30 grains of pollen were scored on each stigma.

### **Validation of the FCR Test for Routine Use**

Twenty samples of pollen of the male fertile lines No. 22, W08024 and ON44-131C grown under field and glasshouse conditions, and with a range of post anthesis histories (see Table 5.1) were tested for germination capacity using the FCR procedure and the *in-vivo* germination test. The results of the two tests were compared to establish the reliability of the FCR procedure for the routine testing of carrot pollen germinability in this study.

### **The Effects of Desiccation and Rehydration on Pollen Viability**

The effects of three factors in the preparation of pollen for storage were examined: duration of pollen dehydration over silica gel (24 or 48 hours) vs. no dehydration; the temperature during the dehydration period (4 or 25°C); and the length of the rehydration period at 30°C and 97% RH (0, 30 or 60 minutes). Pooled samples of freshly dehisced pollen were collected from glasshouse grown plants of W08024 and No. 22 male fertile. For each line, randomly drawn sub-samples of pollen were brushed onto 96 microscope slides, with 4 replicate slides exposed to each possible treatment combination. Following the rehydration component of the treatments, the pollen on each slide was assessed for germination capacity using the FCR test.

**Table 5.1** – Source and post anthesis history of pollen samples used for validation of the FCR procedure. Note: aged pollen was exposed to ambient laboratory conditions after dehiscence for the time periods specified in the table.

Line	Field / glasshouse grown plants	Post anthesis history	No. of samples
WO8024	Field	Tested freshly dehisced pollen	2
ON44-131C	Field	Tested freshly dehisced pollen	2
No. 22	Field	Tested freshly dehisced pollen	1
No.22	Glasshouse	Tested freshly dehisced pollen	2
WO8024	Glasshouse	Tested freshly dehisced pollen	2
No. 22	Glasshouse	Aged 1 hour	2
WO8024	Glasshouse	Aged 1 hour	2
WO8024	Glasshouse	Aged 2 hours	2
WO8024	Field	97% RH @ 30°C for 1 hour	1
WO8024	Field	97% RH @ 30°C for 4 hours	1
WO8024	Field	60°C for 1 hour	1
WO8024	Field	Desiccated over silica gel @ 18°C for 48 hours, stored 1 year @ -80°C, rehydrated for 30 minutes @ 97 %RH and 30°C	2

### **The Effect of Storage Temperature on Pollen Longevity**

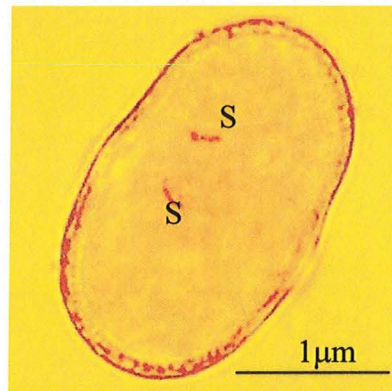
Sub-samples of pollen drawn from pooled samples of freshly dehisced pollen collected from glasshouse grown plants of No. 22 and WO8024 were tested for germination capacity using the FCR procedure. The remaining pollen was desiccated at 18°C for 48 hours. After desiccation, 4 sub-samples of pollen from each line were rehydrated for 30 minutes at 30°C and 97% relative humidity, and viability tested using the FCR procedure. The remaining pollen was randomly divided into 28 samples (WO8024) and 20 samples (No. 22). Each sample was sealed in a 1.5ml Eppendorf centrifuge tube. For each line, the samples were divided equally into 4 sets, with each set packed in silica gel in a sealed plastic container. The four sets of pollen

samples were stored at 4 temperatures, 25, 4, -20 and -80°C. At time intervals of 3, 12, 30, 60, 120, 180 and 365 (WO8024) and 12, 60, 180 and 365 (No. 22) days after desiccation, 4 tubes of pollen of each line were removed from storage, rehydrated at 30°C and 97% RH for 30 minutes and tested for germination capacity using the FCR procedure.

### 5.3 - Results

#### Examination of the Nuclear Number of Carrot Pollen at Anthesis

The vegetative nuclei could not be detected in acetocarmine stained carrot pollen. Despite this, the presence of 2 distinctive spindle shaped sperm nuclei was confirmed (Figure 5.1), which indicates that carrot pollen is released at anthesis in a trinucleate state.

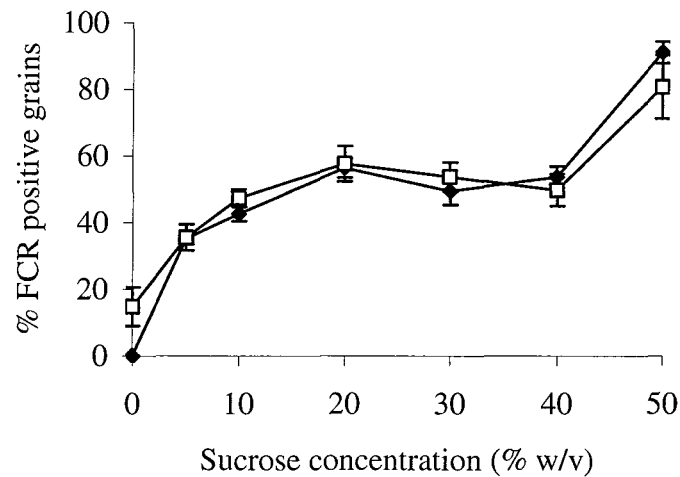


**Figure 5.1** – A carrot pollen grain mounted in acetocarmine showing 2 sperm nuclei (S) indicative of trinucleate pollen. The vegetative nucleus could not be detected in acetocarmine stained pollen. Image photographed with a Leica 300F digital camera mounted on a Leica Leitz DM RBE microscope (Leica, Heerbrugg, Switzerland) at 1000x magnification with oil immersion.

#### Evaluation of Viability Tests for Use with Carrot Pollen

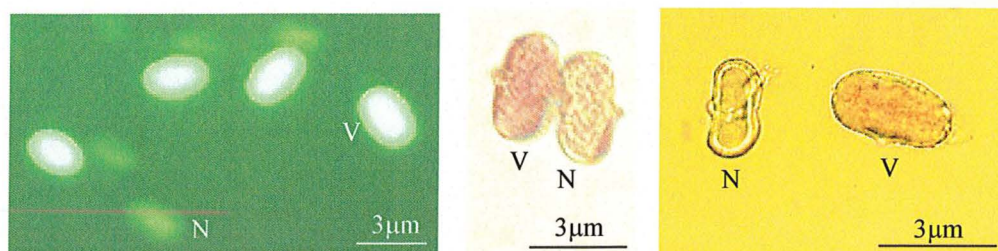
The concentration of sucrose in the FDA staining solution significantly ( $P < 0.001$ ) affected the staining responses of pollen grains of WO8024 and No. 22, with very similar responses from both lines (Figure 5.2). Whilst all grains of WO8024 burst when placed in FDA solution with no sucrose, 15% of pollen grains of No. 22 remained intact and fluoresced. The initial plateau in the relationship occurred at a concentration of 20% sucrose, where approximately 57% of grains of both lines

fluoresced. At 50% sucrose, 81 and 91% of pollen of No. 22 and WO8024 fluoresced respectively.

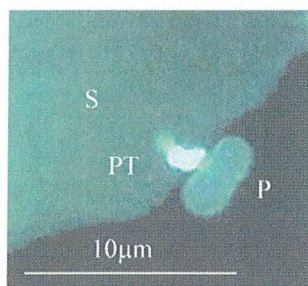


**Figure 5.2** – The relationship between the concentration of sucrose in the FDA solution and percentage of pollen grains of WO8024 (◆) and No.22 (□) scored as positive for viability in the FCR test. For both lines, differences in %FCR positive grains across the range of sucrose concentrations are significant ( $P < 0.001$ ). LSD ( $P < 0.05$ ) No. 22 = 14.56; WO8024 = 8.27. Error bars indicate standard errors,  $n=4$ .

The staining responses of carrot pollen in the Acetocarmine, MTT and FCR viability tests are illustrated in Figure 5.3. Figure 5.4 shows carrot pollen germinating on the stigmatic surface in the *in-vivo* germination test. Of the three tests of pollen viability, the FCR test using FDA in 20% sucrose solution most reliably estimated the capacity of pollen grains to germinate *in-vivo* (Table 5.4). The acetocarmine stain test provided no indication of capacity for *in-vivo* germination, whilst the MTT tests markedly overestimated *in-vivo* germination.



**Figure 5.3** – Viable (V) and nonviable (N) carrot pollen as determined by a) the FCR test; b) MTT staining; and c) acetocarmine staining. Images photographed with a Leica 300F digital camera mounted on a Leica Leitz DM RBE microscope (Leica, Heerbrugg, Switzerland). Lighting and filter details are given in Section 5.3.



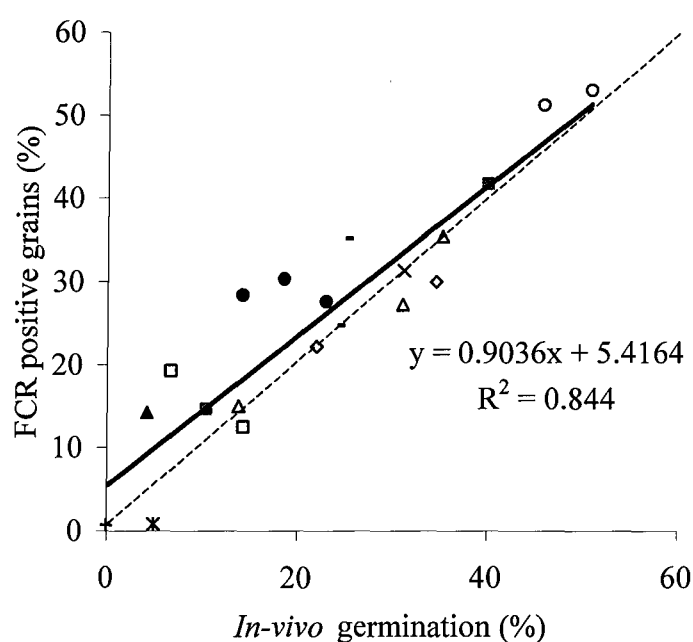
**Figure 5.4** – *In-vivo* germination of a carrot pollen grain (P) on the stigmatic surface (S) 24 hours after hand pollination as seen under a fluorescence microscope following staining with aniline blue. The grain has germinated and produced a pollen tube (PT). The pollen tube can be seen entering the stigmatic surface but is then obscured by the non-cleared stigmatic tissue. Image photographed with a Leica 300F digital camera mounted on a Leica Leitz DM RBE microscope (Leica, Heerbrugg, Switzerland) at 100x magnification. Lighting and filter details are given in Section 5.3.

### Validation of the FCR Test for Routine Use

There was a close linear relationship ( $P < 0.0001$ ) between the results of the FCR test and *in-vivo* germination for samples of pollen from No. 22, W08024 and ON44-131C with a range of histories and *in-vivo* germination percentages between 0 and 51% as illustrated in Figure 5.5. For some pollen samples of relatively low viability there was a tendency for the FCR test to overestimate *in-vivo* germination by up to 14%. This trend was not isolated to a particular line of pollen or pollen history.

**Table 5.4** – A comparison of *in-vivo* percentage germination of carrot pollen samples with the results of the acetocarmine, MTT and FCR viability tests. Note samples a and b refer to pollen collected on 2 separate days during the flowering period. The figures in italics indicate standard errors, n=4.

Pollen source	Viability test							
	<i>In-vivo</i>		Acetocarmine		MTT		FCR	
WO8024a	10.8	<i>1.4</i>	96.7	<i>2.3</i>	26.1	<i>7.6</i>	19.2	<i>3.0</i>
WO8024b	14.3	<i>7.1</i>	94.7	<i>1.5</i>	38.3	<i>6.0</i>	12.5	<i>3.9</i>
No.22a	14.2	<i>4.8</i>	NA	NA	51.0	<i>15.9</i>	28.4	<i>5.1</i>
ON44-131Ca	10.4	<i>2.1</i>	97.7	<i>6.5</i>	40.7	<i>11.5</i>	14.6	<i>2.1</i>
ON44-131Cb	41.7	<i>11.8</i>	97.7	<i>2.3</i>	52.0	<i>4.8</i>	40.0	<i>4.9</i>



**Figure 5.5** – The relationship between pollen viability estimated by the FCR procedure and *in-vivo* germination capacity for pollen samples of the lines WO8024 (□, Δ, ◇, ○, -, ×, +, ✕), No. 22 (●, ▲) and ON44-131C (■). The pollen samples were tested fresh from the field (□, □) or glasshouse (●, ○) or, for glasshouse pollen exposed to the following post anthesis treatments prior to viability testing: ageing under laboratory conditions for 1 (▲, Δ) or 2 hours (◇); exposure to 90%RH at 30°C for 1 (×) and 4 (✕) hours; exposure to 60°C for 1 hour (+); dehydrated and stored at -80°C for 1 year (-). The broken line indicates a 1:1 relationship. Each symbol is the mean of 4 replicate test samples.



## **The Effect of Desiccation and Rehydration Conditions on Pollen Viability**

Pollen of WO8024 and No. 22 responded in a similar way to desiccation and rehydration. Failure to dehydrate pollen after collection resulted in very low (close to 0%) viability after 24 hours irrespective of storage temperature or rehydration treatment. The following discussion of results excludes treatments that were not dehydrated.

Pollen viability was maintained during desiccation over silica gel at 4 or 18°C for up to 48 hours, but the duration of the rehydration stage (97% relative humidity at 30°C) significantly affected pollen viability (Tables 5.5a and 5.5b). The interaction between duration of desiccation, storage temperature during desiccation and duration of rehydration also significantly ( $P < 0.001$ ) affected pollen viability. For all treatments except 24 hours desiccation at 4°C, rehydration for 30 minutes significantly ( $P < 0.001$ ) improved pollen viability. Rehydration for longer than 30 minutes was generally detrimental to pollen viability. The treatment combinations that best maintained pollen viability were 24 hours desiccation at 4°C followed by no rehydration; 24 hours desiccation at 18°C followed by 30 minutes rehydration; and 48 hours desiccation at 4 or 18°C followed by 30 minutes rehydration.

**Table 5.5** – The effects of duration and temperature of desiccation over silica gel and duration of rehydration at 30°C and 97% relative humidity on viability (%) of pollen of the male fertile lines of WO030 (top) and No. 22 (bottom).

Desiccation time (hours)	Desiccation temp (°C)	Rehydration (minutes)	Mean viability (%)	Standard error (n=4)
0	-	-	54.5	3.5
24	4	0	47.8	5.4
24	4	30	38.5	3.9
24	4	60	33.1	2.6
24	18	0	28.5	5.4
24	18	30	47.5	3.7
24	18	60	26.0	1.8
48	4	0	28.5	7.2
48	4	30	48.6	3.9
48	4	60	27.6	3.4
48	18	0	16.3	2.6
48	18	30	47.3	4.2
48	18	60	34.0	3.6
LSD (P<0.05)			9.24	

Desiccation time (hours)	Desiccation temp (°C)	Rehydration (minutes)	Mean viability (%)	Standard error (n=4)
0	NA	0	32.6	2.0
24	4	0	36.0	2.9
24	4	30	31.8	4.3
24	4	60	29.2	4.8
24	18	0	0.0	0.0
24	18	30	32.3	1.1
24	18	60	19.5	5.0
48	4	0	5.4	2.1
48	4	30	32.0	3.6
48	4	60	2.5	0.7
48	18	0	0.0	0.0
48	18	30	28.5	0.8
48	18	60	16.9	2.2
LSD (P<0.05)			8.51	

## The Effect of Storage Temperature on Pollen Longevity

The temperature at which pollen was stored significantly ( $P<0.0001$ ) affected its longevity (Table 5.6). Over 365 days, the viability of pollen of WO8064 and No.22 stored at  $-80^{\circ}\text{C}$  did not decline significantly. Over the same time period, the viability of pollen stored at 18, 4 and  $-20^{\circ}\text{C}$  declined markedly ( $P<0.0001$ ) to 0, 4.2 and 7.5% respectively for WO8024. By day 365, no pollen of No. 22 stored at 18, 4 or  $-20^{\circ}\text{C}$  retained viability.

**Table 5.6** – The effect of storage temperature on the longevity of pollen of the carrot lines No. 22 and WO8024 (results as % viability). Data were analysed as an ANOVA design. Due to the lack of randomisation between temperature treatments, significant differences between temperatures were determined at the 0.01 level of probability.

Line	Storage temperature ( $^{\circ}\text{C}$ )	Time of storage (days)								LSD ( $P<0.05$ ) (time)
		0	3	12	30	60	120	180	365	
WO8024	18	52.4	-	14.4	-	8.9	6.2	2.2	0.0	9.2
	4	52.4	37.5	32.0	28.5	21.3	18.6	14.7	4.2	13.2
	-20	52.4	-	51.1	36.8	37.4	-	19.7	7.5	9.0
	-80	52.4	52.4	53.0	50.5	53.2	51.5	48.4	43.9	11.4
	LSD ( $P<0.01$ ) (temperature)		11.9	13.2	9.6	11.8	4.8	7.9	12.4	
No. 22	18	60.0		20.8		9.8		0.7	0.0	10.0
	4	60.0		55.0		34.4		24.1	0.0	8.0
	-20	60.0		56.9		46.0		26.5	0.0	6.5
	-80	60.0		59.4		55.9		53.5	50.0	17.9
	LSD ( $P<0.01$ ) (temperature)			20.3		10.3		15.3	8.9	

## 5.4 - Discussion

The experiments that are reported in this chapter were conducted so that practical recommendations for the collection, storage and viability testing of carrot pollen could be developed. The observation that carrot pollen is in a trinucleate state at anthesis has important implications for this objective; most trinucleate pollens are difficult to germinate *in-vitro*, have relatively short life spans and are more difficult to store compared to binucleate pollen (Hoekstra, 1979; Shivanna and Rangaswamy, 1992).

The FCR test, based on a carrier solution with 20% sucrose, provided an accurate estimate of the results of *in-vivo* germination of freshly dehisced carrot pollen but was a much simpler and faster test to perform. The results of the MTT test and acetocarmine staining bore little resemblance to the *in-vivo* germination capacity, generally overestimating it by a large margin. Whilst MTT testing has been used successfully in some studies to estimate pollen viability (Firmage and Dafni, 2001), in others, as appears to be the case in carrot, it has been observed that the loss of germination capacity occurs prior to the loss of respiration. Although the acetocarmine stain test of pollen viability can distinguish between abortive and non-abortive pollen in some species based on the presence of cytoplasm (Kearns and Inouye, 1993) the finding that it does not indicate germination capacity in carrot pollen is consistent with reports from a range of other species (Janssen and Herman, 1976).

In addition to reliably estimating the germination capacity of freshly dehisced field and glasshouse pollen, the FCR test provided good estimates of the viability of pollen samples that had been aged, stored or exposed to temperature or humidity stresses. This indicates that the loss of germination capacity in carrot pollen in a range of circumstances is largely due to a loss of membrane integrity, or impaired functioning of esterase enzymes. It was frequently observed that the FCR solution surrounding low viability pollen samples fluoresced. Given that the samples contained little other plant tissue, this suggests that the loss of viability was probably associated with membrane damage rather than a loss of esterase activity. Overall, FCR testing was

observed to slightly overestimate the *in-vivo* germination capacity, particularly for samples of low viability. A similar result was reported by Heslop Harrison *et al.* (1984). As in any histochemical test of pollen viability, a positive result establishes the possibility but not the certainty of effective function, whilst a negative should establish the certainty of non-function.

The role of sucrose in the FCR test is to provide a medium with appropriate water potential for normal functioning of the pollen grain. The response of carrot pollen to increasing sucrose concentration in the FDA carrier solution over the range 0 to 40% was consistent with the responses of a range of other species of pollen including *Impatiens sultanii*, *Cucurbita pepo* and *Setaria palmifolia* (Heslop Harrison and Heslop Harrison, 1970). Without the addition of sucrose, most pollen grains burst and, between 0 and 40%, a concentration of 20% sucrose produced the highest percentage of fluorescing grains. Although it was repeatable, the large (20-30%) increase in the percentage of fluorescing carrot pollen grains when the sucrose concentration was increased to 50% is unusual compared with the responses of other species reported in the literature. The use of 20% sucrose more accurately estimated *in-vivo* germination than 50% sucrose but it is possible that under the conditions of the 50% sucrose FDA solution an increased number of pollen grains were in a functional state. The FCR test scores pollen placed directly in a fluid medium. This means that the grains imbibe virtually instantaneously. The initial inflow of water is driven by the water potential gradient between the solution and the pollen grains. If uncontrolled, this inflow causes a rapid dilation of the grain (Heslop Harrison, 1979). Accordingly, one may expect that the components of the plasma membrane in the dehydrated grain would be placed under immediate stress, which may impact on their ability to reconstitute a normal membrane thereafter. The reduced water potential gradient when a 50% sucrose solution is used in place of a 20% sucrose solution may reduce the stress placed on the plasma membrane during water uptake and therefore increase the likelihood of a functional pollen grain following rehydration. The positive response of dehydrated carrot pollen to rehydration in humid air observed in this work is consistent with such an explanation.

Desiccation is a necessary step in the storage of most pollen types and, in this work, was shown to be necessary for storage of carrot pollen. During desiccation, carrot

pollen could be held at 25°C over silica gel for 48 hours without a detectable loss of viability. Over longer storage periods the viability of desiccated pollen was particularly sensitive to temperature. Although -20°C was satisfactory for short-term storage (up to 12 days) maintenance of pollen germinability for up to one year was only achieved with storage at -80°C. Desiccated pollen samples required rehydration to regain the capacity to germinate. Thirty minutes at 30°C and 97% relative humidity proved satisfactory for this purpose, but care should be taken in using this treatment because longer periods of exposure were damaging to pollen germinability.

In summary, FCR testing with a FDA solution containing 20% sucrose appears to provide a reliable method of estimation of the germination capacity of carrot pollen. For optimum viability, carrot pollen should be collected soon after anthesis. Samples that are not for immediate testing should be placed in desiccating conditions at the time of collection. After 24 to 48 hours desiccation at the ambient laboratory temperature (25°C) the pollen should be transferred to low temperature (-80°C) storage. Stored samples should be rehydrated for 30 minutes at 30°C and 97% relative humidity prior to use.

## Chapter 6

### Some Limiting Factors for Seed Set in Hybrid Carrot Seed Crops

*In variety trials at Cambridge (Chapter 3) seed set was considerably lower in the male sterile lines compared to open pollinated lines. This chapter examines mother plant resource limitation, pollination and pollen viability as causes of low seed set in hybrid carrot seed crops produced in southern Tasmania.*

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#### **6.1 - Literature Review**

Many plants produce mature seeds from only a proportion of their functional female flowers. Plant ecologists have advanced several reasons for surplus flower production. These hypotheses fall into three broad categories (Stephenson, 1981): a) uncertainties surrounding pollination, fruit/seed predation and resource availability; b) the male role of hermaphroditic flowers in pollination; and c) the improvement in seed quality acquired by selective abscission. Generally, the proportion of flowers that produce mature seeds increases with more favourable conditions (Bouwmeester *et al.*, 1995). Thus the fraction of the reproductive potential that is realised often depends upon variables such as the extent of pollination and fertilisation, seed predation and the capacity of the maternal parent to provide the resources necessary for seed development.

Many studies of cultural practices for carrot seed production have been made. The effects of time of sowing (Ilic, 1997), planting density (Harrington, 1951; Gray, 1981; Gray and Steckel, 1983a; Gray and Steckel, 1983b; Noland *et al.*, 1988; Oliva *et al.*,

1988), nutrition (Hawthorn, 1952; Austin and Longden 1966 cited in George, 1985), irrigation (Steiner *et al.*, 1990) and timing of harvest (Hawthorn *et al.*, 1961; Gray, 1979; Sandin, 1980; Gray and Steckel, 1983b; Tucker and Gray, 1986) on the yield of carrot seed crops have been studied, with optima determined for each. These studies have been predominantly conducted with open pollinated varieties so it is unclear if many of the recommendations are applicable for hybrid seed production. Use of cultural practices determined for open pollinated seed crops may contribute to lower than desired yields from hybrid seed crops in some circumstances, but does not account for the large seasonal variation in seed set in certain varieties observed by industry (Dalrymple pers comm; Garewall pers. comm<sup>1</sup>). Seasonal variation in seed yield of up to 150% is shown in the data presented by Gray and Steckel (1983a) for open pollinated Chantenay carrots grown at the same location in 3 seasons with a standard planting density and uniform cultural practices, suggesting a strong environmental component.

Although there is some evidence suggesting that hybrid carrot seed set is primarily limited by processes during pollination (Erickson and Peterson, 1978; Erickson and Peterson, 1979; Erickson *et al.*, 1979; Galuszka and Tegrek, 1987; Galuszka *et al.*, 1989) both pollination and assimilate availability may limit yield (Bierzychudek, 1981; Zimmerman and Pyke, 1988). This review examines the processes between flowering and the beginning of seed development that may limit seed yields in hybrid carrot.

### **Female Flower Fertility**

Within the Apiaceae there is a widespread tendency to produce a proportion of male only flowers, characterised by the absence of the ovary structures (Braak and Kho, 1958; Quagliotti, 1967; Koul, 1993). In male sterile carrot lines, female sterility causes sexless flowers that are incapable of setting seed. Differences in the level of expression of female sterility between wild and cultivated carrot varieties have been

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<sup>1</sup> Mr Max Dalrymple, Production Manager, South Pacific Seeds, South Australia, Australia.  
Mr Chad Garewall, Production Manager, North Pacific Seeds, San Joaquin, USA



days after anthesis (Tian *et al.*, 1995). Thus the duration of stigmatic receptivity does not always determine the window for pollination.

The duration of stigmatic receptivity and fertilisation potential of the ovules can potentially have a significant effect on seed yields, particularly if they are of a short period. Such conditions necessitate close coordination of the flowering of the parent lines for successful pollination. In a range of hybrid carrot crosses, male sterile plants often bloomed later than their male fertile partners did (Erickson and Peterson, 1979).

### ***Pollination***

Pollination is the transfer of pollen from the anther to the stigma. Inadequate pollination of open pollinated carrot seed crops by naturally occurring insect populations was first established by Hawthorn *et al.* (1955) in the United States. This study of pollination in the cultivar Chantenay demonstrated that higher yields were obtained from plants caged with honeybees (876 kg/ha) compared with open pollinated plants (682 kg/ha). Native pollinator populations contribute significantly to pollination of carrot seed crops (Bohart and Nye, 1960; Ritchie Bell, 1971; Sinha and Chkrabarti, 1992) but their reliability is affected by pest control measures and seasonal variation in climatic conditions (Goyal *et al.*, 1989). The recommendation that honeybees should be introduced to flowering carrot seed crops has been widely adopted by commercial producers with up to 15 to 20 hives/ha placed around the borders of hybrid carrot seed crops in the US (Rubatzky *et al.*, 1999). In Australia fewer hives, typically 5 to 8/ha are used, but the size of the hives used at each location is unclear. It is also unclear if the lower stocking rates used in Australia restrict seed yield. Carrot flowers are considered to be relatively unattractive to honeybees (Free, 1970; Delaplane and Mayer, 2000), and they tend not to visit the plants if more attractive forage sources are located nearby (Galuszka *et al.*, 1989).

Low hybrid seed yields and variation in yield between hybrid crosses with petaloid CMS appear to be partly due to differing attractiveness of certain parent lines to pollinators. Honeybees were reported to most willingly forage on male fertile lines, followed by brown anther lines and white petaloid lines, with green petaloid lines

least favoured (Galuszka and Tegrek, 1987). In caging trials non-random foraging between hybrid seed parents was observed in hybrids with petaloid CMS (Erickson *et al.*, 1979) but not in hybrids with brown anther CMS (Rodet and Torre Grossa, 1991). Studies demonstrating differences in foraging between carrot lines have identified several factors that may affect the attractiveness of carrot lines to honeybees. These include variations in nectar and aroma production (Erickson and Peterson, 1979; Galuszka, and Tegrek, 1987), reduced ultra violet reflectance (Erickson and Peterson, 1979), and changes to floral morphology (Erickson *et al.*, 1982) and colour (Galuszka and Tegrek, 1987). Erickson and Peterson (1979) also noted differences in height between some male fertile and male sterile lines, which they suggested might modify pollinator foraging patterns. It is unclear if breeding since the time of these studies has addressed the issue of pollinator attractiveness, although seed production in general appears to have been a relatively minor consideration in carrot breeding programs.

Despite correlations of differences in seed yield between hybrid lines with insect visitation rates (Erickson *et al.*, 1979), it is unclear if inadequate pollination limits seed yield in individual lines. Given that inadequate pollination is often suggested as a potential cause of low seed yield in hybrid carrot, it is surprising that there are apparently no studies that have attempted to demonstrate this directly. One possible reason for this is the difficulty in producing conclusive evidence of pollination limitation of seed yield in plants that produce large numbers of flowers (Zimmerman and Pyke, 1988).

Other variables including the ratio of male fertile to male sterile plants within hybrid crops, distance between male sterile and male fertile plants and environmental conditions during flowering affect the pollination of hybrid seed crops (Williams and Free, 1974; Mendham and Salisbury, 1995; Pu *et al.*, 1999). Hybrid carrot seed crops are usually grown using the strip method of production in which blocks of rows of the male fertile and male sterile lines are alternated. There have been few studies of optimum parent line ratios and arrangement for carrot seed crops under field conditions. In commercial practice male sterile to male fertile ratios between 4 to 1 and 2 to 1 and male sterile block widths between 4 to 8 rows are appropriate in most hybrid crosses (Rubatzky *et al.*, 1999; Fulton, 1999).

Reductions in yield from hybrid seed crops have been observed when conditions at flowering are unfavourable for pollinator activity (Mendham and Salisbury, 1995). Honeybees and various Diptera are generally considered the most important pollinators of carrot seed crops (Bohart and Nye, 1960; Sinha and Chkrabarti, 1992; Abrol, 1997). Honeybees forage optimally at temperatures between 16 and 32°C (Anon, 2003). Blowflies (*Calliphora* and *Lucilia* spp.) were largely inactive in terms of pollination of onions at 16°C, but increased in activity between 16 and 26°C (Currah and Ockendon, 1984). Wind velocities greater than 40km per hour prevent honeybee foraging (Anon, 2003).

### ***Pollen Viability***

Factors affecting pollen viability and longevity were examined in detail in Chapter 5. Inbreeding depression of pollen production and viability has been reported from a number of normally outbreeding plant species including *Brassica rapa* (Aslam *et al.*, 1990) and *Mimulus guttatus* (Carr and Dudash, 1997). In each instance the extent of loss of male function was related to the number of generations of inbreeding. Laboratory studies indicate that low pollen viability and longevity due to environmental stresses such as high and low temperature, humidity and moisture deficit could limit seed yield in a range of field crops, for example, onion (Chang and Struckmeyer, 1975; Ockendon and Gates, 1975), tomato (Abdul-Baki, 1995) and rice (Virmani and Ilyas-Ahmed, 2001).

There appear to have been no published reports of pollen viability in carrot, presumably due to a lack of testing procedures for this trinucleate species, but it seems probable that pollen viability in inbred male lines would be lower than outcrossed lines. Strong inbreeding depression has been observed for a range of other agronomic traits in carrot (ISSI, 2002). Male fertile carrot lines with poor selfing ability exhibited poor outcrossing ability in cage trials (Erickson *et al.*, 1979). Although this could indicate low pollen viability it may also be due to a lack of attractiveness to pollinators or an incompatibility reaction between seed parents.

### ***Incompatibility Reactions and Pollen Tube Growth***

In incompatibility reactions trinucleate pollen is inhibited at the germination stage or during initial pollen tube growth in the stigma. This reaction is under sporophytic control (Mulchay and Mulchay, 1983 cited in Reynolds, 1995). Lewis (1965 cited in Reynolds, 1995) failed to find examples of incompatibility between pollen donors and maternal seed parents within the Apiaceae and concluded that incompatibility did not occur in this family. Observations of low germination of pollen on the stigmas and pollen with short tubes and irregular development in carrot plants (Braak and Kho 1958) suggests that this conclusion requires revision, although other factors such as unfavourable temperature for pollen tube growth (Vasil, 1987) may have been involved.

### **Factors Affecting Seed Set After Fertilisation**

In many open and self-pollinated field crops such as canola (Evans, 1984; Habekotte, 1993), *Lupinus angustifolius* (Pate *et al.*, 1980) cereals (Judel and Mengel, 1982) faba bean (Peat, 1983 cited in Bouwmeester *et al.*, 1995) and caraway (Bouwmeester *et al.*, 1995) post fertilisation resource limitation has been shown to restrict seed yields. In temperate crops the availability of assimilates is generally most limiting of seed yields (Fenner, 1985; Wardlaw, 1996; Habekotte, 1993; Bouwmeester, 1995a; Lee, 1988). Post fertilisation assimilate limitation can reduce seed yields through abortion of developing seed, or impaired seed filling. Although seed size does vary considerably (Fenner, 1992), it is generally considered the least plastic yield component in seed crops (Weiner, 1988), with seed number usually affected to a greater extent.

Because of the primary focus of this review on seed set, only the role of resource limitation in seed abortion is considered. However, it should be noted that low seed numbers due to impaired pollination can often be compensated for in terms of total seed yield by increased seed size (Mendham and Salisbury, 1995). With the exception of Bouwmeester *et al.*'s, (1995) study of caraway and studies in open pollinated carrot and fennel by Gray *et al.* (1986) and Peterson (1990), the role of seed abortion in

determination of yields of seed crops of the Apiaceae appears to have received relatively little attention.

### *Causes and Timing of Seed Abortion*

The mechanisms of seed abortion are not fully understood. There is some evidence of chemical inhibition of aborting seeds by those that are more mature (Lee, 1988) but the results of most studies conform to the 'source-sink' model. Neither mechanism should be considered to be mutually exclusive (Sage, 1986 cited in Lee, 1988). In the source-sink model of seed abortion, individual seeds are 'sinks' for resources, competing with each other and other reproductive and vegetative sinks for limited photosynthate, nutrients and water provided by 'sources' (the leaves, roots and storage structures). Although phloem transport can occur over long distances, seeds are generally supplied with photosynthate from nearby leaves (Lee, 1988; Wardlaw, 1990). In addition to assimilate sourced from leaves, umbels of fennel are photosynthetically active and therefore likely to contribute assimilate to seed growth (Peterson, 1990); a similar occurrence in carrot is probable, but the significance of the contribution is unclear.

In the early stages of development, seeds are relatively weak sinks for resources and appear to rely heavily on current assimilate (Pate *et al.*, 1980; Wardlaw, 1990; Yamagata *et al.*, 1987 cited in Bouwmeester *et al.*, 1995; Bouwmeester *et al.*, 1995; Mendham, 1995). As a consequence young seeds are susceptible to abortion, particularly when conditions are unfavourable for photosynthesis. Assimilate limited seed abortion occurs within the first three weeks of fertilisation in many plants including canola (Habekotte, 1993; Mendham and Salisbury, 1995), beans (Sage, 1990), perennial ryegrass (Mares Martins and Gamble, 1993), onion (Chang, 1976a) and caraway (Bouwmeester *et al.*, 1995). Relationships between photosynthetically active radiation levels during the flowering period and seed set have been established for a number of crop species under field conditions including canola (Leterme 1988b cited in Mendham and Salisbury, 1995) and caraway (Bouwmeester *et al.*, 1995). In addition to incident light levels, other environmental parameters including temperature and moisture stress have been shown to cause seed abortion through

disruption of assimilate supply (Chang and Struckmeyer, 1975; Cheikh and Jones, 1994; Abdul-Baki, 1995).

### ***Seed Abortion in Carrot and Other Apiaceae***

With the exception of studies by Bouwmeester *et al.* (1995), there appear to be few published accounts of the role of seed abortion in seed yield determination of plants of the Apiaceae. There have been no studies of the role of seed abortion in determination of seed set of male sterile carrot lines but it is conceivable that it could play a role, particularly in low vigour lines. In carrot and other Apiaceae the staggered flowering pattern of the umbels results in the simultaneous occurrences of umbel initiation, floral development and various stages of seed development within the one plant. Competition within and between umbel orders was negatively correlated with seed quality in the secondary, tertiary and quaternary umbels of the open pollinated Nantes carrots (Steiner *et al.*, 1990) but the possibility of similar effects on seed yield was not examined. Carrot seed from higher order umbels decreases in size, with the growth differences notable soon after fertilisation (Gray and Steckel, 1983a). Shading or umbel removal treatments applied around the time of flowering to plants of the open pollinated carrot cv. Chantenay grown in polythene tunnels affected seed size but not seed number (Gray *et al.*, 1986). In all treatments including the non shaded or untrimmed controls, the seed yields were very low for an open pollinated variety (4.5g/plant at a density of 10 plants/m<sup>2</sup>), suggesting that factors not typically limiting in the field may have had an effect. A 50% leaf removal treatment applied to fennel plants at flowering did not reduce the yield of oil derived from the seeds compared with untrimmed control plants (Peterson, 1990).

### **Conclusion**

Environmental, genetic and cultural factors affect the size of carrot plants, the number of umbels and flowers they produce and hence their capacities for seed yield. Data from Chapter 3 indicate that much of this potential may be unrealised in hybrid carrot seed production due to low levels of seed set. In contrast to open or self-pollinated

crops in which yields are most commonly resource limited, studies of hybrid seed yield limitation often focus on pollination. Whilst the published data generally support this approach, unequivocal evidence of pollination limitation of seed yield in hybrid carrot has not been published. In a range of crops low pollen viability, pollen-pistil interactions and seed abortion have been shown to limit seed yield. The role of these variables in determination of carrot seed yields, particularly hybrid carrot seed yields remains unclear. In the following sections, the alternative hypotheses that seed yield is limited by assimilate source strength or pollination in hybrid carrot seed crops produced in southern Tasmania are tested.

## **6.2-Materials and Methods**

### **Plant Material and Environmental Conditions**

The hybrid crosses WO030 and No. 22 were used in these studies on the basis that: a) the seed set and yield of their male sterile lines in preliminary variety trials (Chapter 3) were at opposite ends of the typical range for Nantes male sterile lines; b) they represent the two CMS systems in commercial use, brown anther (No.22) and petaloidy (WO030); and c) they were of commercial interest to Australian carrot seed producers.

The resource allocation, supplemental hand pollination and field based pollen viability experiments reported in this chapter were conducted on plants grown using the seed to seed method. Plots of No. 22 located in trial grounds at the University Farm, Cambridge were used in both seasons. These were grown on raised beds, at a density of 12 plants/m<sup>2</sup> and a 2:4 male fertile to male sterile row ratio. For WO030 the first season of experiments was located in plots within the University Farm trial grounds. The planting arrangement was the same as used for No. 22. In 2001–02 a commercial crop located at Tea Tree was used. The crop was grown at a density of 25 plants/m<sup>2</sup> with a male fertile to male sterile row ratio of 3:6. Raised beds were not used. Other cultural details were as outlined in Chapter 2.

Data presented on the synchrony of flowering of the parent lines in both hybrids were obtained from records kept for the variety trial at Cambridge in 1999–2000. This trial was described in Chapter 3.

Daily maximum and minimum temperatures and rainfall data for the periods of the resource partitioning and pollination experiments are shown in Table 6.1. Long-term averages for the Hobart Airport (less than 15km from all trial sites) were given in Chapter 2 (Figure 2.2). The December to February periods were notably warmer and drier than average and colder and wetter than average in 2000–01 and 2001–02 respectively.



**Table 6.1** – Climatic conditions during the pollination and resource partitioning experiments in 2000–01 and 2001–02. Source. Australian Bureau of Meteorology records for the Hobart Airport weather station, site no. 94008.

Month	2000 - 01				2001 - 02			
	Mean daily min. temp. (°C)	Mean daily max. temp. (°C)	No of rainfall days (<0.2mm)	Total precipitation (mm)	Mean daily min. temp. (°C)	Mean daily max. temp. (°C)	No of rainfall days (<0.2mm)	Total precipitation (mm)
December	11.1	21.8	13	35.4	11.2	19.8	17	52.8
January	13.1	24.0	7	10.4	12.0	20.6	11	97.6
February	13.7	25.4	2	3.2	11.7	21.3	10	33.8

## Modification of Resource Availability for Seed Development

### *The Effect of Shading on Seed Yield and Quality*

Three treatments, consisting of shading with 30% or 50% shade rated shadecloth® (70% and 50% transmission of light) and an unshaded control were applied to both sets of parents from peak bloom to harvest (approximately 10 weeks). Within the shading treatments, the shadecloth was positioned horizontally 1m above the plants in each plot in such a way as to completely shade approximately 50 male sterile plants from 9am to 3pm (Eastern Australian Standard Time). In all experiments the six treatments were arranged in 4 replicate plots in a randomised complete block design.

In 2001–02, leaf photosynthetic rates of plants in the 50% shaded and control plots were measured at 9am, 12noon and 3pm (Eastern Standard Time) at full bloom and 3 weeks after full bloom. On each occasion, 2 randomly selected plants from each replicate were sampled at each time. Measurements were made on the terminal leaflet of the distal leaf on the first secondary branch using an ADC LCi portable photosynthesis system (ADC Bioscientific Ltd, Hoddleston, UK). After recording, the portion of the leaflet that was enclosed in the leaf chamber was removed and placed in a sealed plastic bag. The sampled area of each leaflet was subsequently scanned and measured using Scion Image Beta V 4.02 image analysis software (Scioncorp,

Maryland, USA). Photosynthetic rate was calculated using the CO<sub>2</sub> uptake rate data and the area of the leaflet used.

In both seasons, measurements were made of pollen deposition in the control and shaded plots on 3 days during flowering to assess whether the shading treatments affected pollination. Each day, single flowers were sampled from six umbellets of the second whorl in the 4<sup>th</sup> secondary umbels of 5 randomly selected plants per plot. Assessment of pollen deposition was undertaken using the procedure described in Chapter 2.

At maturity, 20 plants were selected at random from each plot and harvested using the small plot thresher described in Chapter 2. Seed cleaning procedures and methods of collection of yield and germination data for each treatment replicate were as outlined in Chapter 2. The results of the experiments were analysed using ANOVA for randomised complete block designs. Fisher's LSD's were used to separate significantly different means at the 5% level of probability.

### ***The Effect of Umbel and Leaf Removal on Seed Yield and Quality***

Umbel and leaf removal treatments were applied to the male sterile lines of No. 22 and WO030. In 2001–01 the treatments were removal of all umbels except the primary umbel and 50% leaf removal. The leaf removal treatment was applied so that every second leaf in order from the base of the plant to the top of the canopy was removed. In 2001–02 the treatments were removal of all umbels except the primary umbel, removal of all umbels except the secondary umbels and 50% leaf removal. In both seasons each treatment was applied to 20 plants selected at random from rows of male sterile plants adjacent to the pollinator line. The treatments were applied approximately 2 weeks prior to flowering of the primary umbel. At the time the treatments were applied, each treated plant was paired to a control (untreated) plant of similar size, position and stage of development. Weekly checks were made to remove any newly forming leaf or umbel buds from plants in the leaf and umbel removal treatments respectively. At maturity the treated and control plants were harvested individually using the modified plant mulcher described in Chapter 2. Seed cleaning

procedures and methods for collection of yield and germination data for each plant were as described in Chapter 2. Statistical analysis of the data from these experiments was based on a t-test for paired samples, with significant differences determined at the 5% level of probability.

## **Pollination Experiments**

### ***Parent Line Flowering Synchrony***

During the 1999–2000 variety trials at Cambridge (Chapter 3) data were collected on the timing of flowering of a number of lines including the parent lines of WO030 and No. 22. In each of the 3 replicate blocks 6 plants of each line were tagged at the time of bolting and, from the time of appearance of the individual flower buds on the umbels, checked twice weekly for stage of flowering. The time of first flowering on the primary umbel and completion of flowering of the first three umbel orders were recorded for each plant.

### ***Duration of Receptivity of the Male Sterile Lines***

The duration of receptivity of flowers of the male sterile lines of No. 22 and WO030 was examined in the field on two occasions in the 1999-2000 season commencing on the 15<sup>th</sup> and 29<sup>th</sup> of December. Four female plants of each line in which the second secondary umbel was about to commence flowering were selected. Eighteen umbellets of similar development from the second whorl of the second secondary umbel were tagged with waterproof labels and allocated for hand pollination on each of the following 9 days (2 umbellets per plant per day). After tagging, the umbels were bagged in fine nylon mesh to prevent insect pollination. Hand pollination commenced when the stigmas were approximately 50% extended, but had not separated (see Figure 6.3). Hand pollination was performed at 2pm each day using pollen collected from field grown plants of the corresponding male line as described in the General Materials and Methods. A fine artists brush was used to transfer the pollen from the cardboard on which it was collected to the stigmas. Within each

umbellet, all of the flowers were pollinated. Four sub-samples of the pollen used for hand pollination each day were collected and viability tested using the FCR procedure (Chapter 5) to confirm that viable pollen had been used. Three days after the final hand pollination (day 12) the mesh bags were removed. The treated umbellets were harvested 30 days later when seed set was easily discernible. The percentage of set flowers was determined for each day of pollination and related to the stage of stigma development.

### ***Supplemental Pollination Experiments***

Experiments involving supplemental pollination of male sterile plants of No. 22 and WO030 were conducted in 2000–01 and 2001–02. In each experiment 12 male sterile plants commencing flowering at peak bloom were selected from rows adjacent to the male fertile line for hand pollination in supplement to natural pollination. Each hand pollinated plant was paired to a control plant of similar size, stage of development and position, that received natural pollination only. As the male sterile line of WO030 segregated into equal numbers of white flower and pale greenish white flower phenotypes, 6 plants of each type were chosen for supplemental hand pollination and matched to control plants of the same flower phenotype.

The large number of flowers on individual carrot plants precluded hand pollination of all umbels within the supplemental pollination treatment. Hand pollinations were performed on the primary and first two secondary umbels in 2000–01 and the first 4 secondary umbels in 2001–02. As an indication of the proportion of the plants receiving supplemental hand pollination, individual plants of No. 22 and WO030 typically produced between 6 and 10 secondary umbels. Hand pollination of flowers at peak receptivity was performed between 10am and 4pm on 3 occasions 2 to 3 days apart to ensure that all targeted flowers were pollinated. The method of hand pollination was as described above with the pollen collected from nearby beds of the male fertile line. On each day a sub-sample of the pollen used for hand pollination was collected, stored and subsequently tested for viability (FCR test) according to the methods determined in Chapter 5.

Within the supplemental pollination experiments samples were collected for examination of pollination levels, seed set 10 days after pollination and seed set at maturity in the hand pollinated and control plants. Details of the protocols for collection of these samples are given in Table 6.2. Pollination levels (%-fertilised stigmas and number of pollen grains per fertilised stigma) were assessed using the procedure outlined in Chapter 2. Early seed set and seed set at maturity were measured in terms of the percentage of flowers setting seed and the mean number of seeds set per flower.

At maturity, the umbels of all plants were collected for determination of seed yield and quality. The umbels of hand pollinated plants were separated into two samples, umbels that had been hand pollinated and the remaining umbels from the plant. Matching samples were prepared for the control plants. The seed from each set of umbels was harvested using a modified leaf mulcher and cleaned as outlined in Chapter 2. Seed yield and germination data were collected for each set of umbels from each plant using the methods detailed in Chapter 2. The data generated from the experiment were analysed for each line using a paired t-test with 12 replicates. Significant differences were determined at the 5% level of probability.

**Table 6.2** – Sampling protocols for comparison of pollination, early seed set and seed set at maturity in supplemental pollination experiments with No.22 and WO030 in 2000-01 and 2001-02.

Season	Umbels receiving supplemental pollination	Samples for pollen deposition	Samples for seed set 10 days after pollination	Samples for seed set at maturity
2000-01	Primary umbel + first 2 secondary umbels	Single flowers from 4 randomly selected umbellets in the 2nd, 4th and innermost whorls of the primary umbel and 2nd and 4th whorls of the 2nd secondary umbel. Collected at 4pm on each day of hand pollination.	Single randomly selected umbellets from the 2nd, 4th and innermost whorls of the primary umbel and 2nd and 4th whorls of the 2nd secondary umbel. Collected 14 days after the final hand pollination.	Collected at harvest. Sampling as for early seed set.
2001-02	First 4 secondary umbels	Single flowers from 6 randomly selected umbellets in the 2nd whorl of the 2nd and 4th secondary umbels. Collected at 4pm on each day of hand pollination.	Two randomly selected umbellets from the 2nd whorl of the 2nd and 4th secondary umbels. Collected 14 days after the final hand pollination.	Collected at harvest. Sampling as for early seed set.

## Pollen Viability Experiments

Pollen samples were collected from the male fertile lines of No. 22 and WO030 using the method described in Chapter 2. Unless otherwise specified, they were transferred to 1.5ml Eppendorf® tubes and desiccated, stored and subsequently rehydrated and tested for viability (FCR test) using the methods determined in Chapter 5.

### *Temporal Variation of Pollen Viability in the Field*

The effect of time of day on pollen viability was examined in the field during the 2001–02 season. Four replicates of 1m long sections of male fertile row (approximately 12 plants) were marked out in separate male fertile rows throughout trial sites for collection of pollen samples. Samples were collected on 8 days during peak bloom in 2000–01 and 2001–02. On each day, a pooled pollen sample was collected at 10am, 1pm and 3pm from all umbels at anthesis on 3 representative plants

in each row section using the method described in Chapter 2. Care was taken to ensure that no plant was sampled twice on the same day.

Daily assessments of pollen viability were made for 15 consecutive days during the period of peak bloom of both hybrids. Four replicates of 1m long sections of pollinator row were marked out adjacent to the sections used for the time of day experiment (above) for collection of pollen samples. On each day, a pooled pollen sample was collected at 1pm from secondary umbels at anthesis in each row section. Temperature and relative humidity at canopy height were recorded during the period of sample collection using Tiny Tag Ultra temperature and humidity loggers (Gemini Data loggers, Port Macquarie, Australia) mounted at canopy height in a Stevenson screen.

### ***The Effects of Temperature and Relative Humidity on the Longevity of Freshly Dehisced Pollen***

The effects of 13 temperature and relative humidity combinations (Table 6.3) on the longevity of freshly dehisced carrot pollen were examined over a 12-hour period. The range of temperatures and relative humidities were generated by placing sealed petri dishes filled with 20ml of saturated salt solutions on a thermogradient table (Terratec, Hobart) set with a gradient of 5 to 55°C. The types and amounts of salts required to generate the desired relative humidities at each temperature were determined using theoretical values from Labuza (2001). The calculated quantities of each salt to produce saturated solutions were increased by 50% to give a slurry. Care was taken to ensure that salt crystals were not exposed to the air above the slurry. A summary of the salts used and humidities generated is given in Table 6.3. Each temperature/humidity treatment was replicated 4 times in 4 separate petri dishes. In each petri dish, a single microscope slide was suspended above the salt slurry. Each microscope slide was covered with pollen drawn from an homogeneous sample of freshly dehisced pollen collected from glasshouse grown male fertile plants of WO030. Four additional slides of pollen were prepared and viability tested using the FCR procedure at the start of the experiment. Each microscope slide exposed to a treatment in the experiment was divided into 5 even sections which were allocated at

random for viability testing at 1, 2, 4, 8 and 12 hours after commencement of the experiment. At each time the relevant samples of pollen were transferred, using a small paintbrush, to another set of microscope slides and tested immediately using the FCR procedure. After natural log transformation, linear equations were fitted to the change in viability of pollen over time for each replicate of all temperature/humidity combinations. Pollen half-lives were calculated from these equations. The effects of different temperatures at a fixed relative humidity of 55%, different relative humidities at a fixed temperature of 25°C and the interaction between temperature and humidity on the half-life of pollen were analysed using the ANOVA function of SAS version 8 (SAS Institute Inc, Cary, USA). Fisher's LSD's were used to determine significantly different means at the 5% level of probability.

**Table 6.3** – A summary of the temperature and relative humidity conditions used to examine the effects of temperature and relative humidity on carrot pollen and the saturated salt solutions used to achieve them. The reported relative humidity values were obtained from Labuza (2001). The small variation between 'constant' relative humidities for treatments in 1 and 3 were considered insignificant in the context of the experiment.

Variable examined	Treatment combination		
	Temperature (°C)	Relative humidity	Saturated Salt Solution
1 – Temperature at constant relative humidity (≈55%)	10	57.36±0.33	Mg(NO <sub>3</sub> ) <sub>2</sub>
	20	54.38±0.23	Mg(NO <sub>3</sub> ) <sub>2</sub>
	25	57.57±0.40	NaBr
	30	56.03±0.38	NaBr
	40	55.48±1.80	CoCl <sub>2</sub>
2 – Relative humidity at constant temperature (25°C)	25	43.16±0.39	KCO <sub>3</sub>
	25	57.57±0.40	NaBr
	25	75.29±0.12	NaCl
	25	84.34±0.26	KCl
	25	100.00	Deionised water
3 - Temperature / relative humidity interaction	10	43.14±0.39	KCO <sub>3</sub>
	25	43.16±0.33	KCO <sub>3</sub>
	30	43.17±0.50	KCO <sub>3</sub>
	10	75.67±0.22	NaCl
	25	75.29±0.14	NaCl
	30	75.09±0.11	NaCl



### ***Inter- and Intra- Plant Variation in Pollen Viability***

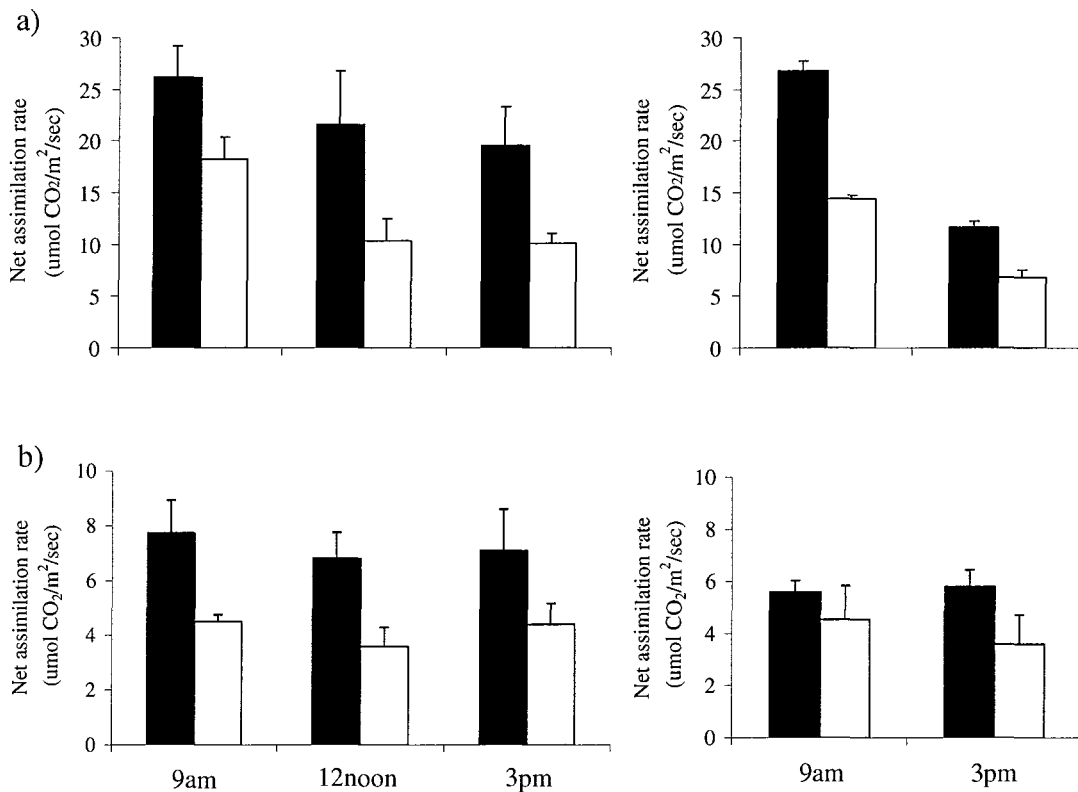
The viability of pollen samples from male fertile flowers located in the 2<sup>nd</sup> and 4<sup>th</sup> whorls of umbellets in the primary and fourth secondary umbels and the terminal tertiary umbels of the 4<sup>th</sup> secondary branches of glasshouse grown plants were tested. At 10am on 4 consecutive days a single, freshly dehisced anther was removed from 10 umbellets in the 2<sup>nd</sup> and 4<sup>th</sup> whorls of the primary umbel of four plants, with the 10 anthers from each whorl pooled into a single sample per whorl per plant per day. Immediately after collection the pooled samples were macerated in FCR test solution (FDA 2mg/ml in 20% sucrose solution) on a microscope slide and assessed for pollen viability using the FCR procedure described in Chapter 5. The process was repeated for each plant approximately 8 and 16 days later for the secondary and tertiary umbels.

Twenty evenly developed plants of each of the pollinator lines of No. 22 and WO030 grown under glasshouse conditions were selected for pollen viability testing. The plants were arranged randomly in a double row on a central bench within the glasshouse 3 weeks prior to the onset of flowering. At 10am on 4 consecutive days, 10 freshly dehisced anthers were collected from the 2<sup>nd</sup> whorl of umbellets of the 2<sup>nd</sup> secondary umbel of each plant. Immediately after collection the samples were macerated in FCR test solution (FDA 2mg/ml in 20% sucrose solution) on a microscope slide and assessed for pollen viability. Between each day of sampling the arrangement of the plants on the glasshouse bench was re-randomised.

## 6.3 - Results

### Modification of Resource Availability for Seed Development

Shading of male sterile plants of No. 22 and WO030 with 50% shade cloth caused a reduction in leaf net assimilation rate of 30 to 52% throughout the day (10am to 4pm) compared with control plants under full sun (Figure 6.1). Reductions in photosynthetic activity due to shading were also observed in a second set of measurements of the same treatments 3 weeks later, although the effect was less apparent for WO030 at this stage (Figure 6.1).



**Figure 6.2** – Leaf photosynthetic rates throughout the day for control, non-shaded male sterile plants (■) and male sterile plants shaded with 50% shade rated shade cloth (□) at peak bloom (left) and 3 weeks after peak bloom (right) in No. 22 a) and WO030 b). Error bars indicate standard errors (N=8). Note times given are Eastern Standard Times.

Across all treatments 42 to 46% and 31% to 34% of flowers of No. 22 and 52% to 62% and 31% to 34% of flowers of WO030 were pollinated in 2000–01 and 2001–02 respectively. Within these ranges, there was no consistent pattern with respect to particular treatments, indicating that the shading treatments had no effect on pollination in comparison with the unshaded control treatment. Although there was a general trend towards reduced seed yield with 50% shading for both lines, the effects of shading on seed yield were not significant in either season (Table 6.4). Furthermore, there were no significant effects of shading on seed size, seed number/plant or the quality parameters of germination percentage and mean germination time in either hybrid (Table 6.4).

**Table 6.4** – The effects of continuous shading with 30 and 50% shade rated shade cloth from 2 weeks prior to anthesis until seed maturity on seed yield and quality in No. 22 and WO030. NS = not significant at  $P < 0.05$ .

Hybrid	Treatment	Mean seed yield / plant (g)		No. of seeds / plant		Seed dry weight (mg)		% Germination		Mean germination time (days)	
		00-01	01-02	00-01	01-02	00-01	01-02	00-01	01-02	00-01	01-02
No. 22	Control	15.2	9.9	6759	4304	2.2	2.3	95.5	93.5	4.9	4.2
	30% shade	16.8	10.5	7594	4422	2.2	2.4	97.2	90.0	5.4	4.5
	50% shade	12.8	8.1	5233	3581	2.5	2.3	96.2	91.1	5.5	4.7
	Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
WO030	Control	5.3	1.9	4560	1508	1.2	1.3	98.1	53.0	5.3	5.2
	30% shade	4.1	1.7	3048	1239	1.3	1.3	95.4	48.0	5.6	5.5
	50% shade	5.8	1.3	4104	1017	1.4	1.3	95.0	53.0	5.3	5.6
	Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Removal of all umbels except the primaries or secondaries from male sterile plants 2 weeks before flowering of the primary umbel had little effect on the yield

characteristics or the quality parameters of % germination and mean time to germination of seed of No. 22 or WO030 in either season (Table 6.5). The only significant effects of these treatments were increases in seed yield and mean seed weight on the primary umbels of No. 22 in 2000-01 from 1.37 to 1.86grams/plant and 3.0 to 3.4mg respectively. Similarly, for both lines removal of 50% of the leaves of male sterile plants 2 weeks prior to flowering had no effect on seed yield or seed quality in either season (Table 6.6).

**Table 6.5** – The effects of removal of all umbel orders except the primary or secondary two weeks prior to flowering of the primary umbel on seed yield and quality in the hybrids No. 22 and WO030. Data for the controls plants, which did not have any umbels removed, refer to the umbel order corresponding to that of the treated plants. NS = not significant at  $P<0.05$ , nr = not recorded.

Hybrid	Year	Treatment	Mean seed yield / plant (g)	No. of seeds / Plant	Seed dry weight (mg)	Germination percentage	Mean germination time (days)
No. 22	2000-01	All orders except 1 <sup>o</sup> removed	1.86	554	3.4	89.6	nr
		Control	1.37	461	3.0	91.2	nr
		Significance	( $P<0.05$ )	NS	( $P<0.05$ )	NS	
No. 22	2001-02	All orders except 1 <sup>o</sup> removed	1.14	306	3.7	81.1	5.6
		Control	1.01	272	3.7	83.4	5.7
		Significance	NS	NS	NS	NS	NS
No. 22	2001-02	All orders except 2 <sup>o</sup> removed	3.86	1279	3.0	89.7	6.4
		Control	4.00	1232	3.2	88.5	6.0
		Significance	NS	NS	NS	NS	NS
WO030	2000-01	All orders except 1 <sup>o</sup> removed	0.35	188	1.9	84.8	6.5
		Control	0.33	208	1.6	83.2	6.5
		Significance	NS	NS	NS	NS	NS
WO030	2001-02	All orders except 1 <sup>o</sup> removed	0.36	187	1.9	70.3	6.2
		Control	0.35	204	1.7	75.7	6.0
		Significance	NS	NS	NS	NS	NS
WO030	2001-02	All orders except 2 <sup>o</sup> removed	1.76	1246	1.4	63.5	6.5
		Control	1.70	1439	1.2	69.8	7.0
		Significance	NS	NS	NS	NS	NS

In contrast to the lack of effect of manipulation of resource availability, large seasonal differences in germination were observed across all resource allocation experiments, with lower levels of each in 2001–02 compared to 2000–01 in both hybrids. The cause of reduced germination of seed of both hybrids in 2001–02 compared to 2000–01 was a high incidence of rudimentary embryos in seeds that failed to germinate. This condition was most evident in WO030 where up to 45% of all seeds had rudimentary embryos.

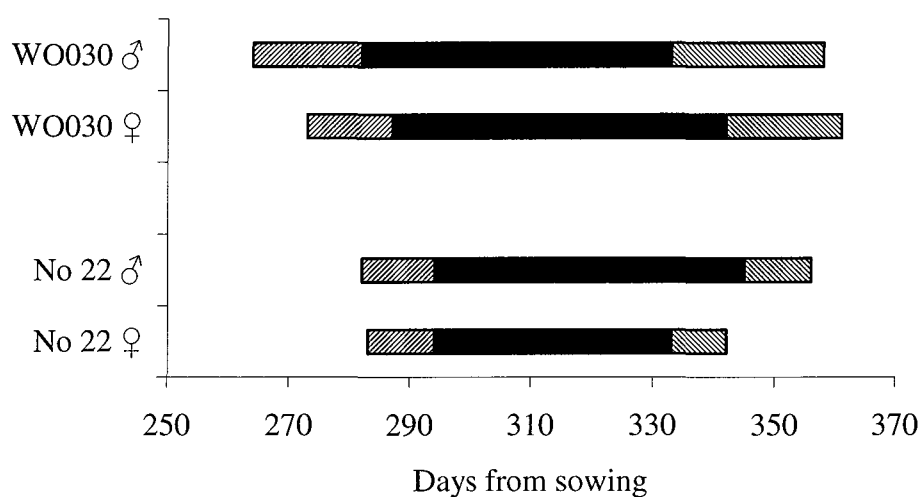
**Table 6.6** – The effects of leaf removal treatments imposed two weeks prior to flowering of the primary umbel on seed yield and quality in the hybrids No. 22 and WO030. NS = not significant at  $P < 0.05$ .

Hybrid	Year	Treatment	Mean seed yield / plant (g)	No. of seeds / plant	Seed dry weight (mg)	Germination percentage	Mean germination time (days)
No. 22	2000-01	50% leaf removal	11.43	4677	2.4	86.2	5.6
		Control	10.38	3841	2.7	85.4	6.1
		Significance	NS	NS	NS	NS	NS
No. 22	2001-02	50% leaf removal	9.99	5017	2.0	89.7	5.8
		Control	11.37	5572	2.0	91.7	5.4
		Significance	NS	NS	NS	NS	NS
WO030	2000-01	50% leaf removal	2.35	1920	1.2	82.8	6.3
		Control	2.70	2478	1.1	83.7	6.5
		Significance	NS	NS	NS	NS	NS
WO030	2001-02	50% leaf removal	2.35	2047	1.2	59.6	6.1
		Control	2.28	1907	1.1	67.9	6.4
		Significance	NS	NS	NS	NS	NS

## Pollination Experiments

### *Parent Line Flowering Synchrony*

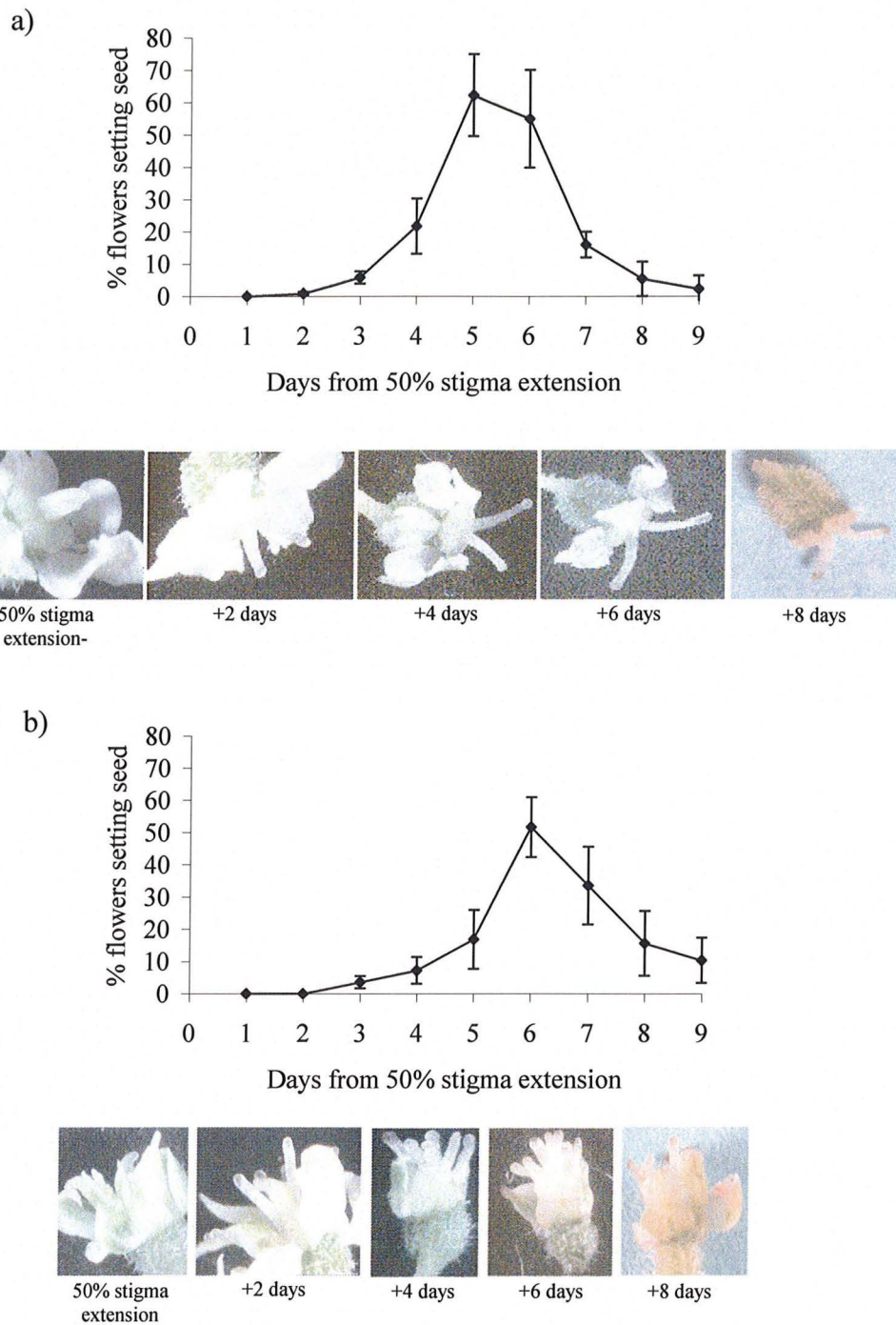
In 1999-00 flowering of the male fertile lines commenced 1 and 9 days prior to flowering of the male sterile lines for No. 22 and WO030 respectively (Figure 6.2). Flowering of the first three orders was completed in the male sterile line 14 days before the male fertile line in No. 22, but 3 days after the male sterile line in WO030. Similar synchrony of flowering was observed in both hybrids in 2000-01 and 2001-02, but flowering continued for approximately 2 weeks longer in 2001-02 compared to the previous two seasons.



**Figure 6.2** – Timing of flowering in the parent lines of WO030 and No. 22. Male fertile and male sterile lines are indicated by ♂ and ♀ respectively. The time periods indicated are anthesis of primary umbels on the first to median plants (▨); anthesis of the primary umbel to completion of tertiary umbel flowering on the median plants (the main period of flowering) (■); and completion tertiary umbel flowering on the median to last plants (▩). The data are based on observations of 18 plants of each parent line.

### ***Duration of Receptivity of the Male Sterile Lines***

Stigmatic receptivity of flowers of the second whorl of umbellets of the 2<sup>nd</sup> secondary umbel lasted at least 6 and greater than 7 days for male sterile plants of No. 22 and WO030 respectively. In No. 22 seed set was highest 5 days after the stigmas of flowers were on average 50% extended but not separated (Figure 6.3). At this stage the stigmas were fully extended with a slight outwards curve from the centre of the flower and the flowers were beginning to drop their petals. For WO030, seed set was highest 6 days after 50% stigma extension. At this stage most petals had dropped, the petaloid structures had begun to wilt and the stigmas were at their most prominent. In the two lines, 50 to 60% of flowers pollinated at the optimum stage of stigma development set seed.



**Figure 6.3** – Duration of receptivity of flowers of the second whorl umbellets of the 2<sup>nd</sup> secondary umbels of the male sterile lines of a) No. 22 and b) WO030. Receptivity was determined on the basis of the percentage of hand pollinated female flowers from each day that set seeds. Figures are the pooled data from tests of 4 plants on 2 occasions. Standard errors are indicated by the bars (N=8). The pictures beneath the graphs show the average flower development in second whorl umbellets at (l to r) 50% stigma extension and 2, 4, 6 and 8 days thereafter. Flower size = approximately 3mm corolla diameter at 50% stigma extension.



### *Supplemental Pollination Experiments*

Pollen used for supplemental hand pollination of No. 22 and WO030 in field trials was collected from male sterile plants within the trial plots of each hybrid cross between 12am and 2pm each day. The viability of pollen used for hand pollination varied widely within and between seasons with ranges of 19.8% to 57.1% and 9.7% to 67.3% for No.22 and WO030 respectively across both seasons (Table 6.7). Pollen viability in both hybrids was generally much lower in 2001–02 than in 2000–01 (Table 6.7).

**Table 6.7** – % Viability (determined by FCR testing) of pollen samples used for supplemental hand pollination in 2000–01 and 2001–02. The values given are the means of 4 replicate samples. ns = not sampled.

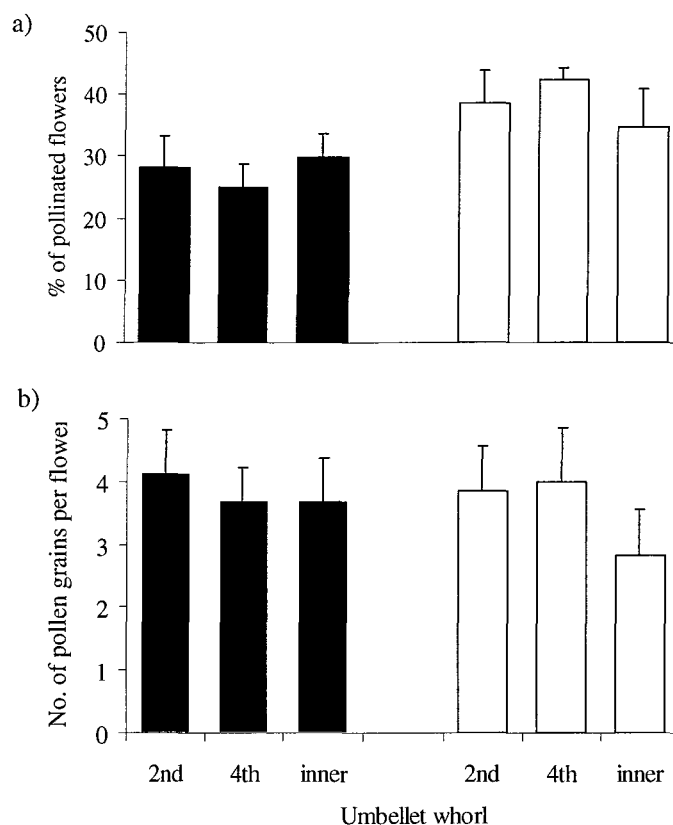
Season	Date of collection	No. 22		WO8024	
		% Viable pollen	S. E.	% Viable pollen	S. E.
2000-01	18/12/00	57.1	3.5	ns	
	19/12/00	31.5	4.0	51.4	6.5
	20/12/00	54.4	8.7	67.3	3.9
	27/12/00	32.0	13.3	55.2	4.6
	1/1/01	41.2	6.2	25.5	9.8
2001-02	19/12/01	19.8	1.6	ns	
	21/12/01	24.8	2.6	ns	
	28/12/01	20.8	5.6	ns	
	30/12/01	20.7	5.0	ns	
	2/1/02	ns		57.4	7.8
	3/1/02	ns		9.7	0.8
	4/1/02	ns		28.4	6.5
	5/1/02	ns		31.9	1.0

For the male sterile lines of No. 22 and WO030 between 0 and 7.6% of flowers of the primary umbel were sexless, with the remainder having ovaries. On the 4<sup>th</sup> secondary umbels of No. 22 and WO030 between 36.3 and 45.0% of flowers were sexless. Natural pollination levels of primary and secondary umbels of male sterile plants of No. 22 and WO030 ranged from 21.3% to 66.0% of all female flowers, with between

3.2 and 5.4 pollen grains deposited per pollinated flower by the end of their period of receptivity. Generally, there was little difference in natural pollination levels between the primary and secondary umbels (Table 6.8) or between umbellet positions within the primary umbel (Figure 6.4). Over two seasons, supplemental hand pollination resulted in 22 to 58% increases in the percentage of pollinated flowers and increased average numbers of pollen grains deposited on pollinated stigmas by 0.6 to 2.5 pollen grains in primary and 4<sup>th</sup> secondary umbels of No.22 and WO030 (Table 6.8). Natural pollination levels of secondary umbels were 26 to 60% lower in 2001-02 than in 2000-01.

**Table 6.8** – Mean pollination levels in control (natural pollination) and supplementally hand pollinated primary and 4<sup>th</sup> secondary umbels of the male sterile parent lines of No. 22 and WO030 in 2000–01 and 2001–02. Flowers were counted as pollinated if at least one of the two stigmas was pollinated. The number of pollen grains / pollinated flower is the total number of grains on both stigmas of flowers of No. 22 and the two main stigmas of WO030. Standard errors are given in italics (N=12).

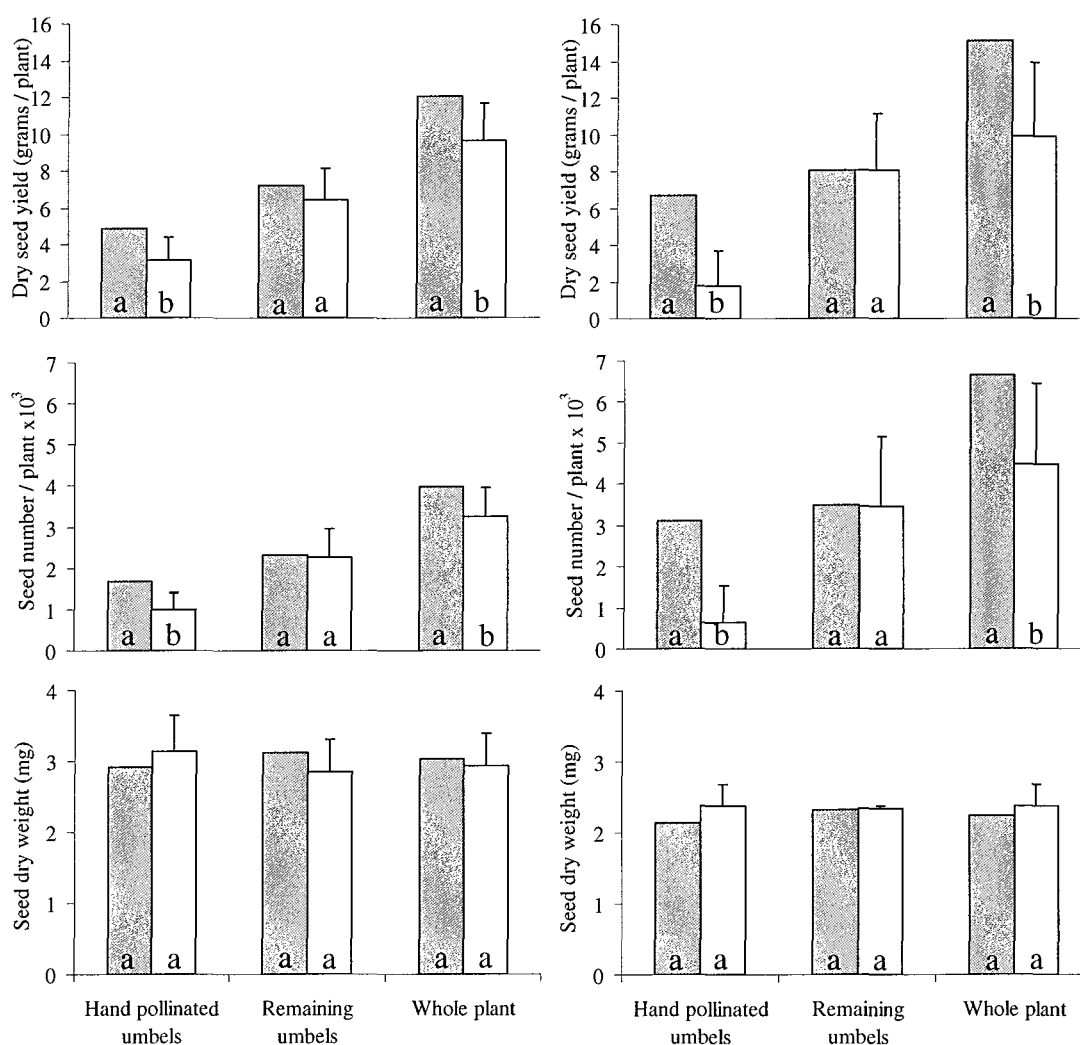
Line	Season	Umbel order	Treatment	% of pollinated flowers		No. of pollen grains / pollinated flower	
No. 22	2000-01	1°	Control	54.1	7.8	5.4	0.1
			Hand pollinated	94.1	2.3	8.0	0.3
		2°	Control	46.4	9.0	4.0	0.3
			Hand pollinated	79.2	6.8	5.2	0.2
	2001-02	2°	Control	34.2	8.8	3.4	0.5
			Hand pollinated	83.0	7.2	4.4	0.2
WO030	2000-01	1°	Control	65.0	11.7	4.6	0.2
			Hand pollinated	87.4	8.6	7.4	0.7
		2°	Control	66.0	9.2	4.8	0.4
			Hand pollinated	95.0	2.9	9.0	0.7
	2001-02	2°	Control	21.3	4.5	3.2	0.1
			Hand pollinated	78.8	6.3	8.2	0.5



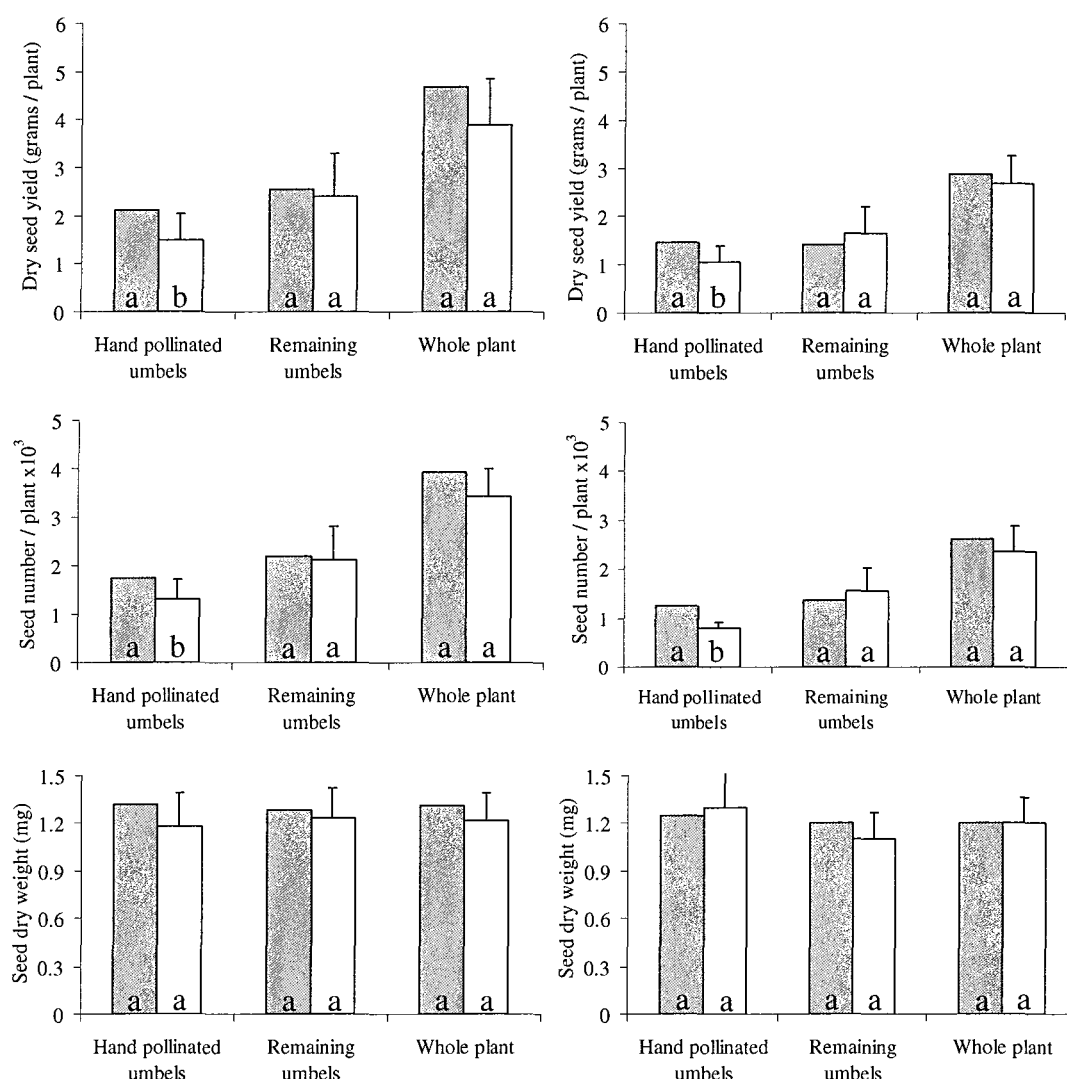
**Figure 6.4** – The level of natural pollination of flowers in the primary umbels of the male sterile lines of No. 22 (■) and WO030 (□) with respect to position within the umbel. Flowers were sampled from the 2<sup>nd</sup>, 4<sup>th</sup> and innermost whorls of umbellets of the primary umbel: a) % pollinated flowers (flowers with at least 1 pollinated stigma); b) mean number of pollen grains deposited on pollinated flowers (both stigmas). Error bars indicate standard errors (N=12).

For both No. 22 and WO030 the seed yields of umbels that were hand pollinated in supplement to natural pollination were significantly higher ( $P < 0.05$  to  $P < 0.001$ ) than the seed yields of the corresponding umbels on control plants that received natural pollination only (Figures 6.5 and 6.6). For WO030, the yield increase from supplemental pollination was 41% in both seasons, whilst for No. 22 increases of 54% in 2000-01 and 284% in 2001-02 were recorded. The increased seed yields were due to a greater number of seeds set on hand pollinated umbels (Figures 6.5 and 6.6). There was no evidence that increased yield from the hand pollinated umbels was at the expense of seed size, or the yield of seed from the remaining (naturally pollinated) umbels on the same plants. Only the primary and first two secondary umbels, and the first four secondary umbels were hand pollinated in 2000-01 and 2001-02 respectively, but supplemental hand pollination significantly increased the total seed

yield of plants of No. 22 by 25% in 2000-01 ( $P<0.001$ ) and 52% in 2001-02 ( $P<0.05$ ) (Figure 6.5). There were trends of increased total seed yield per plant with supplemental pollination in both seasons for WO030 (20% and 7% in 2000-01 and 2001-02 respectively) but the differences were not significant (Figure 6.6).

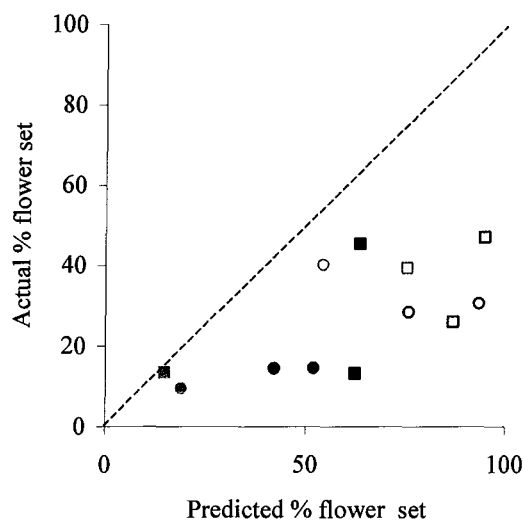


**Figure 6.5** – A comparison of the yield parameters mean dry seed yield/plant, mean seed number/plant and mean seed dry weight for plants receiving supplemental hand pollination (■) and naturally pollinated control plants (□) in the male sterile line of No. 22 in 2000–01 (left) and 2001–02 (right). The values of each yield parameter are shown for the hand pollinated umbels, the remaining (non-hand pollinated) umbels and the whole plant (all umbels) from the plants receiving supplemental hand pollination and the corresponding umbels (based on position on the plant) from the control plants. Significantly different means ( $P<0.05$ ) are indicated by the letters in the columns. The error bars indicate the 95% confidence intervals for differences between the hand pollinated and control treatment means ( $N=12$ ).

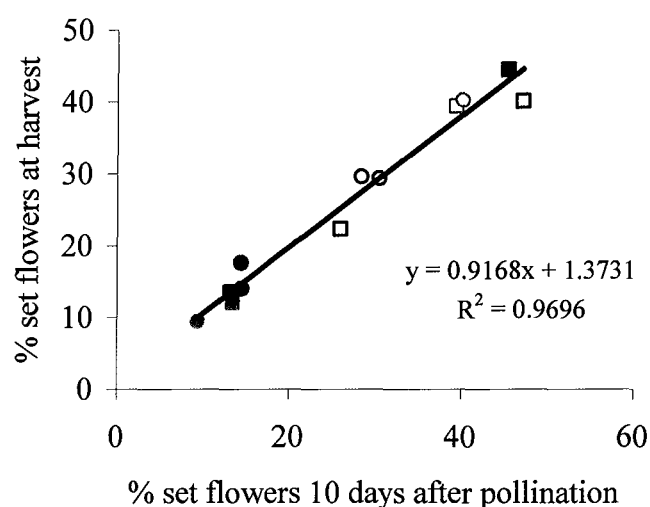


**Figure 6.6** – A comparison of the yield parameters mean dry seed yield/plant, mean seed number/plant and mean seed dry weight for plants receiving supplemental hand pollination (■) and naturally pollinated control plants (□) in the male sterile line of WO030 in 2000–01 (left) and 2001–02 (right). The values of each yield parameter are shown for the hand pollinated umbels, the remaining (non-hand pollinated) umbels and the whole plant (all umbels) from the plants receiving supplemental hand pollination and the corresponding umbels (based on position on the plant) from the control plants. Significantly different means ( $P < 0.05$ ) are indicated by the letters in the columns. Error bars indicate the 95% confidence intervals for differences between the hand pollinated and control treatment means ( $N=12$ ).

Across both lines and seasons the seed set 10 days after pollination was overestimated by the potential maximum seed set based on pollination levels and average pollen viability (see the legend for Figure 6.7 for an explanation of this calculation) by up to 63%. In most instances the actual seed set was less than 50% of this calculated potential (Figure 6.7). Theoretically, it is possible to set two seeds per carrot flower. The average numbers of seeds that matured in male sterile flowers for hand pollinated and control treatments of No. 22 were 1.1 and 1.0 in 2000–01 and 1.3 and 1.1 in 2001–02. The average number of seeds set in the male sterile flowers of WO030 in the hand pollinated and control treatments were 1.2 and 1.1 in 2000–01 and 1.1 and 1.0 in 2001–02. There was a highly significant linear relationship between seed set 10 days after anthesis and seed set at maturity for hand pollinated and control male sterile umbels ( $P < 0.0001$ ) (Figure 6.8). This relationship was not significantly different to a 1 to 1 relationship ( $P = 0.12$ ), indicating that very few seeds aborted from 10 days after pollination.



**Figure 6.7** – Potential maximum seed set (based on pollination rates and average pollen viability) vs actual seed set for the primary and secondary umbels of the male sterile lines of No. 22 (circular symbols) and WO030 (square symbols) within the supplemental hand pollination (open symbols) and control treatments (filled symbols) imposed in the 2000–01 (black symbols) and 2001–02 (red symbols) seasons. Potential seed set was calculated using the equation  $S = P(1 - (1 - V)^N)$ , where  $S$  = the predicted maximum percentage of flowers setting seed;  $P$  = percentage of flowers that were pollinated;  $V$  = average pollen viability during the pollination period; and  $N$  = the average number of pollen grains deposited on the flowers.



**Figure 6.8** – The relationship between the percentage of set flowers 10 days after pollination and the percentage of flowers maturing seeds for the primary and secondary umbels of the male sterile lines of No. 22 (circular symbols) and WO030 (square symbols) within the supplemental hand pollination (filled symbols) and control treatments (open symbols) imposed in the 2000–01 (black symbols) and 2001–02 (red symbols) seasons.

There were no significant differences in the germination percentage or rate of germination of seed from umbels receiving supplemental hand pollination or the remaining (non hand pollinated) umbels on the same plants compared with seed from the corresponding umbels of control plants (Tables 6.9 and 6.10). The germination of WO030 was lower in 2001–02 than 2000–01. The main difference was that 15 to 18% of seeds within the samples from 2001–02 had rudimentary embryos, which failed to germinate within the 14-day test period. In 2000–01, rudimentary embryos were not observed in seed samples from WO030.

**Table 6.9** - % Germination and mean time to germination of seed lots of No. 22 from the control and supplemental (hand) pollination treatments applied in 2000–01 and 2001–02. NS = not significant at  $P < 0.05$ .

Season	Umbels	Treatment	% Germination (day 14)	Mean germination time (days)
2000-01	Hand pollinated	Supplemental pollination	96.6	8.0
		Control	91.9	7.8
			NS	NS
	Remainder	Supplemental pollination	92.6	7.8
		Control	91.6	7.9
			NS	NS
	All (whole plant)	Supplemental pollination	94.2	7.9
		Control	91.7	7.9
			NS	NS
2001-02	Hand pollinated	Supplemental pollination	95.6	7.5
		Control	90.4	7.8
			NS	NS
	Remainder	Supplemental pollination	91.6	7.3
		Control	86.9	7.5
			NS	NS
	All (whole plant)	Supplemental pollination	93.4	7.4
		Control	88.5	7.6
			NS	NS



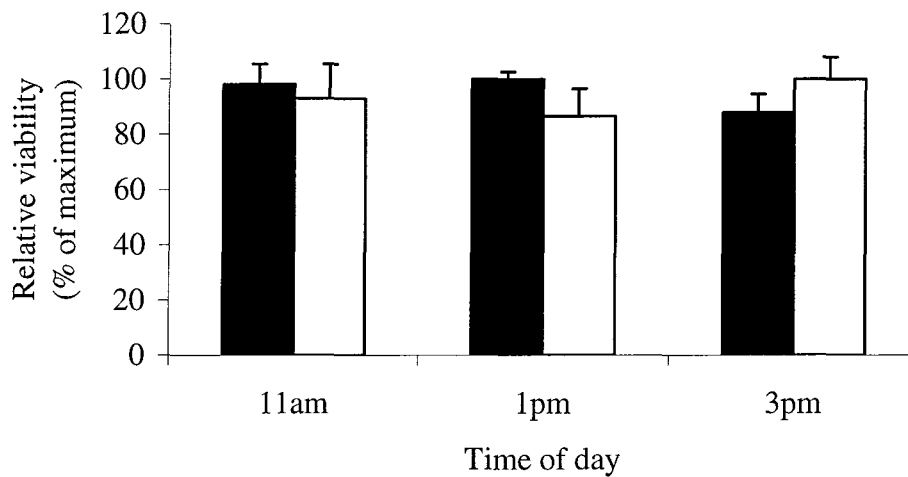
**Table 6.10** - % Germination and mean time to germination of seed lots of WO030 from the control and supplemental (hand) pollination treatments applied in 2000–01 and 2001–02. NS = not significant at  $P < 0.05$ .

Season	Umbels	Treatment	% Germination (day 14)	Mean germination time (days)
2000-01	Hand pollinated	Supplemental pollination	93.5	8
		Control	87.8	7.8
			NS	NS
	Remainder	Supplemental pollination	95.3	8.2
		Control	90.8	7.8
			NS	NS
	All (whole plant)	Supplemental pollination	94.5	8.1
		Control	89.6	7.9
			NS	NS
2001-02	Hand pollinated	Supplemental pollination	77.5	9.4
		Control	76.1	9.5
			NS	NS
	Remainder	Supplemental pollination	76.8	9.4
		Control	73.1	9.2
			NS	NS
	All (whole plant)	Supplemental pollination	77.1	9.4
		Control	74.3	9.3
			NS	NS

## Pollen Viability Experiments

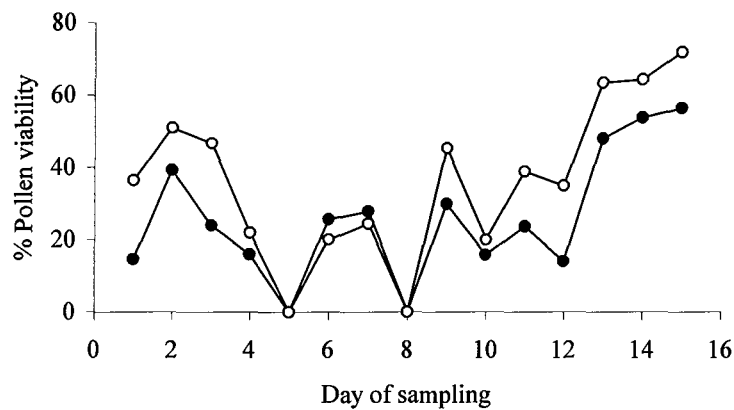
### *Temporal Variation in Pollen Viability in the Field*

There was no significant difference between the mean viability of pollen samples of No. 22 and WO030 collected from the field at 11 am, 1 pm or 3 pm (Figure 6.9).

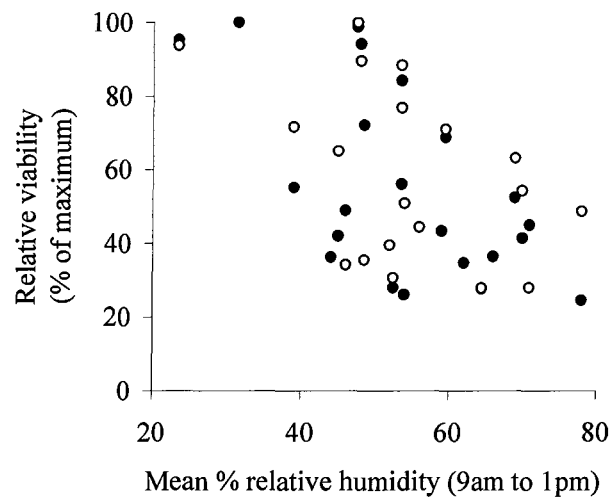


**Figure 6.9** – Mean relative viability (viability expressed as a percentage of the maximum viability observed) of pollen samples of No. 22 (■) and WO030 (□) collected at 11am, 1pm and 3pm on 8 days during peak bloom in 2000-01 and 2001-02. Error bars indicate standard errors (N=8).

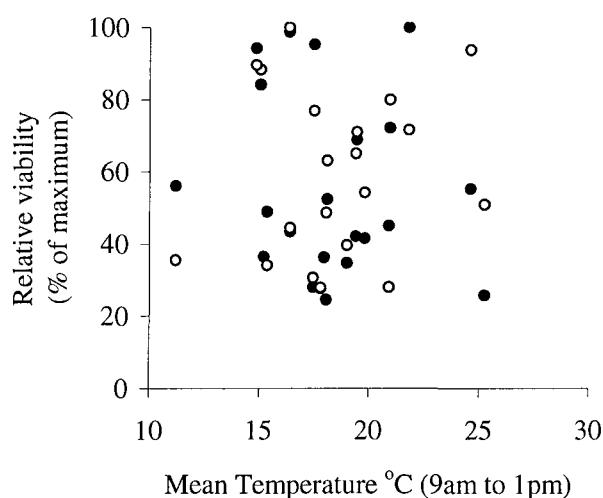
Despite the lack of variation within days, the viability of pollen samples from the male fertile lines of No. 22 and WO030 collected at 1pm on each day over a 15 day period varied widely between days (14 to 56% for No. 22 and 20 to 72% for WO030). Similar patterns of change over time were observed in both lines (Figure 6.10). A significant relationship ( $P < 0.001$ ) was observed between the viability of pollen samples collected at 1pm and the average humidity conditions at the trial site from 9am to 1pm on the day of sampling (Figure 6.10). This relationship was described by the equation  $y = -0.98x + 110.70$ , ( $R^2 = 0.27$ ) where  $y$  = pollen viability and  $x$  = mean relative humidity. For the same samples, no significant effect of mean temperature between 9am and 1pm on the day of collection on pollen viability was observed (Figure 6.11) but inclusion of both mean temperature and mean humidity into the pollen viability model slightly improved its goodness of fit ( $R^2 = 0.33$ ). This model was described by the equation  $y = -1.01x - 0.19z + 145.74$ , with  $x$  and  $y$  as above and  $z$  = mean temperature (9am to 1pm) on the day of sampling.



**Figure 6.10** – Daily variation in viability of pollen collected from the secondary umbels of field grown plants of the male fertile lines of No. 22 (●) and WO030 (○) over a 15 day period during peak bloom (2<sup>nd</sup> to 16<sup>th</sup> of January, 2002). Note 0% viabilities were recorded on days 5 and 8 for samples collected after morning rainfall.



**Figure 6.11** – Viability of pollen samples collected from the field at 1pm plotted against the mean relative humidity from 9am to 1pm on the day of collection. The viability of each pollen sample is expressed as a relative viability; that is,  $100 \times (\text{individual sample viability} / \text{highest observed sample viability for that line})$ . Pollen samples were collected from the male fertile lines of No. 22 (●) and WO030 (○). Samples were collected in 2000–01 and 2001–02.



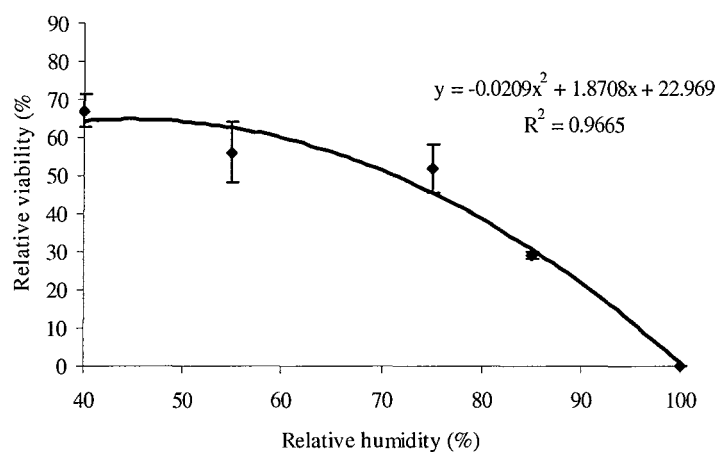
**Figure 6.12** - Viability of pollen samples collected from the field at 1pm plotted against the mean temperature from 9am to 1pm on the day of collection. The viability of each pollen sample is expressed as a relative viability; that is,  $100 \times (\text{individual sample viability} / \text{highest observed sample viability for that line})$ . Pollen samples were collected from the male fertile lines of No. 22 (●) and WO030 (○). Samples were collected in 2000–01 and 2001–02.

### ***The Effects of Temperature and Relative Humidity on the Longevity of Freshly Dehiscent Pollen***

Under laboratory conditions the half-lives of glasshouse grown pollen (WO030) exposed to 55% relative humidity and temperatures from 10 to 30°C did not differ significantly (243 to 210 minutes). In contrast, pollen held at 55% relative humidity and 40°C had a significantly ( $P < 0.001$ ) shorter half-life of 110 minutes.

There was a marked decline in carrot pollen longevity with increasing relative humidity from 40 to 100% at 25°C, characterised by the data presented in Figure 6.13 for pollen viability after 1 hour of exposure to the treatments. The half-lives of pollen samples decreased from 200 minutes for 40% relative humidity to 19 minutes for 100% relative humidity at 25°C (Table 6.11). The interaction between temperature and relative humidity was significant ( $P < 0.001$ ), with a combination of high

temperature and high relative humidity having the most damaging effect on pollen longevity (Table 6.11).



**Figure 6.13** – The effect of varying relative humidity at 25°C on the longevity of freshly dehisced carrot pollen one hour after exposure to the humidity treatments. The relationship between relative humidity and relative pollen viability is significant ( $P < 0.001$ ) and is described by the equation  $y = -0.02x^2 + 1.87x + 22.97$  ( $R^2 = 0.97$ ), where  $y$  = relative pollen viability and  $x$  = relative humidity. The error bars indicate standard errors ( $n=4$ ).

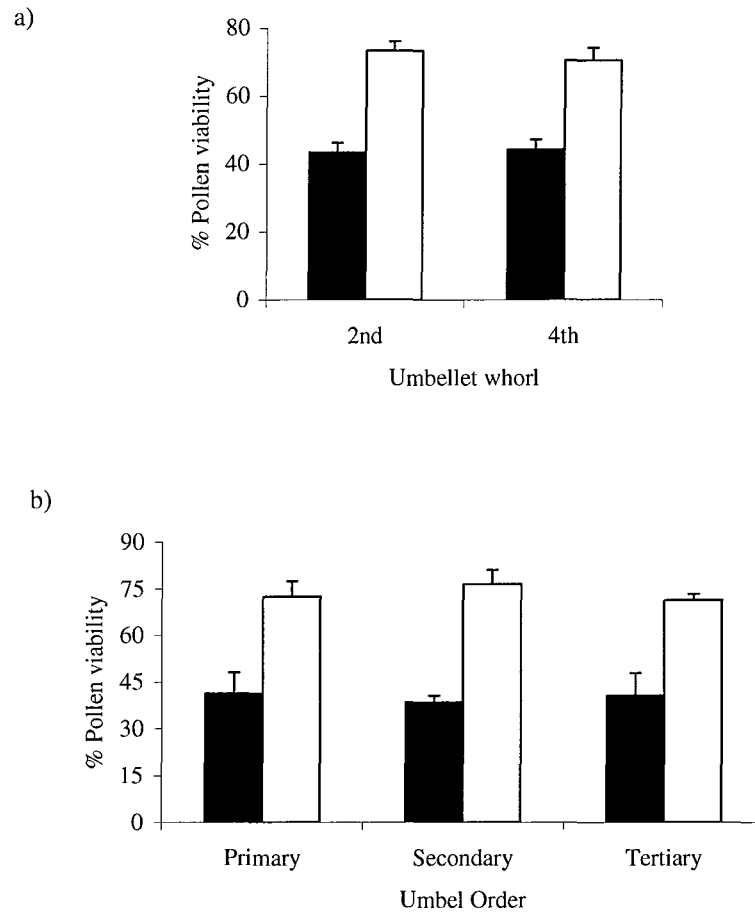
**Table 6.11** – The half-lives of freshly dehiscid carrot pollen samples exposed to a range of temperature and humidity conditions.

Variable examined	Treatment combination		Half-life (minutes)
	Temperature (°C)	Relative humidity (%)	
Temperature	10	55	243
	20	55	239
	25	55	211
	30	55	210
	40	55	110
	LSD (P<0.05)		96
Humidity	25	40	200
	25	55	186
	25	75	139
	25	85	80
	25	100	19
	LSD (P<0.05)		43
Temperature / humidity interaction	10	40	255
	25	40	200
	30	40	211
	10	75	165
	25	75	94
	30	75	72
	LSD (P<0.05)		90

### *Inter- and Intra- Plant Variation in Pollen Viability*

There was no evidence of variation in the viability of pollen taken from different positions within the primary, secondary or tertiary umbels (2<sup>nd</sup> and 4<sup>th</sup> whorl umbellets) (Figure 6.14a) or from different umbel positions within the plant (primary to tertiary umbels) (Figure 6.14b) for either WO030 or No. 22. In contrast, there was significant variation in pollen viability among individual plants in each line ( $P<0.05$ ), with the pollen viability of plants of No. 22 ranging from 29.7 to 58.1% and WO030 ranging from 64.0 to 95.8%. Mean pollen viabilities for each line, determined from these data showed that the differences in pollen viability between the two male fertile lines consistently observed throughout all field and glasshouse trials were significant under glasshouse conditions ( $P<0.001$ ). Under the glasshouse conditions, WO030 had a mean pollen viability of 75.0% compared to 39.4% for No. 22. For both lines, throughout all experiments, there was a trend of higher pollen viability from

glasshouse grown plants compared to field grown plants (refer to Tables 6.7 and 6.12). This trend was most apparent for WO030.



**Figure 6.14** – Intra-plant variation in pollen viability within the male fertile lines of No.22 (■) and WO030 (□). a) Comparison of pollen viability from flowers of the 2<sup>nd</sup> and 4<sup>th</sup> whorl of umbellets of the 4<sup>th</sup> secondary umbel. Samples collected from the 2<sup>nd</sup> and 4<sup>th</sup> whorls of the primary and 4<sup>th</sup> tertiary umbels (not shown) showed the same pattern of no difference in pollen viability with respect to umbellet position. b) Comparison of pollen viability from the primary, 4<sup>th</sup> secondary and distal tertiary umbel on the 4<sup>th</sup> secondary branch.

**Table 6.12** – Mean pollen viabilities of individual plants of No. 22 and WO030 sampled at 10am on 4 consecutive mornings.

Plant No.	Line	
	No. 22	WO030
1	58.1	95.8
2	52.2	85.2
3	48.1	84.0
4	46.9	80.7
5	43.4	76.0
6	41.4	75.7
7	41.4	75.5
8	41.2	75.5
9	40.9	75.4
10	39.0	74.8
11	38.9	74.7
12	37.4	74.6
13	34.9	69.5
14	34.8	68.6
15	33.0	67.4
16	33.0	67.2
17	32.5	65.9
18	31.2	64.0
19	30.7	
20	29.7	
Line mean (all plants)	<b>39.4</b>	<b>75.0</b>
LSD (P<0.05) (among plants)	14.7	16.4
LSD (P<0.05) (between lines)	3.4	



## 6.4 - Discussion

Over 3 seasons 9.4 to 64.0% of female flowers on the primary and secondary umbels of the male sterile lines of a range of Nantes hybrids matured seeds under field conditions in southern Tasmania. In contrast, in preliminary trials (Chapter 3) open pollinated carrot lines matured seeds from greater than 85% of hemaphrodite flowers in the primary and secondary umbels and between 55 and 86% of flowers set seed in field grown plants of the cultivar Amsterdam Forcing (Braak and Kho, 1958). Therefore, by comparison, seed set of the male sterile lines in this study was generally low. The experiments reported in this chapter demonstrate that in some hybrid varieties seed yield is limited by inadequate pollination of the male sterile line. The results of the experiments indicate that factors operating around the time of fertilisation and early seed development may also significantly restrict seed yield.

Supplemental hand pollination increased the yield of primary and secondary umbels of the male sterile lines of No. 22 by 54 to 284% and WO030 by 41% in both seasons. This resulted in significant increases in total plant yield of 25 to 52% for No. 22 and non-significant trend of yield increases of between 7 and 20% in WO030. Although smaller and of less vigour than No. 22, supplemental pollination of a greater proportion of umbels of WO030 may have resulted in a significant increase in total seed yield in this variety. Inadequate pollination has previously been suggested as a cause of unreliable seed yields in hybrid carrot based on pollinator foraging behaviour and comparison of seed set in different male sterile lines (Erickson and Peterson, 1978; Erickson and Peterson, 1979; Erickson *et al.*, 1979; Galuszka and Tegrek, 1987; Galuszka *et al.*, 1989; Rodet and Torre Grossa, 1991). However, the potential for increased pollination to improve hybrid seed yield under field conditions does not appear to have been conclusively demonstrated prior to this study. Given that the yields and seed set of No. 22 and WO030 were typical of a range of male sterile lines in preliminary trials in Tasmania, South Australia and New Zealand, the potential for improved yield of hybrid carrot seed crops from increased pollination may be widespread.

Seed yields of field crops are often reported to be limited by the availability of resources, particularly assimilate, to the developing seeds (Judel and Mengel, 1982; Evans, 1984; Fenner, 1985; Bouwmeester *et al.*, 1995; Mendham and Salisbury 1995). Early seed development is generally thought to rely heavily on current assimilate (Wardlaw, 1996) and relationships between seed yield and incident light levels during flowering and seed filling have been documented in a number of crops under field conditions (Leterme, 1988b in Mendham and Salisbury, 1995; Bouwmeester *et al.*, 1995). In the current work, there are several lines of evidence that suggest that seed yield was not restricted by source strength. Firstly, supplemental hand pollination increased the number of seeds set on plants without any effect on seed size or seed quality. Secondly, treatments applied to the male sterile line during the period from pre-flowering to seed maturation, including 30% shading and umbel and leaf removal generally had little effect on seed yield, seed size or seed quality. Finally, although 50% shading from flowering to seed maturation caused up to 51% reduction in leaf photosynthetic rates between 10am and 4pm in the 3 week period from full bloom, yields did not decrease significantly in either WO030 or No.22.

During the pollination experiments, male sterile plants of No. 22 and WO030 produced between 0 and 8% sexless flowers in the primary umbel and 36 to 45% sexless flowers in the 4<sup>th</sup> secondary umbel. Similar levels of sexless or male only flower production have previously been reported in cultivated and wild carrots (Braak and Kho, 1958). Within the male sterile lines under study, 21 to 66% of female flowers were pollinated in 2000–01 and 2001–02. Given the previously mentioned levels of seed set observed in open pollinated varieties, pollination rates within No. 22 and WO030 were relatively low. Asynchrony of flowering of the parent lines, inadequate pollinator levels, discriminatory foraging of the honeybees and arrangement and ratio of the parent lines with respect to pollinator foraging patterns have previously been suggested as causes of low seed yield in open pollinated and hybrid carrot seed crops (Hawthorn *et al.*, 1955; Erickson and Peterson, 1978; Erickson and Peterson, 1979; Erickson *et al.*, 1979; Galuszka and Tegrek, 1987; Galuszka *et al.*, 1989; Rodet and Torre Grossa, 1991). In the field, flowering of the first three umbel orders of the male sterile lines of WO030 and No. 22 coincided with the peak flowering of their corresponding male fertile lines, indicating that nicking of the parent lines did not limit seed yield. Under typical field conditions for southern

Tasmania individual flowers had a peak period of receptivity lasting 1 to 2 days when the stigmas were fully extended, turgid and separated. Lower levels of seed set were observed for several days prior to and after this stage. Within individual umbels, flowering lasts 7 to 10 days (Rubatzky *et al.*, 1999). The spread of flowering across the first three umbel orders in both male sterile lines under study lasted approximately 50 days. Based on these observations, seed set was unlikely to be restricted by a narrow time window for effective pollination of individual flowers, umbels or the plant as a whole.

In addition to low rates of pollination, pollen viability of the male sterile line was observed to be highly variable, ranging from 19.8 to 57.1% for No. 22 and 9.7 to 67.3% for WO030 on different sampling days. In comparison with reports of pollen viability for some other crop plants including onion (Chang and Struckmeyer, 1975), cabbage (Hodgkin and Lyon, 1986; Jandurova and Pavlick, 1995) and tomato (Abdul-Baki, 1992), the viability of carrot pollen in this study, particularly from No. 22 was relatively low. Overall, the viability of pollen of both male sterile lines was markedly lower in 2001–02 than 2000–01. Combined with the lower rates of pollination observed in 2001-02 this may explain the lower percentage of secondary umbel flowers setting seed set in 2001 – 02 compared to 2000 – 01 (14.5 and 9.4% for WO030 and No. 22 in 2001 – 02 compared to 45.4 and 14.5% respectively in 2000 – 01).

Daily variation in viability of pollen samples collected from the field at 1pm was associated with the prevailing relative humidity conditions prior to sampling (9am to 1pm on the day of sampling). Given that pollen dehiscence commences between 9 and 10am on most days, this effect was likely to have been mainly on post anthesis longevity. In laboratory trials the half-life of freshly dehisced glasshouse grown pollen of the male sterile line of WO030 was, at most, 4 hours, over a range of temperature and humidity conditions typical of those encountered in the field. In confirmation of field observations, pollen longevity was shown to be particularly sensitive to increasing relative humidity. The daily variation in pollen viability in the field was not directly associated with the mean temperature on each day prior to sampling, which ranged from 11 to 25°C. Despite this, incorporation of mean temperature and humidity data from 9am to 1pm produced a slightly better model of

daily pollen viability at 1pm. The data collected from laboratory studies were consistent with these observations. Temperatures between 10 and 30°C had little effect on pollen longevity at a fixed relative humidity of 55%, but elevated temperature (30°C) and high humidity 75% interacted to markedly reduce the half-life of carrot pollen. High temperature (40°C) at 55% relative humidity reduced pollen longevity, but field temperatures did not get this high during the sampling periods. Based on the effect of humidity on pollen longevity, the generally lower viability of pollen in 2001-02 compared to 2000-01 was probably due in part to the higher average relative humidity in 2001-02 compared to 2000-01 (seasonal climate data sourced from the Australia Bureau of Meteorology).

Similar responses to temperature and humidity have previously been demonstrated in a number of binucleate and trinucleate pollens (Chang and Struckmeyer, 1975; Hoekstra and Bruinsma, 1975a; Hoekstra and Bruinsma, 1975b), but longevity is generally much shorter for members of the trinucleate group. Although trinucleate pollens are characterised by a short life span the half-lives of carrot pollen appear very short, even within this group. For example, half-lives of around 3 hours and several days were reported for species of the Asteraceae held at 30°C and 97% or 60% relative humidity respectively (Hoekstra and Bruinsma, 1975a) compared to 1.2 hours for pollen of WO030 held at 30°C and 70% relative humidity.

Despite the overall effect of temperature and humidity on daily pollen viability at 1pm, there was considerable spread in the data around the values predicted from the temperature and humidity model. This variation is presumably the effect of environmental conditions on pollen development and viability before anthesis, or the effect of other environmental parameters on post anthesis longevity.

In spite of the variation in pollen viability between sampling days there did not appear to be a consistent pattern of viability change throughout the day in the field. A number of factors may have affected this including variation between days in terms of environmental conditions at different stages of the day, the rate with which pollen was removed from the male fertile line by pollinators and the pattern of anther dehiscence throughout the day.

Under field and laboratory conditions pollen viability was notably higher in the male fertile line of WO030 compared to the male fertile line of No. 22 (75% compared to 39% in glasshouse conditions). Intra-plant variation in pollen viability at anthesis was not observed within the first three umbel orders of glasshouse grown plants but significant inter-plant variation occurred in both lines, suggesting a genetic restriction on pollen viability. Inbreeding depression is known to cause loss of male function in plants (Aslam *et al.*, 1990; Carr and Dudash, 1997). Given the strong inbreeding depression reported in carrot for a range of agronomic traits (ISSI, 2002) it is plausible that inbreeding depression would restrict pollen viability in carrots. Samples of pollen collected at anthesis from glasshouse grown plants of WO030 were generally of markedly higher viability than pollen collected from field grown plants at approximately 10am, when the anthers commenced dehiscence each day. Although not as obvious, a similar pattern was often observed for No. 22. This also indicates that environmental conditions encountered in the field during flowering may restrict carrot pollen viability at anthesis as well as pollen longevity.

Predictions of seed set based on measurements of pollen viability and pollination rates overestimated the percentage of male sterile flowers that matured seeds by 0 to 63% in both lines over the two seasons that pollination was studied. Furthermore, in most treatments the majority of flowers set one seed, despite the presence of two ovules in each flower. Seed set did not change between 10 days after pollination and maturity, indicating that the limiting processes exerted an effect soon after pollination. The FCR testing procedure for pollen viability, upon which the predictions of seed set were based, was demonstrated to reliably predict pollen germination on stigmas under favourable glasshouse conditions (Chapter 5), but not necessarily under more stressful field conditions. In addition to pollen germination, a host of variables including impaired pollen tube growth, aborted ovules or seed abortion in the first 10 days following pollination may restrict the proportion of flowers maturing seeds. Preliminary attempts to examine pollen tube growth and fertilisation were unsuccessful as the tissue clearing and aniline blue fluorescence techniques, which had previously been used successfully in fennel (Reynolds, 1995) proved unsuitable for use with carrot. Further research effort is required in this area to identify the causes of yield limitation after pollination.

In contrast to data collected from a range of Australian production locations between 1998 and 2001, the primary cause of failed germinants in seed of WO030 and No. 22 collected from the resource partitioning and pollination experiments was seeds with rudimentary embryos. Although the cause of this problem is relatively unclear, two interesting observations about the occurrence of rudimentary embryos can be made. Firstly, 2001–02 was the only season in which significant numbers of rudimentary embryos were observed from Tasmanian trial sites, and their occurrence coincided with a particularly cool, wet summer season. Similarly, rudimentary embryos were observed in seed lots produced at Methven (New Zealand) in a cooler and wetter than average summer season (Chapter 3). Secondly, none of the treatments imposed in this series of experiments reduced the incidence of rudimentary embryos with respect to the comparative control treatments.

In summary, the research reported in this chapter provides a clearer understanding of some of the variables restricting seed yield in European type hybrid carrot seed crops. In southern Tasmania sub-optimal yields of No. 22 and, to a lesser extent WO030, were partly due to low rates of pollination of the male sterile line and variable pollen viability. Given that the yields of these lines were typical of many hybrids grown in Tasmania, South Australia and New Zealand these findings may have broad applicability.

Carrot pollen from the inbred male sterile line of WO030 was short lived under typical field conditions, and was particularly sensitive to humidities above 55%, high temperatures (40°C) and combinations of elevated temperature and humidity. This explained a significant portion of the daily variation in pollen viability that was observed in the field. Significant seasonal variation in pollen viability, as observed in this work, may be an important factor in seed yield variability in individual varieties between seasons and production locations. Further study of pollination and pollen viability in hybrid carrot systems with a view to developing management strategies for both would be a valuable approach for improving hybrid carrot seed yields. Although low rates of pollination and pollen viability limited seed yield, other factors exerting an effect between pollination and early seed development were also involved.

Further research effort is required to identify the yield limiting processes operating during this period of reproduction.

## Chapter 7

### **Pollinator Foraging Patterns and Parent Line Arrangement in Relation to Hybrid Seed Yield**

*A low rate of pollen transfer to the male sterile line was identified as one factor limiting hybrid seed yields, particularly within the brown anther F1 hybrid cross No. 22 (Chapter 6). This chapter reports a single season of experimental work examining factors affecting pollen transfer. There were two avenues of investigation. The first examined the effects of different male fertile and male sterile row arrangements on pollination and seed yields of No.22 within the strip method of hybrid seed production. In the second, the vectors of pollination in trial plots of No. 22 were identified and their foraging behaviour examined in relation to transfer of pollen to the male sterile line.*

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#### **7.1 - Literature Review**

##### **Pollen Vectors in Carrot**

Hybrid seed production requires a vector for transport of pollen from the male fertile to male sterile parent lines. Carrots are largely entomophilous plants, being well suited to insect pollination through several adaptations. Although the individual flowers are inconspicuous they are densely aggregated into umbels, creating a strong visual cue for pollinator insects (Koul *et al.*, 1993). At anthesis, the flowers produce a distinctive



aroma comprised of components described variously as carrot like, sweet, fruity and foul, which are thought to attract pollinators (Erickson and Peterson, 1979). Insects visiting the carrot flowers are rewarded with pollen and/or nectar. The nectar, secreted from the stylopodium, is exposed and, like the anthers, reflects ultraviolet light, which is also likely to provide a visual attraction (Ritchie Bell, 1971; Erickson and Peterson, 1979).

It has been argued that the opportunity for wind pollination in carrots is limited by properties of the carrot pollen, which would largely prevent it from becoming, or remaining airborne (Koul *et al.*, 1993). Whilst a number of experiments have demonstrated improvements in seed yield of 33 to 1500% when carrot plants are exposed to insect pollination, (Hawthorn *et al.*, 1955; Pankratova *et al.*, 1957 cited in Crane, 1972; Sinha and Chkrabarti, 1992; Abrol, 1997) up to 9062 seeds were set per plant when all insects were excluded (Sinha and Chkrabarti, 1992). Carrot flowers are strongly protandrous, but there may be a significant opportunity for self pollination from one umbellet to another by wind action and a greater opportunity for cross pollination by rubbing together of umbels on adjacent plants (Hawthorne *et al.*, 1960). While this may explain results from insect exclusion trials, the weight of evidence indicates that insects are the main vectors of pollen transfer in hybrid carrot seed production.

### **Insect Pollinators of Carrot Seed Crops**

In many families and genera of plants, there is a highly specialised relationship 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 nearly all insects and the inflorescence can easily support large insects (Bohart and Nye, 1960). On the basis of carrot inflorescence structure, Ritchie Bell (1971) concluded that

published lists of insect visitors to the flowers of umbels could be taken as a fair indication of the number and diversity of actual pollinators.

Studies of pollinator visitation to carrot seed crops have been made in the United States (Bohart and Nye, 1960; Treherne, 1923 cited in Free, 1970), India (Singh, 1983 cited in Sinha and Chkrabarti, 1992; Kolesnic, 1989 cited in Sinha and Chkrabarti, 1992; Sinha and Chkrabarti, 1992; Abrol, 1997) and Russia (Pankratova, 1959 cited in Crane, 1972). As with other reports for the Apiaceae (Ritchie Bell, 1971), a large number of pollinator species were observed in each study, for example 334 species of insects representing 71 families (Bohart and Nye, 1960). Although members of the Diptera and Hymenoptera were generally regarded to be the most significant pollinators in each study, the importance of each of these, and of individual species or families, varied between locations. Singh (1983 cited in Sinha and Chkrabarti, 1992) reported that honeybees *Apis mellifera* and *A. cerana indica* were the most important pollinators, but Kolesnik (1989 cited in Sinha and Chkrabarti, 1992) observed that unless honeybee colonies were located within 500m of the crop wild bumblebees were the principal pollinators. Sinha and Chkrabarti (1992) observed that Dipterans were the dominant pollinators, but that the relative levels of activity of individual species varied throughout the day and with climatic conditions.

Blowflies (*Calliphora* sp.) gave better crossing rates between inbred lines of brussel sprouts than honeybees because they were not as selective in their foraging (Faulkner, 1978). Blowflies (*Calliphora* and *Lucilia* sp.) satisfactorily pollinated hybrid onion seed parent lines grown in cages (Currah and Ockendon, 1984). On the basis of seed yield per insect, the solitary bee *Osmia rufa* was more efficient than a range of Dipterans for pollination of carrots, onions and brassicas (Schittenhelm *et al.*, 1997). In most instances, a range of complementary species appears more effective for pollination than an individual species alone (Wilson *et al.*, 1991).

There have been no reported surveys of the pollinators of carrot seed crops in Australia. In the related crop fennel (*Foeniculum vulgare*) grown in Tasmania, A.

*mellifera* was the dominant pollinator species but various native bees, moths, butterflies and beetles also contributed to pollination (Giudici, 1991).

### **Recommendations for Pollination of Carrot Seed Crops**

Populations of natural pollinators in carrot seed crops are often lower than optimal and may limit seed yield (Hawthorn *et al.*, 1955). Because of their efficiency as pollinators and ease of manipulation for use in commercial seed production, honeybees are often introduced into carrot seed crops to increase pollinator numbers. Honeybee populations of 9.6 foragers / m<sup>2</sup> of plants were determined to be optimal for pollination of open pollinated carrot plants in cages (Hawthorne *et al.*, 1960). Stocking rates of 10-15 hives/ha were recommended for hybrid carrot seed crops to achieve an average of 9.6 foragers / m<sup>2</sup> of plants (Mayer and Lunden, 1983 cited in Delaplane and Mayer, 2000). In the United States 15–20 hives/ha are commonly placed around the border of hybrid carrot seed fields (Rubatzky *et al.*, 1999). Stocking rates used in Australian crops of 5-8 hives/ha (Fulton, 1999; Manning, 2003) appear relatively low, although it is unclear if the size of the hives on which the recommendations were based were the same in each instance.

Habitat manipulation by adding suitable attractants for desirable pollinator species has been suggested as a means of improving seed yields. Odour lures placed in hybrid carrot seed crops in Canterbury, New Zealand increased fly activity within a short distance of the lures but did not significantly increase seed set in the crop as a whole (Evans *et al.*, 1995). Weekly sprays of rose water during flowering were reported to increase seed set by 7 to 20% in the onion cultivars Texas Grano and Local White (Al-Sahaf, 2002). Several honeybee attractants that contain sugars, attractive oils or components of the Nansonov pheromone used by bees to orientate low odour food resources have been marketed. The results from use of attractants have generally been poor or mixed (Mayer *et al.*, 1989; Loper and Roselle, 1991). No change in bee

foraging behaviour or crop seed yield was recorded following sprays of the food supplement Beeline<sup>®</sup> in carrot seed crops (Beletti and Zani, 1981).

## **Factors Affecting Pollinator Visitation Rates to Carrot Seed Crops**

### ***Competition from Other Plants for Pollinators***

Carrot crops do not appear to be particularly attractive to honeybees. Other nearby nectar sources often cause a marked reduction in bee counts and seed yields in carrot seed crops (Hawthorn 1955; Delaplane and Mayer 2000). Competition from other nectar sources can be minimised by locating carrot crops away from competing crops, in areas with a diversity of habitats capable of supporting many different species of pollinators, or by ensuring that significant numbers of carrot umbels are in flower at the time of introduction of the honeybees (Free, 1965; Delaplane and Mayer, 2000). In many seed production areas, where potential production sites are limited by the available land area and distance requirements for isolation, competition for pollinators is likely to be a significant problem.

### ***Pesticides***

Night-time applications of synthetic pyrethroid insecticides based on alpha-cypermethrin have been used to manage insect pests in Australian carrot seed crops during flowering. Applied in this manner, such chemicals are non-toxic to honeybees (Manning, 2003) but they may repel them foraging for periods ranging from 1–3 days (Moreti *et al.*, 1988; Kakar, 1994; Manning, 2003). Furthermore, synthetic pyrethroid insecticides may be toxic to other potential pollinator species, notably those within the Diptera and Coleoptera.

## **Pollinator Foraging Patterns**

Dipteran insects are largely facultative pollinators in that, for most, their feeding on flowering plants is opportunistic. In contrast honeybees and other bee species are obligate pollinators, for whom pollen and nectar collection is needed to supply the energetic and nutritional requirements for growth and maintenance of the colony (Free, 1970). Whilst honeybees forage for both pollen and nectar, most Dipteran species forage for nectar only (Currah and Ockendon, 1984). In addition to pollination that occurs during feeding, Dipteran species may also cause pollination by accident through their use of flowers as a platform for basking and attraction of mates.

The differences in motives of honeybees and Dipteran pollinators reflect the observations that Dipterans such as *Lucilia* and *Calliphora* have a more random foraging pattern across hybrid seed parent lines than honeybees (Faulkner, 1978; Currah and Ockendon, 1984). Given the documented importance of honeybees as pollinators of carrots, and their widespread use because of their ease of management, the following section examines some of the principles of honeybee foraging and implications for hybrid carrot seed production.

### ***Foraging Patterns of Honeybees***

Optimal foraging theory predicts that animals will forage efficiently, minimising the amount of energy that is expended (Dafni, 1992). Based on optimal foraging theory, three hypotheses have emerged which generally explain the foraging pattern of pollinators in the field (Delaplane and Mayer, 2000). These are; a) feeding rate maximisation, where foragers appear to maximise energy harvest per unit effort; b) minimal uncertainty foraging, in which the flower type least often empty of reward is visited; and c) individual constancy foraging, where individuals discriminately visit only one flower morph (Wells and Wells, 1986). Pollinators appear to be able to alternate between these behaviours in response to changing foraging conditions

(Zimmerman, 1988). For *A. mellifera*, individually constant foraging appears to provide a superstructure under which the sub-classifications of optimal diet and minimal uncertainty foraging may sometimes occur (Wells and Wells, 1986).

### ***Foraging Cues and Discriminatory Foraging***

Pollinators rely on visual and olfactory stimuli to locate and discriminate between flowers and their rewards (Free, 1970; Pernal and Currie, 2000). Visual stimuli including flower colour, flower form and pigmentation patterns are important in attracting pollinators from a distance. In general, inflorescences with a large number of open flowers have higher nectar rewards and honeybees preferentially land on these (Zimmerman, 1988; Delaplane and Mayer, 2000).

At closer range or after alighting on an inflorescence, the presence of stamens, nectar guides and nectar abundance are cues used to locate food (Pernal and Currie, 2000). Pollen deposition on stigmas has been found to increase towards an asymptotic value as a function of nectar level in a number of species (Zimmerman, 1988). In addition to its abundance, the most important factor influencing the attractiveness of nectar is its sugar concentration. When the sugar content of nectar is below an estimated 20%, the energetics of water evaporation to produce honey make its collection uneconomical (Free, 1970). Honeybees appear to have a preference for sugar solutions of 30–50% (Waller, 1972 cited in Silva and Dean, 2000) and especially those with a high sucrose content (Woodrow, 1968 cited in Silva and Dean, 2000). For a range of inbred and open pollinated carrot lines, nectars with 20-30% sugars, that are high in fructose and glucose but low in sucrose have been reported (Erickson and Peterson, 1979). For many species including honeybees, the use of odour is most important for close range orientation, when foragers inspect the flowers prior to and after alighting. This inspection allows insects to discriminate between floral species, decide whether or not to land, and may influence the expression of foraging behaviour (Pernal and Currie,

2000). Differences in aroma production between male fertile and male sterile carrot lines were observed by Erickson and Peterson (1979).

Generally, discriminatory behaviour by pollinators is thought to limit inter-specific foraging activity but, with the differences that are often apparent between CMS hybrid seed parents, there is great potential for discrimination between parent lines within a hybrid. Discriminatory foraging by honeybees between male fertile and cytoplasmically male sterile lines has been reported for many species including sunflowers (Singh *et al.*, 2000), cauliflowers (Funari *et al.*, 1994), brussel sprouts (Faulkner, 1978) and carrots with petaloid male sterility (Erickson and Peterson, 1978; Erickson *et al.*, 1979). In contrast to reports for petaloid lines, in a cage trial honeybees foraged randomly between the parent lines of a hybrid in which the male sterile line had brown anther CMS (Rodet and Torre Grossa, 1991). Seed set in the tertiary umbels of male sterile plants of the petaloid type was reported to be 10 to 11% lower than for male fertile and brown anther plants (Litvinova and Federova, 1990). This discrepancy may be partly due to the closer appearance of the brown anther flower form to the perfect flower (Erickson *et al.*, 1982).

### ***Rates of Movement between Flowers***

Predictions from optimal foraging theory state that animals forced to forage in resource poor habitats tend to spend more time at each forage site than do animals in resource rich habitats (Pyke *et al.*, 1977 cited in Delaplane and Mayer, 2000). Thus, as the pollen and nectar rewards in flowers of a plant increase, the total number of blossoms visited by the pollinators should also increase (Zimmerman, 1988), increasing the rate of pollen dispersal. Although the number of flowers visited per plant is not independent of the number of blossoms available, pollinators have been shown to consistently visit more flowers on high rather than low reward quality inflorescences (Zimmerman, 1988).

### ***Directional Foraging***

Having encountered a patch of profitable flowers, honeybees tend to forage in a more or less straight line. This behaviour limits the chance of revisiting a flower recently emptied of its nectar (Cresswell *et al.*, 1995). Insects may also forage more or less in straight lines when there is a corridor effect within hybrid crops due to height differences between the male fertile and male sterile lines (Pierre *et al.*, 1999). Such height differences are common in hybrid carrot crosses involving an F1 male sterile line and inbred male fertile line (Erickson and Peterson, 1979).

### ***Foraging Range and Area Fidelity***

Where possible bees prefer to forage near their hives (Free, 1970; Gary and Witherell, 1977 cited in Delaplane and Mayer, 2000; Tewari and Singh, 1983). This preference is enhanced by the tendency of successful foragers to recruit more bees when working near rather than far food sources (Boch, 1956 cited in Free, 1970) and is more apparent during weather conditions that are unfavourable for foraging (Nevkryta, 1957 cited in Free, 1970).

Bees generally forage in a manner that increases the likelihood of encountering other rewarding flowers in a site that has been profitable. After visiting a highly rewarding flower bumblebees and honeybees fly shorter distances before visiting another flower than those that have visited a low reward flower (Pyke, 1978; Waddington, 1980 cited in Delaplane and Mayer, 2000). As well as restricting the area of foraging on a single flight, honeybees tend to maintain narrow foraging ranges over many visits (Free, 1970; Rodet and Torre Grossa, 1991).



### ***Pollen Carryover***

Pollen carryover is the acquisition of pollen from a flower by a pollen vector and its carriage and deposition onto a succession of subsequently visited flowers until all the pollen has been deposited, packed in corbiculae (bees) or lost from that part of the body of the insect that contacts the stigmas (Williams, 2001). The density and reward quality of flowers within a patch affects the distance of pollen carryover through an impact on the distance of intra- and inter-floral movements of foraging bees and hence the length of the foraging route. Studies of pollen flow within crop plants generally describe highly leptokurtic distributions of pollen from the source plants, with levels decreasing rapidly within a few metres of the source to a low level at which they remain for a much longer distance (Free, 1970; Williams, 2001).

### **The Effects of Parent Line Arrangement on Hybrid Seed Yields**

Hybrid vegetable seed crops involving a male sterile line are usually produced using the strip method of production in which the male sterile and male fertile lines are grown in alternating rows, or blocks of rows for the length of the field. The area occupied by the male fertile line does not yield hybrid seed and should be minimised for efficient production, but this must be balanced against ensuring that there is adequate pollen within the crop and that male sterile plants are located close enough to the male fertile plants to be pollinated. Hence, for efficient production there is a need to establish the optimum ratios of male fertile to male sterile plants and width of male fertile and male sterile blocks or strips within the field. Both variables are likely to be influenced by factors such as crop type, cultivar and pollen vector (Schittenhelm *et al.*, 1997; Rubatzky *et al.*, 1999). Because of the variables involved, optimum parent line arrangements for individual hybrids may vary between different locations. Studies on a number of hybrid seed crops including onion (Williams, 2001), rape (Pu *et al.*, 1999) and sugar-beet (Matsebera *et al.*, 1994) recommend male fertile to male sterile row ratios ranging from 1:9 to 1:4. In bee pollinated crops such as oilseed rape and

sunflower, yield reductions have been reported when the distance from male sterile to male fertile plants within the crop exceeded 3m (Lavigne *et al.*, 1998; Singh *et al.*, 2001). Male fertile to male sterile ratios of 1:4 with 8 rows of male sterile plants per bed are commonly used for hybrid carrot seed production in the United States (Rubatzky *et al.*, 1999). Lower yields of male sterile carrot plants positioned 1.5m compared to 0.9m from the male fertile line were reported in cage trials (Rodet and Torre Grossa, 1991). The distance of outcrossing from the male fertile line varied with pollinator species for carrots grown in cage trials (Schittenhelm *et al.*, 1997). Pollen transfer to male sterile lines dropped sharply within the first metre from the male fertile line when red mason bees *Osmia rufa* and blowflies of the genus *Calliphora* were the pollinators but was maintained for more than 2 metres by blowflies of the genus *Lucilia*. Currently, male fertile to male sterile ratios of 1:2 to 1:3 and male sterile block widths of 3.6m are used for hybrid carrot seed production in Tasmania but studies to identify optimal ratios have not been conducted.

## **Conclusion**

Under Tasmanian production conditions the rate of pollen transfer to the male sterile line has been shown to be inadequate for optimum hybrid seed yield, particularly for the variety No. 22 (Chapter 6). This review examined a number of factors that may contribute to this deficiency. Although there is evidence from the literature that members of the Hymenoptera and Diptera are the most important pollinator species for carrot seed crops, the range, importance and frequency of visitation of pollinator species in Australian carrot seed crops has not been examined.

Male fertile and male sterile carrot lines appear to differ in a number of characters that are generally considered important foraging cues for pollinators. Non-random foraging of honeybees was observed in hybrid crosses involving petaloid lines but there is evidence to suggest that this problem is less evident when brown anther lines are used within the hybrid cross.

The Australian carrot seed industry, which is largely based on contract multiplication of seed lines for overseas vegetable breeding companies, has little opportunity to influence the CMS type or varieties grown. The greatest potential for improved pollination of carrot seed crops in Australia may therefore lie in the manipulation of pollinator populations and the arrangement of the parental lines within the carrot fields. Experiments conducted with honeybees, red mason bees and blowflies in caged plots suggest that seed yields from male sterile plants decrease over relatively short distances from the male fertile line. Studies of the effects of parent line arrangement in open fields of carrot, where a range of pollinator species may be present, have apparently not been reported.

## 7.2 - Materials and Methods

### Plant Material and Climatic Conditions

Experiments reported in this chapter were conducted on the University of Tasmania Farm at Cambridge in the 2001–02 season. Climatic data during the period between pollination and seed maturation are given for the trial site in Table 7.1. Compared to the long term averages, conditions during this period were cooler and wetter than average (for comparison see Figure 2.2 in Chapter 2). All experiments were conducted on the brown anther hybrid carrot cross No. 22, which was grown by the seed to seed method using standard commercial practices. Cultural details were as described in Chapter 2. When 10% of the primary umbels of the male sterile line had commenced flowering, hives of honeybees (*Apis mellifera* L. *ligustica*) were placed along the edge of the trial plots at a density of 6 hives/ha.

**Table 7.1** – Climatic conditions during the period from flowering to seed harvest and on the four individual days that the insect pollinators were surveyed in the parent line arrangement trials. Data extracted from the records for the nearest weather station, Hobart airport (site no. 94004). Source Australian Bureau of Meteorology.

Month	Mean daily minimum temperature (°C)	Mean daily maximum temperature (°C)	Precipitation (mm)	No. of rain days (>0.2mm precipitation)
December	11.2	19.8	52.8	17
January	12.0	20.6	97.6	11
February	11.7	21.3	33.8	10

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Date	Daily minimum temperature (°C)	Daily maximum temperature (°C)	Mean wind speed (km/hr 9am to 3pm)
23/12/01	13.0	20.7	7.0
31/12/01	11.3	22.2	13.5
4/1/02	10.5	19.4	22.5
6/1/02	9.8	20.9	11.0

## **The Effects of Parent Line Arrangement on Pollination and Seed Yield**

Two separate experiments were conducted in adjacent trial plots 20m apart. Experiment 1 examined the effect of the ratio of male fertile to male sterile rows on cross-pollination and hybrid seed yield. Experiment 2 examined the effect of the width of the male sterile block on cross-pollination and hybrid seed yield. In each experiment the plants were grown on raised beds in 10m long rows, with 0.8m between adjacent rows.

In Experiment 1 the treatments were male fertile to male sterile row ratios of 1 to 4, 2 to 4 and 4 to 4. Each treatment consisted of 4 central rows of the male sterile line, with 1, 2 or 4 rows of male fertile line on each side (Figure 7.1). Two rows of male sterile plants were grown on either side of the plots for buffering and to maintain the correct parent line ratios. The treatments were arranged in a randomised complete block design with 4 replicates, giving 12 plots (ie sets of the row arrangements) in total. In Experiment 2 the treatments were central male sterile bed widths of 2, 4 and 8 rows with a constant male fertile to male sterile ratio of 1 to 2 across all treatments (Figure 7.2). Buffer rows of male sterile lines were grown between adjacent plots in both experiments, with the number of buffer rows set to maintain the appropriate male fertile to male sterile row ratio for the treatment. The treatments were arranged in a randomised complete block design with 4 replicates, giving 12 plots in total.

The level of pollination was recorded by measurement of pollen deposition on the stigmas as described in Chapter 2. Samples were collected on 4 days over the 2 week period of peak bloom. For each treatment in each replicate of Experiment 1, 4 flowers were removed from the second whorl umbellets of 5 randomly selected secondary umbels in each row of the male sterile block (Figure 7.1). In Experiment 2, samples were collected from the 8 row treatment only. In each replicate 4 flowers were removed from the second whorl umbellets of 4 randomly selected umbels from rows 1, 2, 4, 5, 7 and 8 of the male sterile block. (Figure 7.2)

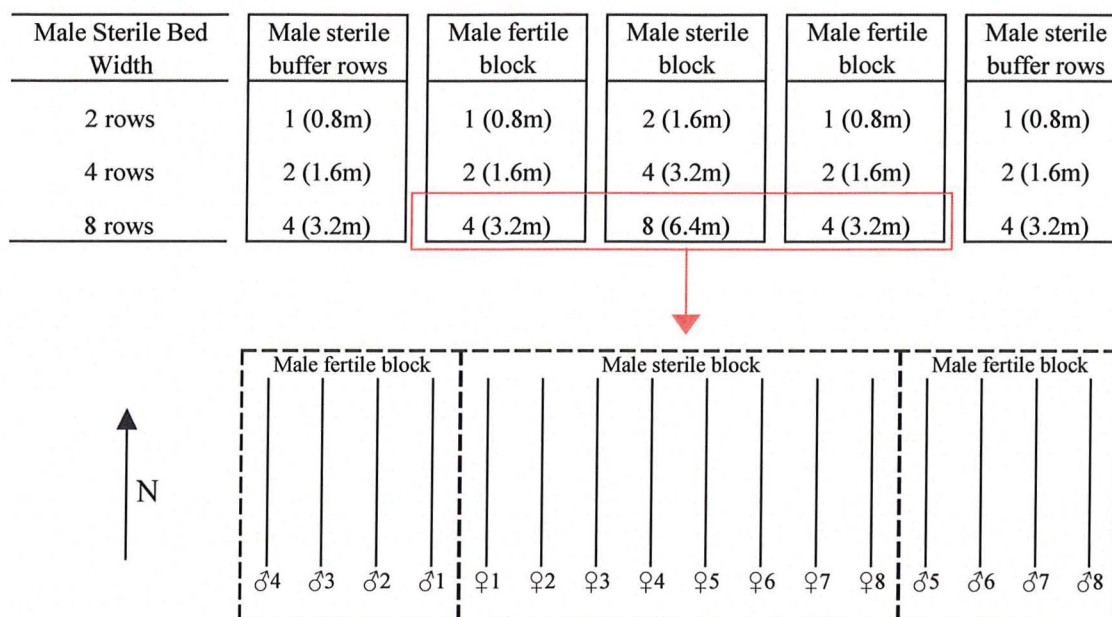
In both experiments, the seed of 10 randomly selected plants from each row of the male sterile block (see Figures 7.1 and 7.2) was harvested from each plot using the small plot thresher described in Chapter 2. After harvest the seed was cleaned, and the mean seed dry yield, seed dry weight and number of seeds per plant determined for each plot using the methods outlined in Chapter 2.

### *Establishment of Yield Potential*

In each replicate of the 4 row treatments of Experiment 2, three randomly selected plants from rows 2 and 3 (Figure 7.2) were hand pollinated in supplement to natural pollination. Freshly dehisced pollen from the male fertile line was used, with each plant pollinated on 3 occasions to ensure that all flowers were hand pollinated during their period of receptivity. The seed of each hand pollinated plant was collected individually at the time of harvest of plants in the parent line arrangement experiments. The handling and assessment of seed lots was the same as for Experiments 1 and 2.

Male fertile to male sterile row ratio	Male sterile buffer rows	Male fertile block	Male sterile block	Male fertile block	Male sterile buffer rows
1 to 4	2 (1.6m)	1 (0.8m)	4 (3.2m)	1 (0.8m)	2 (1.6m)
2 to 4	2 (1.6m)	2 (1.6m)	4 (3.2m)	2 (1.6m)	2 (1.6m)
4 to 4	2 (1.6m)	4 (3.2m)	4 (3.2m)	4 (3.2m)	2 (1.6m)

**Figure 7.1** – Male fertile and male sterile row arrangements for the 1 to 4, 2 to 4 and 4 to 4 male fertile to male sterile row ratio treatments applied in Experiment 1. Within the figure the plots have been divided into 5 different sections consisting of 2 outer sections of buffer rows and 2 male fertile blocks surrounding the central male sterile block in which samples were taken. The figures in each section indicate the number of rows in that section for the specified row ratio treatment. The width in metres of each section in the different treatments is indicated by the figures in brackets.



**Figure 7.2** – Male fertile and male sterile row arrangements for 2, 4 and 8 row male sterile block treatments in Experiment 2. Within the figure the plots have been divided into 5 different sections consisting of 2 outer sections of buffer rows and 2 male fertile blocks surrounding the central male sterile block in which yield and pollination samples were taken. The figures in each section indicate the number of rows in that section for the specified row ratio treatment. The width in metres of each section in the different treatments is indicated by the figures in brackets. The sections within the red box were used in the surveying of pollinators within the 8 row male sterile block treatment. The expanded detail of this section shows the individual rows in each section: male fertile (♂) rows 1 to 4 on the western side of the plot; male sterile (♀) rows 1 to 8 in the centre of the plot; and male fertile rows 5 to 8 on the eastern side of the plot.

## Pollination Vector Studies

Pollination vector studies were undertaken within 4 replicate plots of the 8 row male sterile block treatment from Experiment 2.

The possibility of wind pollination was examined on 4 days throughout the peak bloom period with moderate wind (10-20 knots) from a settled direction. On each day at 9am,

glass microscope slides with one surface coated in a film of vaseline were positioned at canopy height in the first male sterile row downwind of the male fertile block in each replicate (row 1 or 8, depending on the wind direction), so that the coated surface faced into the wind. They were retrieved for examination at 9am the following morning. The trapped pollen was observed under a light microscope at 100x magnification. For each slide, the number of carrot pollen grains in 20 fields of view was counted.

At peak bloom, surveys of insect visitors to the male sterile rows 1, 2, 4, 5, 7 and 8 and the male fertile rows 1 and 5 (Figure 7.2) were made on 4 days between the 23<sup>rd</sup> of December 2001 and the 6<sup>th</sup> of January 2002 with favourable conditions for pollination (Table 7.1). Observations were made at three stages of the day, 10am to 12noon, 12noon to 2pm and 2pm to 4pm, which covered the normal period of pollen availability on the male fertile line. On each occasion, three randomly selected secondary umbels were observed per row in each replicate for 4 minutes. Care was taken to maintain at least three metres between the observer and the umbels under observation. During the 4-minute periods of observation any insects landing on, or crawling onto flowers in the umbels under observation were recorded and identified. Most insects could be identified in the field. Specimens that could not be identified in the field were collected and subsequently identified with reference to insect collections or taxonomic keys (CSIRO, 1991a and b).

The levels of carrot pollen on the 8 major insect species visiting the carrot flowers were examined to confirm their potential as vectors of pollination. On each day, during their peak period of activity 10 individuals of each species were collected from rows 1, 2, 4, 5, 7 and 8 in the male sterile block using an insect net, placed in 1.5ml Eppendorf<sup>®</sup> tubes and frozen at -20°C. Pollen loads were analysed in the laboratory using a modified version of the method developed by MacGillivray (1987). Individual insects were placed in 1.5ml Eppendorf<sup>®</sup> tubes into which 300µl of melted glycerin jelly (50g of melted gelatin in 150ml of glycerin diluted with 175ml of deionised water) had been pipetted and allowed to set. The storage tube from which the insects had been retrieved was flushed with 400µl of xylene, which was subsequently added to



the centrifuge tube containing the insect. After the addition of xylene, the centrifuge tubes were agitated on a vortex mixer for three minutes to displace the pollen load from the insect. The insects were then removed and the tubes centrifuged at 15 000 rpm for 1 minute, after which the xylene was decanted. The pollen impregnated glycerol gelatin pellets were removed from the centrifuge tubes with the aid of a fine hooked needle, placed on microscope slides, heated to melting and covered with cover slips. Light pressure was applied to spread an even film of glycerol jelly over the slide surface beneath the cover slip. After the slides had set they were examined under a light microscope at 100x magnification. Counts of the total number of carrot pollen grains were made from 10 randomly selected fields of view for each slide. The total number of pollen grains collected from each individual insect was calculated from the ratio of the slide area examined in 10 fields of view to the total area of the cover slip.

In preliminary trials, scanning electron microscope examination of washed insects showed that a single wash was effective in removing almost all of the pollen that could have effected pollination from the bodies of species under examination. To avoid confounding of the results of pollen loads from honeybees available for pollination with pollen deposited in the corbiculae, the hind legs were excised from all bee samples prior to the commencement of the washing procedure.

### ***Insect Movement Patterns***

The foraging patterns of the three most prominent pollinators within the trial plots, the hoverfly *Eristalis tenax* (Diptera: Syrphidae), the blowfly *Calliphora ruficornis* (Diptera: Calliphoridae) and honeybee *Apis mellifera* L. *ligustica*, (Hymenoptera: Apidae) were examined on the same four days that pollinator surveys were made. Observations were made during the peak period of activity for each insect within the trial plots. For *C. ruficornis* and *E. tenax* this was between 10am and 12noon and, for *A. mellifera*, between 2pm and 4pm. Each day, 10 individuals of each species were located on plants in rows 1, 4, 5 and 8 of the male sterile blocks and rows 1 and 5 in

the male fertile blocks, and the destination of their next 10 moves to other umbels recorded in terms of plant gender and row position. At the same time 5 individuals of each species were collected in an insect net from each of these rows for measurement of pollen loads using the method described above.

### **Data Analysis**

Pollination and yield differences between treatments were examined in both experiments using ANOVA analysis for randomised complete block designs. Fishers LSD's were used to separate means that were significantly different at the 5% level of probability. The effects of distance from the male fertile line on pollen deposition and seed yield of the male sterile line (Experiment 2) were examined using polynomial regression analysis.

### 7.3 - Results

#### The Effects of Parent Line Arrangement on Pollination and Seed Yield

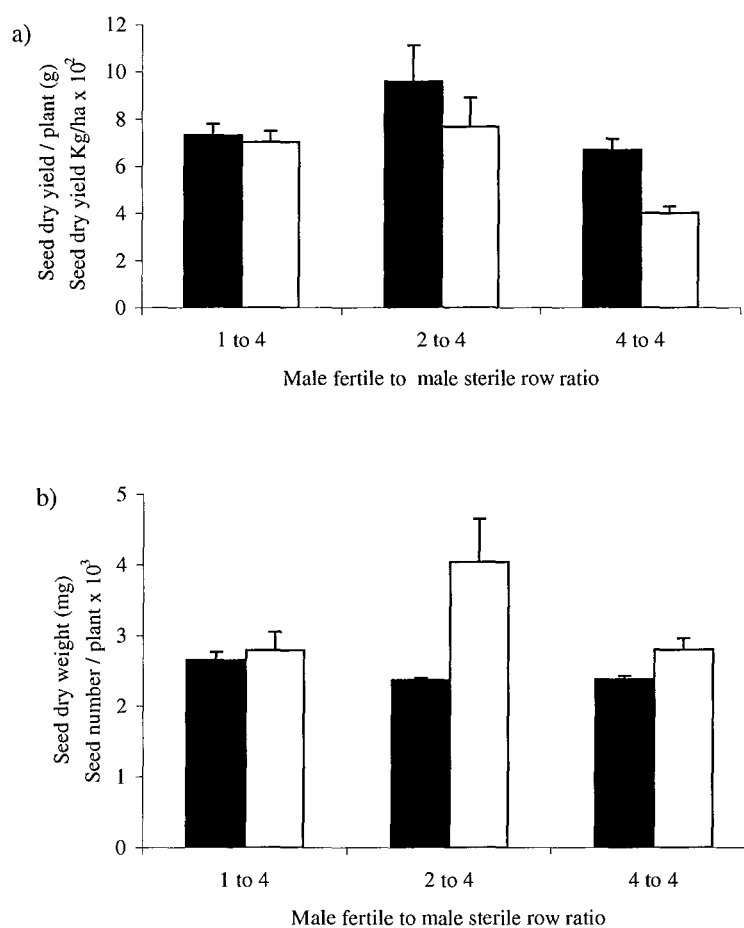
The range of the parent line row ratios and male sterile block widths trialed in this work did not significantly change the percentage of pollinated stigmas and the number of pollen grains deposited on pollinated stigmas (Tables 7.2 and 7.3). Similarly, there were no effects of parent line row ratio or male sterile block width on hybrid seed yields or the number of seeds per male sterile plant when these were averaged across all rows in the male sterile blocks. The hybrid seed yield per total hectare including male fertile and male sterile plants (Figure 7.3) was significantly lower with a 4 to 4 male fertile to male sterile ratio (402 kg/ha) compared to ratios of 1 to 4 (704 kg/ha) and 2 to 4 (767 kg/ha). The average hybrid seed yield per plant for all treatments in both experiments was markedly lower than the yield from plants that received supplemental hand pollination, but seed sizes were slightly higher (Table 7.4).

**Table 7.2** – The effect of male fertile to male sterile row ratio on the pollination of flowers of the male sterile line of the hybrid No. 22. The figures presented are pooled data from the 4 male sterile rows within the male sterile blocks. Standard errors are given in italics (N=4).

Male fertile to male sterile row ratio	% Pollinated stigmas		Pollen grains / pollinated stigma	
1 to 4	23.8	<i>10.5</i>	1.8	<i>0.4</i>
2 to 4	22.3	<i>1.3</i>	1.7	<i>0.3</i>
4 to 4	19.5	<i>2.9</i>	1.8	<i>0.3</i>
LSD (P<0.05)	NS		NS	

**Table 7.3** – The effect of the distance to the nearest male fertile plants on the pollination of flowers of the male sterile line of the hybrid No. 22. Standard errors are given in italics (N=4).

Distance from male fertile row (m)	% Pollinated stigmas		Pollen grains / pollinated stigma	
0.8	15.9	<i>4.4</i>	1.6	<i>0.4</i>
1.6	16.1	<i>4.2</i>	1.8	<i>0.3</i>
3.2	12.4	<i>3.9</i>	1.5	<i>0.5</i>
LSD (P<0.05)	NS		NS	

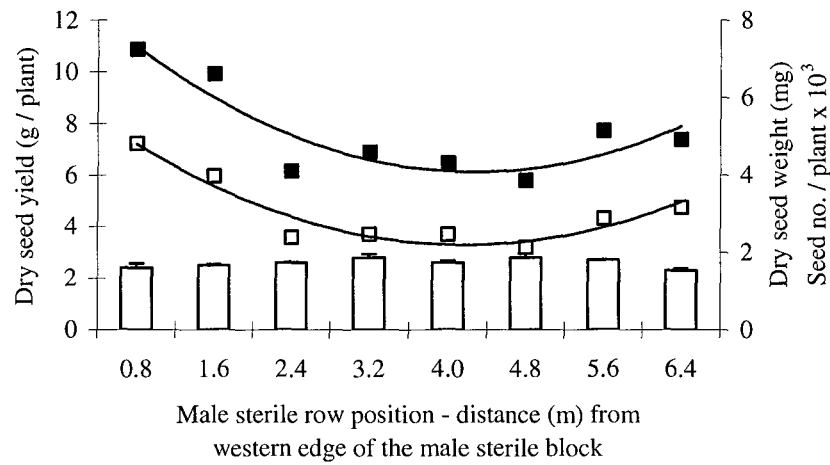


**Figure 7.3** – The effects of male fertile to male sterile row ratios of No. 22 on (a) the yields of hybrid seed per plant (■) and per ha of crop (□); and, (b) seed size (■) and the number of hybrid seeds per male sterile plant (□). A significant effect of male fertile to male sterile row ratio on yield/ha was observed, LSD (P<0.05) = 249.5kg. Differences in the other variables were not significant.

**Table 7.4** – The effects of male sterile bed width and supplemental hand pollination on the seed yield characteristics of the hybrid cross No. 22. Differences between the male sterile block width treatments and supplemental hand pollination, and male sterile row position were analysed using ANOVA. Significant differences are indicated by the LSD values given in the table (N=4) Note differences in seed yield characteristics were also non significant when the data for supplemental hand pollination was removed from the ANOVA analysis.

Variable	Mean dry yield/plant (g)	Seed dry weight (mg)	Number of seeds/plant
Female block width (m)			
1.6	7.83	2.6	3037
3.2	6.27	2.7	2395
6.4	7.64	2.6	3035
Supplemental hand pollination	15.04	2.2	6654
LSD (P<0.05)	5.14	0.3	3949

Although there was no significant effect of male sterile block width on average yields per plant for the entire block, there were significant parabolic relationships between row position within the 8 row wide male sterile blocks and seed yield ( $P<0.05$ ) and number ( $P<0.01$ ) per plant (Figure 7.4).



**Figure 7.4** – Relationships between male sterile row position and seed yield per plant (■), seed number per plant (□) and seed size on a dry weight basis (columns) within 8 row wide male sterile beds with a 2:4 male fertile to male sterile row ratio. Male fertile blocks were located on both sides of the male sterile block (see Figure 7.2). The lines fitted to the seed yield and seed number data show significant relationships ( $P < 0.05$  and  $P < 0.01$  respectively) between these variables and male sterile row position. For seed yield the relationship was described by the equation  $y = 0.25x^2 - 2.73x + 13.44$  ( $r^2 = 0.82$ ), where  $y$  = the seed yield per plant (g) and  $x$  = the distance in meters from the western edge of the male sterile block. For seed number per plant the relationship was described by the equation  $y = 0.15x^2 - 1.52x + 6.17$  ( $r^2 = 0.91$ ), where  $y$  = the seed number per plant and  $x$  = the distance in meters from the western edge of the male sterile block. Data points for each variable are the means of 4 replicates. Error bars for seed weight indicate standard errors ( $N=4$ ).

## Pollination Vector Studies

During peak bloom, windborne fluxes of carrot pollen of less than 4 grains per  $\text{cm}^2$  of trap surface per day were observed in the reproductive canopy of the first rows of male sterile plants downwind from the adjacent male fertile blocks. Over the same period, insects visited individual secondary umbels in flower approximately 15 times an hour between 10am and 4pm. Eight species of Diptera, Hymenoptera and Coleoptera visited

carrot flowers on a regular basis, accounting for greater than 85% of all visits, whilst a number of other species, particularly members of the Coleoptera and Hymenoptera visited infrequently (less than 0.03 visits per hour) (Table 7.5). The three most prevalent species, the hoverfly *Eristalis tenax*, the blowfly *Calliphora ruficornis* and the honeybee *Apis mellifera* accounted for 20, 20 and 15% of all visits respectively. For *A. mellifera*, this level of activity corresponded to an average density of between 0.9 and 7.0 individuals / m<sup>2</sup> of plants throughout the day. A large proportion of *A. mellifera* from hives placed in the trial plots foraged other crop and weed species further afield as evidenced by their flight paths and, in some instances, corbiculae loaded with yellow pollen, rather than the white pollen of carrot. Whilst the total rate of visitation of all species of pollinators combined, and *C. ruficornis* and *E. tenax* individually, decreased throughout the period from 10am to 4pm, the activity of *A. mellifera* was highest between 2pm and 4pm (Figures 7.5a and b).

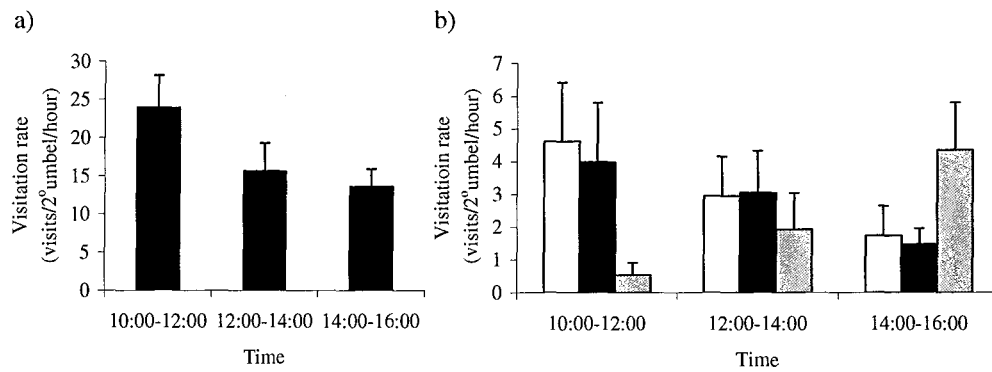
The presence of carrot pollen on individuals of the 8 most frequent visitors was confirmed. There were large variations in mean individual pollen loads between species, ranging from 406 grains for *A. mellifera* to 4 grains for *Musca sp.* (Table 7.5).

For all pollinator species combined, and the three major pollinators individually, there were only small differences between average daily visitation rates to male fertile or male sterile plants located in the central rows of their respective blocks (Figure 7.6). Despite this, pollinator visitation rates to the male sterile plants were affected by row position (Figure 7.7). Within the male sterile block, the average visitation rate to the outside rows (rows 1 and 8), 7.8 visits / secondary umbel / hour, was considerably lower than the average visitation rates to inner rows 2 and 7 and 4 and 5 of 19 and 22 visits / secondary umbel / hour respectively.

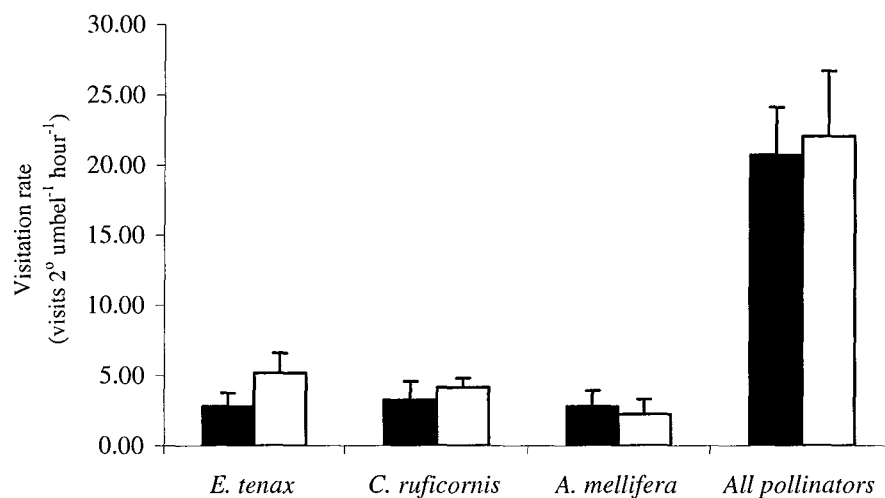
**Table 7.5** – Pollinator visitation rates and pollen loads throughout the day (10am to 4pm) within plots of the carrot hybrid No. 22 with a male sterile bed width of 6.4m (8 rows), male fertile bed width of 3.2m (4 rows) and male fertile to male sterile ratio of 1:2. The visitation rate data were collected from secondary umbels and have been weighted to account for visits to both the male fertile and male sterile lines within the hybrid cross. Pollen load data for each species were determined for equal numbers of insects collected from each of the male sterile rows 1, 2 and 4 (see Figure 7.1). Pollen load data for *A. mellifera* are based entirely on nectar foraging individuals as very few pollen foragers were observed on the male sterile line. Standard errors for visitation rates and pollen loads are given in italics; N=4.

Insect Order	Family	Genus / Species	Visitation rate (visits 2° umbel <sup>-1</sup> hour <sup>-1</sup> )		Pollen load (grains / insect)	
Diptera	<i>Syrphidae</i>	<i>Eristalis tenax</i>	3.12	<i>0.79</i>	89	<i>16</i>
Diptera	<i>Calliphoridae</i>	<i>Calliphora ruficornis</i>	3.09	<i>0.82</i>	26	<i>13</i>
Hymenoptera	<i>Apidae</i>	<i>Apis mellifera</i>	2.28	<i>0.67</i>	406	<i>103</i>
Diptera	<i>Calliphoridae</i>	<i>Calliphora</i> sp.	1.87	<i>0.62</i>	6	<i>2</i>
Diptera	<i>Muscidae</i>	<i>Musca</i> sp.	1.22	<i>0.66</i>	4	<i>4</i>
Diptera	<i>Calliphoridae</i>	<i>Calliphora stygia</i>	0.83	<i>0.41</i>	6	<i>2</i>
Coleoptera	<i>Scarabidae</i>	<i>Phyllotocus rufipennis</i>	0.65	<i>0.50</i>	372	<i>123</i>
Coleoptera	<i>Cantharidae</i>	<i>Chauliognathus lugubris</i>	0.03	<i>0.03</i>	176	<i>121</i>
Other			2.19	<i>0.68</i>	-	-
Total visits / hour			15.28	<i>2.12</i>	-	-



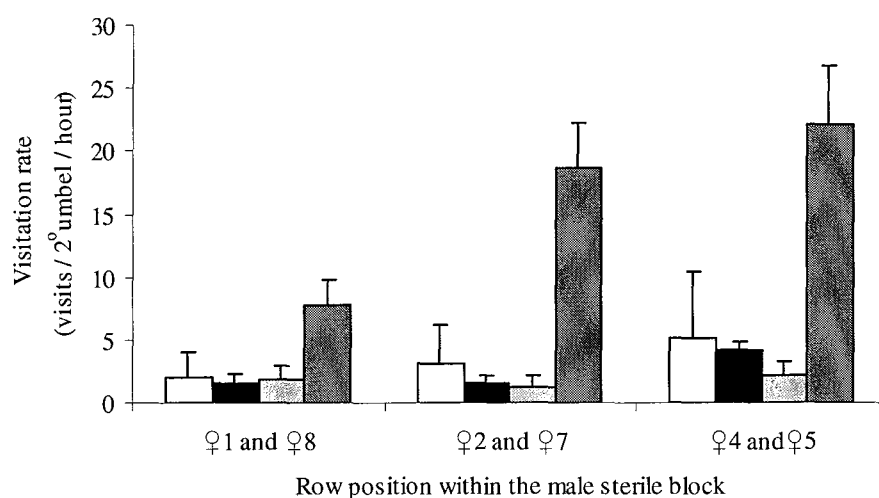


**Figure 7.5** – Visitation rates of a) all pollinators and b) *E. tenax* (□), *C. ruficornis* (■) and *A. mellifera* (▨) to plots with 8 row male sterile blocks at two-hourly intervals from 10am to 4pm. Each data point is the mean of four days of observations of the male fertile and male sterile lines weighted to give average visitation rates to the plot as a whole. Standard errors are indicated by the error bars (N=4).



**Figure 7.6** – Average pollinator visitation rates to male fertile (■) and male sterile (□) secondary umbels located in the central rows of the male fertile (♂2) and male sterile (♀4) beds (see Figure 7.2). Standard errors are indicated by the bars (N=4).

The same trend was evident for *C. ruficornis*, with averages of 2.8 and 7.8 visits/secondary umbel/hour and *E. tenax*, with 2.0 and 5.2 visits/secondary umbel/hour to the outside (rows 1 and 8) and central (rows 4 and 5) rows of the male sterile block respectively (Figure 7.7). In contrast, visitation rates of *A. mellifera* to the male sterile rows did not appear to be affected by row position



**Figure 7.7** – Average visitation rates of *E. tenax* (□), *C. ruficornis* (■), *A. mellifera* (▒) and the total population of pollinators (all species combined) (■) to secondary umbels in male sterile rows 1 and 8, 2 and 7 and 4 and 5, (0, 0.8 and 2.4 m from the edges of the male sterile block respectively -see Figure 7.2). Standard errors are indicated by the error bars (N=4).

For each of the three major species of pollinator foraging on the male fertile line, selfing (male fertile to male fertile) moves were far more common than crossing (male fertile to male sterile) moves, accounting for greater than 80% of all moves (Figure 7.8). This was mainly due to a large proportion of movements between different umbels within the same row of plants. *E. tenax* showed the greatest propensity for crossing to the male sterile line, with 16.6% of moves being crossing moves, compared to 8.5% and 6.6% for *A. mellifera* and *C. ruficornis* respectively.

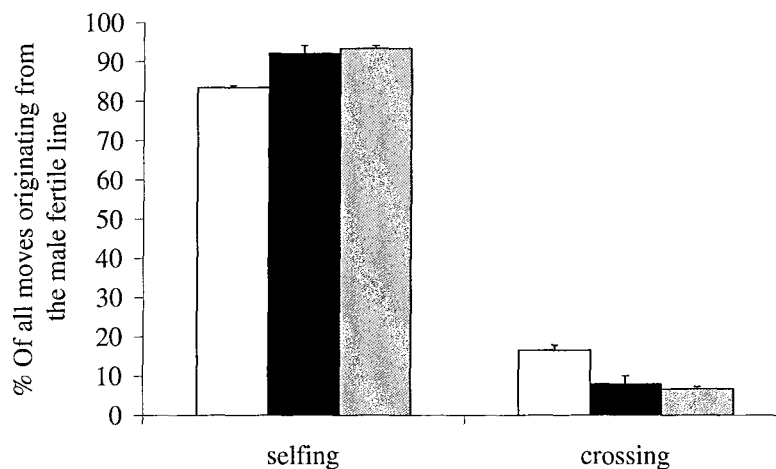
Two distinct foraging behaviours amongst individuals of *A. mellifera* were observed; nectar foraging and pollen foraging. Nectar foragers were characterised by comparatively slow movement from flower to flower across the umbel and probing of the stylopodium of the flowers for nectar. Pollen feeders did not probe the flowers. They were characterised by the presence of pollen in the corbiculae located on the hind legs, comparatively rapid movement from flower to flower across the umbel surface and vigorous use of the fore- and mid-legs to collect pollen into the corbiculae. During the 4 days of observations across the male fertile and male sterile blocks, 77% of individuals were foraging for nectar and 23% were foraging for pollen. Nectar feeders were apparent on umbels of both the male fertile and male sterile lines, whereas individuals that were, or had recently been, foraging pollen were almost exclusively observed on umbels of the male fertile line. Nectar feeders had a male fertile to male sterile crossing rate of 7.1% of moves originating from the male fertile line compared to 2.5% for pollen feeders. The crossing movements of pollen feeders to the male sterile line were usually followed by a rapid return to the male fertile line.

The possibility of discrimination between the hybrid parent lines in the foraging behaviour of the three predominant pollinator species was studied using the pooled movement data collected from the outer rows of the male sterile and male fertile (♀1 and ♀8 and ♂1 and ♂5 – see Figure 7.2) blocks. Under these conditions there was an equal opportunity (in terms of flight distance and direction) for movement between two adjacent rows of the opposite (crossing moves) or same parent lines (selfing moves). For *E. tenax* and *C. ruficornis* the probability of selfing or crossing movements

between rows was equal, indicating that they did not discriminate between the two parent lines during row crossing movements. Pollen feeding *A. mellifera* crossed almost exclusively between male fertile rows. Nectar feeders also showed evidence of discrimination between parent lines when moving between rows, with 69% selfing moves and 31% crossing moves.

Overall, the majority of male fertile to male sterile crossing moves made by each species were between adjacent rows of plants on the outside of the male fertile and male sterile beds, separated by 0.8m (Table 7.6). Whilst only 8.3% and 18% of crossing moves of *C. ruficornis* and *A. mellifera* originated from the inner rows of the male fertile block, 33.5% of crossing moves of *E. tenax* were from these rows.

In all observed instances, *A. mellifera* crossed from a male fertile to a male sterile plant via a move to the outside male sterile row adjacent to the male fertile block from which the move originated (♀1 and ♀8) (Table 7.6). In contrast, 22.3% and 20% of male fertile to male sterile crossing moves of *C. ruficornis* and *E. tenax* resulted in arrival into the male sterile block at the second row in from the edge of the block (♀2 and ♀7). Neither species was observed to venture further than two rows (1.6m) into the male sterile block on the initial crossing move from the male fertile line.



**Figure 7.8** – The percentages of selfing and crossing moves of *E. tenax* (□), *C. ruficornis* (■) and *A. mellifera* (▨) originating from the outside rows of the male fertile beds adjacent to the male sterile blocks. The data, collected over 4 days at the peak time of activity for each species, are based on observations of 10 randomly selected individuals of each species originating on the male fertile rows adjacent to the male sterile bed (denoted ♂1 and ♂5 Figure 7.2). Standard errors are indicated by the bars (N=4).

After crossing to the male sterile block, most individuals of the three major species of pollinator studied continued with moves to other male sterile plants. On average, 37, 15, and 12% of individuals of *E. tenax*, *C. ruficornis* and *A. mellifera* respectively followed the initial crossing move into the male sterile block with a move back to the male fertile block. When foraging within the male sterile beds, all three pollinator species displayed a bias towards remaining in a single row of plants compared to crossing between rows (Table 7.7). *E. tenax* and *A. mellifera*, with 31.8 and 35.5% of moves resulting in a change of rows respectively, moved more freely between male sterile rows than *C. ruficornis*, for which 8.4% of moves resulted in a change of rows.

**Table 7.6** – Origin and destination of crossing moves from the male fertile ( $\sigma$ ) to male sterile line ( $\varphi$ ) for *C. ruficornis*, *E. tenax* and *A. mellifera*. The row positions  $\sigma$ 1 and  $\varphi$ 1 are adjacent male fertile and male sterile rows with  $\sigma$ 2 to  $\sigma$ 4 and  $\varphi$ 2 to  $\varphi$ 3+ being the subsequent male fertile and male sterile rows moving into the respective blocks or away from the other parent line (see Figure 7.2). Standard errors of the means are indicated by the figures in italics (n=4).

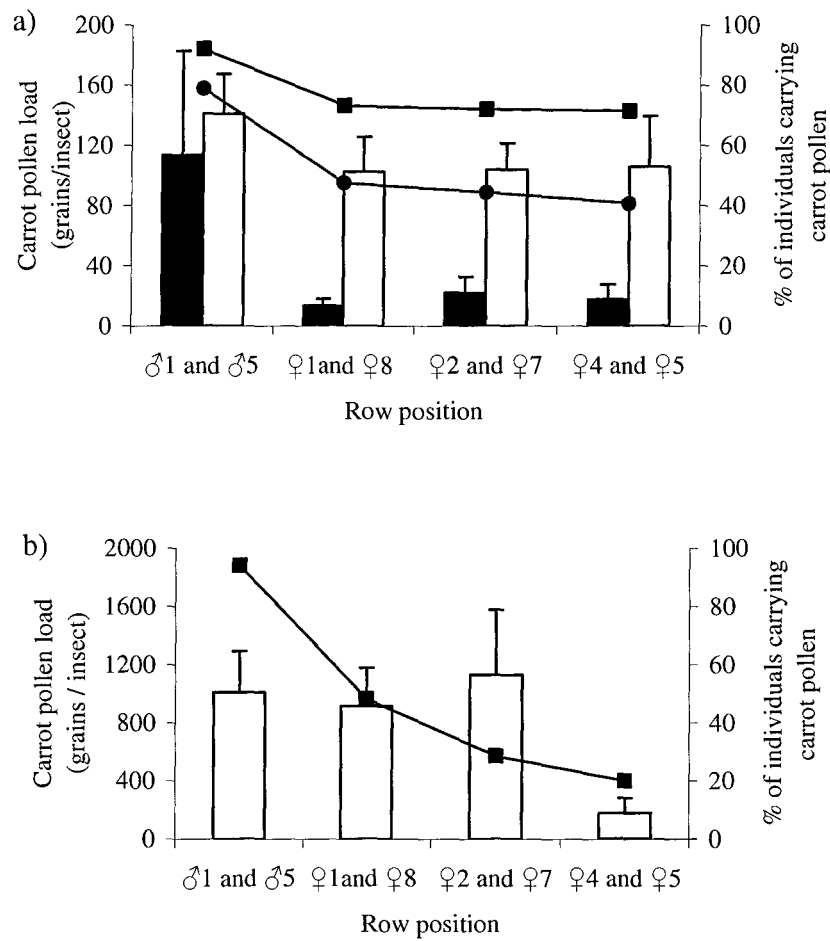
Origin of $\sigma$ to $\varphi$ move	% of all crossing moves ( $\sigma$ to $\varphi$ )					
	<i>E. tenax</i>	<i>C. ruficornis</i>	<i>A. mellifera</i>			
$\sigma$ 1 or $\sigma$ 5	66.5	<i>16.5</i>	91.7	8.3	82.0	<i>10.8</i>
$\sigma$ 2 or $\sigma$ 6	30.0	<i>13.0</i>	8.3	8.3	18.0	<i>10.8</i>
$\sigma$ 3 or $\sigma$ 7	3.5	<i>5.0</i>	0.0	<i>0.0</i>	0.0	<i>0.0</i>
$\sigma$ 4 or $\sigma$ 8	0.0	<i>0.0</i>	0.0	<i>0.0</i>	0.0	<i>0.0</i>
Destination of $\sigma$ to $\varphi$ move						
$\varphi$ 1 or $\varphi$ 8	80.0	<i>2.4</i>	77.7	22	100.0	<i>0.0</i>
$\varphi$ 2 or $\varphi$ 7	20.0	<i>2.4</i>	22.3	22	0.0	<i>0.0</i>
$\varphi$ 3 to $\varphi$ 6	0.0	<i>0.0</i>	0.0	<i>0.0</i>	0.0	<i>0.0</i>

**Table 7.7** – The distribution (as percentages) of distances travelled across the male sterile block in selfing movements of *C. ruficornis*, *E. tenax* and *A. mellifera*. A distance of 0m describes a movement between two umbels in the one row of male sterile plants, whilst 0.8, 1.6, 2.4 and 3.2+m refers to movements between male sterile plants 1, 2, 3 or 4+ rows apart. Standard errors of the means are indicated by the figures in italics (N=4).

Distance (m)	<i>E. tenax</i>	<i>C. ruficornis</i>	<i>A. mellifera</i>
0	66.5 <i>2.3</i>	91.6 <i>1.2</i>	68.2 <i>2.0</i>
0.8	29.2 <i>1.4</i>	4.9 <i>1.8</i>	20.4 <i>5.9</i>
1.6	2.6 <i>2.4</i>	3.5 <i>0.4</i>	9.1 <i>1.4</i>
2.4	1.7 <i>1.6</i>	0.0 <i>0.0</i>	0.5 <i>0.5</i>
3.2+	0.0 <i>0.0</i>	0.0 <i>0.0</i>	1.8 <i>1.6</i>

Of individuals of *C. ruficornis*, *E. tenax* and *A. mellifera* trapped on the male fertile line ( $\sigma$ 1 and  $\sigma$ 5), 78, 92 and 94% respectively were carrying carrot pollen (Figure

7.7). These figures declined sharply for individuals trapped on the adjacent male sterile rows 0.8m away (♀1 and ♀8) to 47.6, 73.3 and 48.3% respectively. Over the 2.4m distance from the outer to central rows within the male sterile block (♀1 to ♀4 and ♀8 to ♀5), the percentages of *C. ruficornis* and *E. tenax* carrying carrot pollen declined slightly from 47.6 to 40.7 % and 73.3 to 70.4% respectively (Figure 7.9a). Over the same distance, the percentage of *A. mellifera* carrying pollen declined from 48.3 to 20% (Figure 7.9b). The average carrot pollen load of individuals of *C. ruficornis* collected from the male fertile rows, 113 grains, was considerably higher than that of individuals collected from the male sterile rows, which ranged from 14 to 22 grains. In contrast, there was little difference in the average pollen loads of individuals of *E. tenax* collected across the male fertile and male sterile rows (103 to 141 grains). Similarly the average pollen loads of *A. mellifera* from the outer male fertile rows (♂1 and ♂5) and the first two rows on the edges of the male sterile block, (♀1, ♀8 and ♀2, ♀7), did not differ greatly (914 to 1129 grains). For *A. mellifera*, the average pollen load of individuals from the inner male sterile rows (♀4 and ♀5), was markedly lower at 170 grains.



**Figure 7.9** – a) The percentage of individuals of *E. tenax* (■) and *C. ruficornis* (●) carrying pollen and the average pollen loads per individual insect of *E. tenax* (white columns) and *C. ruficornis* (black columns) with respect to the row of plants in which they were captured; b) The percentage of nectar foraging individuals of *A. mellifera* (■) carrying pollen and their average pollen loads per insect (columns) with respect to the row of plants in which they were captured. Data points are the means of four days of sampling. Error bars indicate standard errors of the means (N=4).



## **7.4 - Discussion**

None of the parent line arrangements of the hybrid cross No. 22 trialed in these experiments altered the average seed yield per plant from the male sterile blocks despite the inadequacy of natural pollination levels, as demonstrated by the results of supplemental hand pollination. The reason for the failure of the row arrangement treatments to impact on yield was because they failed to alter the level of pollen transfer to the male sterile plants. Thus, a male fertile to male sterile row ratio of 1 to 4 was equally as effective as 1 to 2 or 1 to 1 in terms of average seed yield per plant within the male sterile blocks, and more effective than 1 to 1 in terms of seed yield per hectare of the parent lines.

Male sterile block widths of around 4m are typically used for commercial hybrid carrot seed production in south-eastern Australia. Wider parent line block widths are often desirable in commercial production for ease of male fertile line removal and harvesting operations. In this work there was no change in yields for male sterile block widths of up to 6.4m (8 rows). This finding is in contrast to previous reports for hybrid carrot seed production, which documented declining seed yields when the male sterile block widths exceeded 2m (Rodet *et al.*, 1992; Schittenhelm *et al.*, 1997). It is possible that under the conditions of the current study, male fertile to male sterile row ratios of less than 1:4 may have maintained yields, given that these were the most extreme treatments applied for each variable. The trend of slightly decreasing yields per male sterile plant with distance from the male fertile lines observed across the 6.4m bed indicates that this bed width may have been close to the maximum bed width for maintenance of yields.

As the results from these experiments discounted pollen levels within the hybrid cross and distance across the male sterile beds as reasons for inadequate pollination, it was hypothesised that the limitation of pollen transfer was linked to the vectors of pollination. Insects were shown to be the dominant vectors, a finding consistent with previous studies (Hawthorn *et al.*, 1955; Sinha and Chkrabarti, 1992; Abrol, 1997).

Even with the assumption that any windborne pollen was viable, the observed levels indicated that it would have been of little consequence to overall pollination. It has been suggested that significant pollen transfer occurs when adjacent plants rub against each other (Hawthorn *et al.*, 1955) but there was little evidence of this in the current work.

Only the 8 most abundant pollinator species were documented in this study but it was apparent that the diversity of pollinators was much less than the 334 species from 71 families in open pollinated crops in Utah (Bohart and Nye, 1960), or the diversity reported for the Apiaceae in general (Ritchie Bell, 1971; Koul *et al.*, 1993). Whilst differences in naturally occurring insect fauna between locations may partly explain this, other factors are likely to be involved. The season in which the current work was undertaken was unusually cool and wet, which may have reduced pollinator diversity. Based on visual observations over 3 seasons some pollinators, for example *C. lugubris* and *P. rufipennis* were certainly less evident in 2001 – 02 than in previous seasons. Differences in the attractiveness to pollinators of hybrid and open pollinated carrot crops (in which previous studies of pollinator diversity have been undertaken) are also possible, but have not been examined. Several studies have documented greater attraction of carrot lines with perfect flowers to honeybees than lines with male sterile flowers (Erickson *et al.*, 1979; Galuszka *et al.*, 1989). Finally, in the current study the survey area was sprayed with the synthetic pyrethroid alpha-cypermethrin 14 days prior to the first day of insect surveys to control an infestation of *N. vinitor*. It is likely that reduced the diversity and numbers of pollinators present during the survey.

The insects that appeared to perform most pollination, based on visitation rates and pollen loads, belonged to the Diptera, Hymenoptera and Coleoptera. Within the Diptera, certain Calliphorids, and to a lesser extent, Syrphids have been reported to be efficient pollinators of vegetable seed crops (Bohart and Nye, 1960; Currah and Ockendon, 1984; Sinha and Chkrabarti, 1992; Abrol, 1997; Schittenhelm *et al.*, 1997). In the current work, *Eristalis tenax* (Serphidae) appeared to be an effective pollinator

but of the 3 Calliphorid species visiting in significant numbers, only *C. ruficornis* appeared to carry sufficient carrot pollen to cause meaningful levels of pollination.

Honeybees (*A. mellifera*) are a preferred pollinator of carrot seed crops because of their ease of management and efficiency (Delaplane and Mayer, 2000). Of the pollinators surveyed, nectar-feeding *A. mellifera* carried the largest carrot pollen loads and, from visual observation, individuals of this species foraged more rapidly and thoroughly across a greater number of flowers than any of the other pollinators studied. Despite its efficiency, the effectiveness of *A. mellifera* was diminished by its apparent lack of attraction to the hybrid parent lines under study. Only a small proportion of workers from the nearby hives were observed to forage within the trial, with the majority flying over it to more distant competing nectar and pollen sources including brassica seed crops and brassica weeds, wild mignonette (*Reseda luteola*; Resedaceae), white clover (*Trifolium repens*; Fabaceae) and prickly box (*Bursaria spinosa*; Pittosporaceae). Carrots have previously been reported to be relatively unattractive to *A. mellifera* (Free, 1965; Delaplane and Mayer, 2000). With a recommended hive density of 6/ha ([Fulton, 1999; Manning, 2003), the average density of *A. mellifera* foraging within the trial plots ranged from 0.9 to 7.0 honeybees/m<sup>2</sup> compared to the recommendation for open pollinated carrots of 9.6/m<sup>2</sup> (Hawthorne *et al.*, 1960). Hive densities used for hybrid carrot seed production in the United States are much higher at 15 to 20 hives/ha (Rubatzky *et al.*, 1999). There may therefore be a case for increasing the stocking rate of *A. mellifera* into crops grown in Tasmania, although it is unclear if this would be effective, given the competition for pollinators from other nectar sources.

Discriminatory foraging between parent lines has been observed in a range of hybrid seed crops (Faulkner, 1978; Erickson and Peterson, 1979; Funari *et al.*, 1994; Singh *et al.*, 2000). Despite the obvious differences between the parental lines of No. 22 in terms of floral characteristics and height, no differences in the visitation rates to central rows of the male fertile and male sterile lines were observed for *C. ruficornis*, *E. tenax*, nectar feeding *A. mellifera* or the pollinator population as a whole. In contrast, the

small population of pollen feeding *A. mellifera* foraged almost exclusively on the male fertile line, which prevented them from effecting cross pollination.

Whilst there was no evidence of any bias in the foraging of *C. ruficornis* and *E. tenax*, nectar collecting *A. mellifera* demonstrated a preference towards individual constancy foraging, irrespective of whether foraging the male fertile or male sterile line. Individual constancy foraging has been shown to extend over multiple foraging flights (up to a few days) in this species (Pierre, 1995). Such behaviour may explain the large proportion of individuals foraging the male sterile line that carried no trace of carrot pollen. Because many nectar feeding honeybees inspected and then rejected flowers of the opposite line from close range without landing, it seems likely that floral rather than height differences were used as cues for distinguishing between the parent lines. Nectar abundance and quality, and the presence or absence of stamens and scent are important close range foraging cues for *A. mellifera* (Pernal and Currie, 2000). In contrast to the observations of Rodet and Torre Grossa (1991), the current work demonstrates that non-random, discriminatory foraging behaviour of *A. mellifera* between parent lines does occur in carrot hybrid crosses involving brown anther CMS. However, the severity of this discrimination appears to be less than that reported by Erickson *et al.* (1979) in hybrid crosses with petaloid CMS.

For all 3 species studied, the foraging pattern was dominated by a preference for foraging within a single row, irrespective of whether it contained male fertile or male sterile plants. This may have been caused either by directional foraging behaviour (*A. mellifera*) (Cresswell *et al.*, 1995), or more likely, by the close proximity of the plants to each other within rows compared to between rows (Delaplane and Mayer, 2000). Such behaviour, combined with the preference of all species for short distance crossing moves limited the opportunity for pollen transfer into the male sterile blocks.

A parabolic relationship was observed between seed yield and row position across the 8 row (6.4m) wide male sterile blocks but this was insufficient to cause significant reduction in the average seed yields per plant within these blocks compared to those

from narrower, 2 or 4 row male sterile blocks. The lack of significant reductions in average yield per plant from the wider blocks was consistent with the fact that there were no significant differences in pollination levels in male sterile plants located 1, 2 or 4 rows (0.8, 1.6 and 3.2m) from the nearest male fertile line. Previous studies of pollination in hybrid carrot found yield reductions when the male sterile plants were located more than 1 to 2m from the male sterile line (Rodet and Torre Grossa, 1991; Schittenhelm *et al.*, 1997). One explanation for this discrepancy is that the previous studies were based on cage trials conducted with single species of pollinators. In the current field study, a number of naturally occurring pollinator species plus *A. mellifera* were involved, with each having different foraging patterns and pollen carryover. *A. mellifera* visited all male sterile rows equally, but the percentage of pollen carriers declined sharply with distance from the male fertile line and their pollen loads decreased at distances beyond 1.6m from the male fertile line. Such patterns of pollen carryover have been widely reported for this species (Free, 1965; Rodet and Torre Grossa, 1991; Williams, 2001) and are responsible for the highly leptokurtic pattern of pollen deposition from the source plants in many bee pollinated crops. In contrast, the major Dipteran pollinators visited the central rows of the male sterile line more frequently than the outer rows, with similar percentages of pollen carriers and pollen loads across all rows. The reason for this visitation pattern was unclear but its effects were to counter the foraging pattern of the honeybees and to contribute to relatively even levels of pollen deposition across all 8 rows of the male sterile block.

In summary, the data presented in this chapter suggest that the strip method of hybrid carrot seed production used in Tasmania is incompatible with the foraging pattern of the major pollinator species for achieving optimal seed yields. Within this system two characteristics of the pollinator foraging patterns particularly limited pollen transfer. These were individual constancy foraging (*A. mellifera*) and a preference for foraging within individual rows rather than crossing between rows (*E. tenax*, *C. ruficornis* and *A. mellifera*). In addition, pollination may have been restricted by a limited diversity of pollinator species, competition from other crop, weed and native species for pollinators and inadequate stocking rates of *A. mellifera*.

On the basis of these yield-limiting factors, several practical avenues may exist to improve seed yields. Where possible, hybrid carrot crops should be isolated spatially or temporally from competing floral sources. Whilst spatial isolation is largely impractical in Tasmanian seed production areas, the potential for managing flowering in carrot crops to avoid competing floral sources has not been examined. There may be benefits from increasing the stocking rate of *A. mellifera* into hybrid crops, and from a staggered introduction of hives to take advantage of the tendency of this species to initially forage close to the hive when introduced to a new area (Free, 1970). Finally, the foraging patterns of pollinators observed in this work were based on a strong tendency to remain within a single row. There may therefore be advantages in arranging both lines into sections within the same rows, or alternatively, altering the inter- and intra-row spacings so that the distances between plants within and between rows were the same.

## Chapter 8

### General Discussion

*This chapter examines the outcomes of the research reported in this thesis. It commences with an overview of the project and a summary of the key areas of investigation followed by discussions of the research outcomes within the contexts of the previous knowledge within the areas studied, practical applications for commercial carrot seed production, and prospective avenues for further research.*

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#### ***Project Overview***

The world carrot seed industry is dominated by proprietary varieties owned by vegetable breeding companies. The industry is highly dynamic, with production contracted to areas around the world where technology and climate combine to give the most reliable yield of high quality seed. During the early to mid 1990s, a marked improvement in overseas production standards led to an increase in the minimum quality requirement for export carrot seed to 85% seed germination. Australian carrot

seed producers have experienced difficulty in consistently meeting this standard, which has placed their share of the market under threat. At the same time a continuing shift towards hybrid varieties within the vegetable industry has increased the challenge for

seed producers to achieve reliable yields and added to the issues of sustainability facing the Australian carrot seed industry.

This project was initiated in 1999 to address the production problems facing the Australian carrot seed industry. Two broad objectives emphasising practical outcomes were defined. The first was to identify the cause of the generally poor germination of carrot seed grown in Australia and, based on an understanding of the factors involved, develop management strategies to overcome the problem. The second was to further the understanding of factors determining seed yield with a view to improving seed production practices. Despite the bias towards hybrid seed production within the project, many of its outcomes, particularly in the area of poor seed germination, are equally applicable to production of open pollinated seed in Australia.

## ***Key Findings***

Preliminary trials demonstrated that low germination and variable yield in Australian hybrid carrot seed crops were largely unrelated issues, resulting in the two distinct research themes. The problem of low germination of seed produced in Australia was shown to be mainly due to the occurrence of seeds lacking embryos, or with embryos that had been damaged in such a way as to prevent germination. Embryoless seeds were previously documented in carrot and other Apiaceae produced in the United States, Europe and Africa (Flemion, 1949; Robinson, 1954; Dean *et al.*, 1989; Simon, 2000). At these locations, feeding of the tarnished plant bugs (*Lygus* sp.), on the developing seeds was shown to be responsible for the condition (Flemion, 1949; Robinson, 1954). The species of insects that were implicated do not occur in Australia. In the current research, feeding of an endemic insect, the Rutherglen bug, *Nysius vinitor*, was shown to cause embryoless seeds or seeds with damaged embryos in carrot. *N. vinitor* occurs in all areas of Australia in which carrot seed is produced (Evans, 1943), with the weight of evidence gathered in this project indicating that it is the major cause of low germination of Australian carrot seed. Although not previously documented as a pest of carrot, this



conclusion is consistent with published reports of obligate seed feeding behaviour of *N. vinitor* on a range of plant species (Kehat and Wyndham, 1972a; Attia, 1974) and its pest status in Australian oilseed crops (McDonald, 1977; Broadley *et al.*, 1986). The finding also confirms earlier industry suspicions of a negative effect of species of *Nysius* on carrot seed production.

The absence of *N. vinitor* in New Zealand explained the markedly higher germination percentage of carrot seed that had been produced there compared to Australia prior to this work. Low levels of embryoless seeds and seeds with embryo damage were observed in seed lines from trials near Winchmore, indicating that there are probably insects in New Zealand that cause similar damage. A closely related species to *N. vinitor*, *N. huttonii*, which is known to feed on seeds (Every *et al.*, 1992), occurs in areas of New Zealand where carrot seed is produced (Eyles and Ashlock, 1969). Given the reported similarities of *N. vinitor* and *N. huttonii* it is interesting that this insect is infrequently reported from carrot seed crops and appears to have only a minor, if in fact any, impact on carrot seed quality.

In preliminary trials 32 to 64% of female flowers of male sterile lines set seeds compared to open pollinated lines which set seeds on approximately 85% of flowers. Two alternative hypotheses were proposed to explain the limited seed set in the male sterile lines: that seed set was restricted by resource (assimilate) availability; or, alternatively, inadequate pollination. These hypotheses were tested on two Nantes hybrid varieties, No. 22 and WO030 because the male sterile lines of these represented the two commercial forms of cytoplasmic male sterility in carrot, and because these varieties seed set at the higher and lower end respectively of a range of male sterile lines grown in preliminary trials. Treatments applied to alter assimilate availability during fertilisation and seed development produced no evidence that seed yield was limited by source strength in either line. In contrast, supplemental hand pollination increased seed yields dramatically in No. 22, and to a lesser extent in WO030. In addition to low rates of pollen transfer, it was demonstrated that low pollen viability under field conditions (typically 20 – 50%) also contributed to low seed yield. Thus, within the varieties that were studied,

inadequate transfer of viable pollen to flowers of the male sterile line limited seed yields. Inadequate pollination has previously been inferred as a limiting factor for hybrid seed yield from studies of pollinator foraging patterns and comparisons of seed set in different male sterile lines in cage trials (Erickson and Peterson, 1978; Erickson and Peterson, 1979; Erickson *et al.*, 1979; Galuszka and Tegrek, 1987; Galuszka *et al.*, 1989; Rodet and Torre Grossa, 1991). However, this study provides the first unequivocal demonstration of pollination limitation of yield in hybrid carrot seed grown under field conditions. Pollen viability testing procedures and data on pollen viability have not been previously published for carrot. The similarity of yields of male sterile lines in southern Tasmanian, South Australian and New Zealand trials, plus the existing literature on pollination in hybrid carrots, indicate that yield limitation from low rates of transfer of viable pollen may be a widespread phenomenon in hybrid carrot seed production.

### ***Nysius vinitor in Relation to Carrot Seed Germination***

*N. vinitor* is a highly mobile pest with a polyphagous feeding habit that enables it to exploit niche habitats provided throughout the season by a number of annual spring and summer weeds and crops. During spring and early summer, *N. vinitor* migrates from over-wintering sites in central Australia to summer cropping regions in the south of mainland Australia as it follows a succession of maturing hosts (McDonald and Farrow, 1988). As a relatively late maturing seed crop, carrots act as a host for *N. vinitor*, during the summer period, when a limited range of alternative hosts are available.

The seasonal patterns of *N. vinitor* migration have been studied on the mainland of Australia (Kehat and Wyndham, 1973; McDonald, 1988; McDonald and Farrow, 1988), but little has previously been written about the insect's occurrence in Tasmania. At the sites studied in southern Tasmania, *N. vinitor* was first observed each season in late spring in the adult stage in carrot seed crops that were commencing flowering, and under weedy hosts, particularly capeweed (*Arctotheca calendula*). The first appearance of *N.*

*vinitor* each season coincided with the first migrations into South Australian carrot seed crops and periods of strong northerly winds favourable for southerly migrations. These observations suggest that the occurrence of *N. vinitor* populations in Tasmania each season may stem from springtime migrations from mainland Australia. In support of this theory, laboratory studies of the cardinal temperatures for survival of *N. vinitor*, (Kehat and Wyndham, 1972b; Kehat and Wyndham, 1974; Attia, 1982) indicate that the insect would probably be unable to survive most Tasmanian winters in any stage of development.

Adults of *N. vinitor* infested carrot seed crops in South Australia and Tasmania in a series of migratory flights over a period of up to approximately 100 days from 2 weeks prior to flowering until harvest. The timing of infestation of carrot seed crops each season appeared to be a function of the stage of development of the crop and several other variables such as the availability of alternative hosts, the timing of development of *N. vinitor* populations on other hosts and the occurrence of suitable conditions for migration.

Despite the long period of infestation relative to the generation time of *N. vinitor* in southern Australian summertime conditions (Kehat and Wyndham, 1972b), the nymphal stages were infrequently observed within carrot seed crops. When they were present, it was at relatively low levels and generally late in the season. Thus nymphal *N. vinitor* appear to make a minor contribution (if any) to the loss of germination of carrot seed. This observation appears consistent with an earlier study of suitable food sources for rearing *N. vinitor* (Kehat and Wyndham, 1972a), which found that a diet of carrot seed was unfavourable for nymphal development.

Following the initial detection of adults in late spring in Tasmania, areas of weedy hosts such as capeweed (*A. calendula*) and wireweed (*P. aviculare*) adjacent to carrot seed fields were used as sites for oviposition and, except for the capeweed plots studied in 2001 – 02 when conditions were unfavourable for survival of the eggs or nymphs, subsequently supported nymphal generations. During the course of the season dispersal of new adults from these populations was a significant source of additional migrants into

nearby carrot seed crops. This dispersal appeared to be highly localised at times, as evidenced in 2000-01, in 2 crops separated by approximately 6 kilometres. In the first, surrounded by areas of *A. calendula* and *P. aviculare* significant infestations of *N. vinitor* were observed, with the timing of the major infestation coinciding with the maturation of a nymphal population on the nearby areas of *P. aviculare*. In the second crop at tea Tree, which was isolated from areas of weedy hosts by grassland, no *N. vinitor* were detected throughout the entire season.

In caging experiments, adult *N. vinitor* reduced the germinability of developing carrot seeds at a rate of 0.04 to 0.11%/insect/plant/day, or up to 2 seeds/insect/day. Seed lines were shown to be susceptible to damage throughout the period from flowering until the mean seed moisture content dropped below 31%. Generally, in commercial crops the mean seed moisture content did not drop below this point until after windrowing. As a consequence of the rate at which damage occurred and length of the period of susceptibility, it was concluded that timely application of control measures for *N. vinitor* was critical to successful carrot seed production in Australia.

The limited information that has been published on the management of *N. vinitor* in field crops focuses on comparatively low value, high volume oilseed crops in which *N. vinitor* feeding appears to reduce the yield but, in general, not the marketability of the harvested product. In these crops control measures are generally reserved for times in which large numbers of insects are visually detected within the crop (McDonald, 1977; Broadley *et al.*, 1986). A more rigorous approach to *N. vinitor* monitoring and management was clearly required in carrot seed crops. The current work addressed this need through a study of the potential for a monitoring system for *N. vinitor* in carrot seed crops based on sticky traps. Sticky traps were chosen because alternative monitoring techniques including visual assessment and sweep netting, as used for *Lygus* bugs in carrot (Krivholavek, 1999), had proven unsuccessful. Sticky traps were also examined as a monitoring tool because of their ease of use, time efficiency and availability. The research that was undertaken demonstrated that sticky traps could be used to detect flights of *N. vinitor* into carrot seed fields and, in small plots, to reliably estimate the

average population density on individual plants. In commercial carrot seed fields it was shown that *N. vinitor* distributions were likely to be non-random, with the highest densities located on the windward edge of the field. Placement of sticky traps around the perimeter of carrot fields therefore appears to be adequate for detection of infestation events, but further work is required to establish the optimum placement of traps for population estimates over whole fields.

## ***Pollination and Pollen viability in Hybrid Carrot Seed Crops***

### **Pollination**

Most previous studies of pollination in hybrid carrot have been conducted on caged plots of plants with an individual pollinator species (usually honeybees) or at most several species of pollinator. Based on these studies, a number of possible causes for low seed yield in hybrid carrot have been identified including asynchronous flowering of the parent lines (Erickson and Peterson, 1979), lack of attraction of the parent lines to pollinators (Erickson and Peterson, 1978; Erickson *et al.*, 1979), discriminatory foraging between parent lines (Erickson *et al.*, 1979; Galuszka, *et al.*, 1989), and the proximity of male sterile rows to the male fertile rows (Rodet and Torre Grossa, 1991; Schittenhelm, *et al.*, 1997). In this project, inadequate rates of transfer of viable pollen to receptive flowers of the male fertile lines were demonstrated under open field conditions in supplemental hand pollination experiments. To explain these results, studies of pollination and pollen fertility were therefore also conducted in open field plots.

In both hybrids under study (No.22 and WO030) the parent lines flowered synchronously and, for No. 22, increasing or decreasing the ratio of male fertile to male sterile plants from the standard commercial ratio of 1:2 had no effect on pollination or seed yield. Therefore, whilst only 21 to 66% of male sterile flowers were naturally pollinated within in this study, there was no evidence that availability of pollen was a limiting factor.

Whilst there was a trend of decreasing seed yields from male sterile plants at increasing distances from the male fertile line, plants of No.22 located adjacent to the male fertile beds (0.8m away) failed to achieve their maximum potential yield as indicated by supplemental hand pollination. Thus factors other than proximity to the male fertile line limited seed yield.

In previous studies of carrot, it has been assumed that the stigmas become receptive when the two styles separate and may remain receptive for a week or longer (Erickson and Peterson, 1979). Under the field conditions encountered in this work, carrot flowers were highly receptive to pollination for 1 to 2 days after the styles had separated and were fully extended. Flowering of individual umbels lasted 7 to 10 days, and the flowering period of the first three umbel orders lasted up to 50 days. Thus, although the duration of receptivity was not as long as previously thought, seed set did not appear to be restricted by a narrow window of time for effective pollination of individual flowers, umbels or the plant as a whole.

The weight of evidence from previous studies indicates that carrots are predominantly insect pollinated (Bohart and Nye 1960; Ritchie Bell, 1971; Goyal, 1989; Sinha and Chkrabarti, 1992; Koul *et al.*, 1993; Abrol, 1997) but it has also been suggested that wind pollination may make a significant contribution to seed yield (Sinha and Chkrabarti, 1992). In the current study the flux of windborne carrot pollen in flowering carrot seed plots was less than 4 grains/cm<sup>2</sup>/day at the canopy level, 0.8m downwind from the male fertile plants. Even with the assumption that the windborne pollen was viable, the very low levels observed would have had little effect on seed yield. Furthermore, there were only minor yield increases in rows of male sterile plants adjacent to the male fertile lines, where it has been hypothesised that wind could cause mechanical transfer of pollen by the rubbing together of flowers (Hawthorne *et al.*, 1960). In the current study, insects were therefore clearly responsible for pollination.

Members of the Apiaceae are characterised by unspecialised flowers that are adapted to pollination by a range of insect species (Ritchie Bell, 1971; Koul *et al.*, 1993). In

previous studies up to 334 species of insects have been reported to visit carrot flowers (Bohart and Nye, 1960; Goyal *et al.*, 1989; Sinha and Chkabarti, 1992). By comparison, the diversity of insect visitors observed in this study was low, with only 8 species visiting in significant numbers. Factors such as the seasonal conditions in which the current survey was undertaken and the attractiveness of the parent lines may have been involved, but the use of pesticides during the flowering period for management of Rutherglen bug was also a probable cause of reduced pollinator diversity. Apart from a short term repellent effect the synthetic pyrethroid chemicals that are currently used are safe for honeybees but are toxic to many naturally occurring insect species (Kakar, 1994; Manning, 2003).

Within the Apiaceae, it has been suggested that, on the basis of the umbel structure and the exposed position of the anthers and stigmas within the flowers, published lists of insect visitors during flowering give a fair indication of the number and diversity of actual pollinators (Ritchie Bell, 1971). In the current work, it was evident that some insect visitors were very inefficient pollinators in terms of the load of pollen they carried and their foraging behaviour. Thus, in hybrid carrot crops at least, the number and diversity of insects visiting at flowering was not necessarily a good indicator of the size or diversity of the effective pollinator population. Furthermore, these observations and the relatively small diversity of visiting insects indicate that in the current study, a small number of insect species were responsible for most pollination.

Of particular interest were the low pollen loads observed on Calliphorid and Muscid flies (blowflies and houseflies), given their use as pollinators in carrot breeding (Ponses, 2000) and the inferences from caging trials and surveys of insect visitation to field crops that they are important or desirable pollinators in hybrid seed crops (Faulkner, 1978; Currah and Ockendon, 1984; Wilson *et al.*, 1991; Sinha and Chkrabarti, 1992). Of the 4 species studied (*Musca domestica*, *Calliphora ruficonis*, *C. stygia* and an unidentified species of *Calliphora*) only *C. ruficornis* (26 pollen grains/insect) carried sufficient carrot pollen to cause meaningful pollination, but compared to the other major pollinators, honeybees (406 pollen grains/insect) and hoverflies (*Eristalis. tenax*) (89 pollen grains/insect) its

loads of carrot pollen were low. The fact that Calliphorids and other blowflies do not discriminate between pollen and nectar sources in the same way that honeybees do is often cited as an advantage in achieving cross-pollination between hybrid parent lines (Faulkner, 1978; Currah and Ockendon, 1984; Ponses, 2000). Despite this difference in foraging behaviour, in the current study, the crossing rate of *C. ruficornis* from the male fertile to male sterile lines was no higher than the crossing rate of honeybees. One possible reason for the discrepancy between the findings of caging trials and the current field study may be the density of insects present. Under caging trials introduction of very high levels of Calliphorids may ensure satisfactory pollination through the weight of numbers of insects present.

The importance of honeybees for pollination of carrot seed crops has been recognised in several studies (Hawthorne *et al.*, 1955; Erickson *et al.*, 1979; Singh, 1983 cited in Sinha and Chkarbarti, 1992; Rodet and Torre Grossa, 1991) and by commercial seed producers who generally place honeybee beehives in or near their carrot seed crops. A density of 9.6 foraging honeybees/m<sup>2</sup> was recommended for optimal pollination of open pollinated crops by Hawthorne *et al.* (1955). In the current study, honeybee densities in flowering hybrid carrot seed plots ranged from 0.9 to 7.0 individuals/m<sup>2</sup>. Whilst stocking rates for honeybees in Australian crops appear to be lower than in crops grown in the United States (Fulton, 1999; Rubatzky *et al.*, 1999), this does not appear to be the core problem. Carrot flowers have previously been reported to be unattractive to honeybees when other forage sources are available (Bohart and Nye, 1960; Free, 1970; Delaplane and Mayer, 2000). In the current study, most worker bees from the hives adjacent to carrot seed plots or fields foraged other plants including brassica seed crops and brassica weeds, wild mignonette (*Reseda luteola*), white clover (*Trifolium repens*) and prickly box (*Bursaria spinosa*) in preference to carrots. Most suitable sites for carrot seed production in Tasmania are also utilised for other seed or fruit crops and have significant populations of summer flowering native flora and weedy species. Whilst there may be a case for increasing the stocking rate of honeybees in crops, management of the effects of competing nectar sources on pollination of carrots is likely to be a significant issue for Tasmanian producers.



In field trials both parent lines of No. 22 received similar levels of visitation from pollinator species. There was no evidence of discrimination between parent lines in the foraging behaviour of the Dipteran species that were studied (*E. tenax* and *C. ruficornis*), but honeybees showed varying degrees of discrimination according to their source of forage. Pollen foragers displayed a strong fidelity to the male fertile line whilst nectar foragers were slightly biased towards foraging constancy on either parent line. Discrimination between parent lines was not reported in a previous study of honeybee foraging in hybrid carrot crosses using brown anther CMS (Rodet and Torre Grossa, 1991), but the current observations indicate that it does occur, although probably with less severity than reported in certain hybrid crosses involving petaloid CMS ([Erickson and Peterson, 1979). Lower levels of discrimination against brown anther lines compared with petaloid lines would be consistent with the floral morphology of the two forms of sterility; brown anther flowers bear closer resemblance to perfect carrot flowers in both form and colour. In addition, the nectariferous tissue of carrot flowers appears to be linked developmentally to the anthers (Erickson *et al.*, 1982). Brown anther flowers, in which the anthers commence development, may therefore produce a greater volume of nectar and compete more strongly with male fertile lines for attention from pollinators compared to petaloid lines in which there is no anther development.

In addition to the effects of discriminatory foraging, cross-pollination was strongly limited by the preference of all of the pollinator species studied for foraging within a single row of plants, instead of crossing between rows. Some pollinator species, including honeybees, are thought to employ this type of foraging pattern to ensure that effort is not wasted revisiting recently foraged flowers (Cresswell *et al.*, 1995). For honeybees this behaviour may be enhanced by a tendency to fly shorter distances between flowers after encountering a rewarding flower, presumably to increase the likelihood of another, similar encounter (Pyke, 1978; Waddington, 1980 cited in Delaplane and Mayer, 2000). On the basis of these observed foraging behaviours, it is likely that the arrangement of the parent lines in commercial production into separate rows reduces the probability of cross pollination. Furthermore, this negative effect may

be increased where the intra-row spacing between plants is less than the inter-row spacing, as it is in commercial carrot seed crops.

### **Pollen Viability**

Although previous studies of low yield in hybrid carrot seed crops have focussed on aspects of pollination and pollinator behaviour, data on carrot pollen viability do not appear to have been published. In the current work, the FCR test (Heslop Harrison and Heslop Harrison, 1970) was shown to reliably estimate the capacity of pollen to germinate *in-vivo* under favourable (glasshouse) conditions. For pollen samples collected from the field at 1pm, viability ranged from 0 to 57.1% for No. 22 and 0 to 67.3% for WO030 on different sampling days during 2000 – 01 and 2001 – 02. Compared to the reported levels of pollen viability in some other field crops such as onion (Chang and Struckmeyer, 1975), tomato (Abdul-Baki, 1992) and brassicas (Hodgkin and Lyon, 1985; Jandurova and Pavlik, 1995) pollen viability in the male fertile lines studied in this work was generally low.

Daily variability in pollen viability at 1pm in the field was related to the mean relative humidity conditions over the period from 10am (the approximate time of commencement of anther dehiscence each day) to 1pm on each day of sampling. This relationship was presumably due to an effect on post anthesis pollen longevity. Although temperature alone (11 to 25°C) did not affect pollen viability in the field, there was a small interaction between temperature and relative humidity with respect to pollen viability.

Under laboratory conditions, carrot pollen was short lived. Freshly dehisced pollen samples from the male fertile line of WO030 had half lives ranging from 19 minutes to 4 hours across a range of temperatures and relative humidities typical of field conditions in southern Tasmania. Longevity of pollen of WO030 was particularly sensitive to increases in relative humidity above 55%, especially when combined with high temperatures (30°C). Over the range of 10 to 30°C, temperature alone had little effect on pollen

longevity but at 40°C pollen longevity decreased. Similar responses to temperature and humidity have been demonstrated in other binucleate and trinucleate pollens (Chang and Struckmeyer, 1975; Hoekstra and Bruinsma, 1975a; Hoekstra and Bruinsma, 1975b), but the half-life of the carrot pollen studied in this work, especially under environmental stress, was particularly short. For example, half-lives of between 3 hours and several days were reported for several other trinucleate species held at 30°C and 97% or 60% relative humidity respectively (Hoekstra and Bruinsma, 1975a) compared to 1.2 hours for pollen of WO030 held at 30°C and 70% relative humidity.

Post anthesis longevity was clearly a factor in the incidence of low viability carrot pollen in this study, but other factors were also involved. After the effects of temperature and humidity conditions on longevity had been accounted for there was still significant variability in daily pollen viability. This variability may have been caused by the effects of environmental conditions on pre-anthesis pollen viability and environmental factors other than temperature and humidity on post-anthesis longevity. Amongst the environmental factors that could vary in the field on a daily basis, low and high temperatures (Chang and Struckmeyer, 1976b; Subedi *et al.*, 1998), light intensity (Yoshida and Tanimoto, 1999) and water stress (Rawson and Subedi, 1996; Sheoran and Saini, 1996) have been shown to affect pollen viability. Further work is required to establish the effects of these factors on the viability of carrot pollen.

In addition to the clear environmental effects, there was evidence of a genetic effect on pollen viability. Pollen viability was consistently higher in WO030 than No. 22 throughout the study (for example 75% compared to 39% in glasshouse conditions) and there was significant inter-plant variation in pollen viability within the two male fertile lines studied. The basis of genetic limitations of pollen viability in carrot is unclear, but it seems probable that inbreeding depression may play a role, particularly where there are varietal differences between male fertile lines. Strong inbreeding depression occurs for a range of agronomic traits in carrot (ISSI, 2002), so it is probable that it would also restrict pollen viability in the same way that it does in other plants (Aslam *et al.*, 1990; Carr and Dudash, 1997).

In many species, loss of function of the vegetative cell membrane appears to be the principal determinant of pollen viability (Heslop Harrison and Heslop Harrison, 1970; Shivanna and Heslop Harrison, 1981; Abdul Baki and Stommel, 1995). The close relationship between *in-vivo* germination and the results of FCR testing, suggests that this also applies to carrot pollen. Negative FCR test results may also indicate impaired functioning of esterase enzymes, but the high levels of fluorescence in the staining solution surrounding non-viable carrot pollen indicate functional esterase enzymes and membrane leakage. Although numerous studies have examined the effects of humidity on pollen longevity, the basis of loss of membrane function under continuous high humidity remains unclear. Correlations between respiratory activity, ATP turnover, relative humidity and longevity have been made (Hoekstra and Bruinsma, 1975; Hoekstra and Bruinsma, 1979). On the basis of this evidence one possible explanation is that rehydration of the pollen grain under high humidity causes re-activation of metabolic activity and a consequent depletion of energy reserves, leading to a loss of ability to maintain normal cell function (Hoekstra and Bruinsma, 1975; Shivanna and Ram, 1993). Such a mechanism may explain the comparatively low longevity of tri-nucleate pollens compared to bi-nucleate pollens. Given that formation of the two sperm cells in tri-nucleate pollens prior to anthesis appears to place a large burden on pollen energy reserves and cause a requirement for exogenous sources of some metabolites for germination (Frankel and Galun, 1977; Mulchay and Mulchay, 1988), longevity of trinucleate pollen may be particularly vulnerable to a resumption of respiratory activity. A link between respiratory activity and pollen longevity would be consistent with the reduction in longevity in carrot pollen with increasing temperatures (10 to 30°C) under high humidity conditions (75% relative humidity) observed in this study.

A probability based prediction of seed set derived from estimates of pollination rates and pollen viability overestimated the seed set of field grown plants by 0 to 100% in this study. One possible explanation for the discrepancy between some predicted and actual seed sets may be that there is a difference between the capacity of pollen to germinate under field conditions and the estimates of pollen viability obtained from FCR testing. As

in any histochemical test of pollen viability, FCR testing establishes the possibility, but not the certainty of pollen function. A test of pollen vigour, for example, viability after exposure to stress conditions may contribute to a more accurate prediction of seed set. In addition, it must be recognised that pollen viability testing establishes the potential for germination, but not necessarily for pollen tube growth in the style or successful fertilisation. Seed set may also be limited by factors during these processes or through abortion of young seed. From the data that were collected it is evident that any post-pollination restriction on seed set occurs in the first 10 days after pollination, as after this point virtually all seeds survived.

### ***Recommended Management Practices for Improved Seed Germination and Yields from Australian Carrot Seed Crops***

The thesis was initiated in an attempt to improve carrot seed production in Australia. The results highlight several opportunities to achieve this objective. In particular, evidence is presented that shows that effective management of Rutherglen bug (*N. vinitor*) is critical for production of seed lines with a high germination percentage. The following guidelines for management of *N. vinitor* in carrot seed crops are tendered on the basis of the information that was collected within this project.

1. The migration of Rutherglen bugs into carrot seed crops during flowering and seed development must be monitored and control strategies implemented in response to infestation. Monitoring and control strategies should be in place from just before flowering (by mid November for European hybrid varieties produced in South Australia and Tasmania) until the crop is harvested.
2. Feeding of *N. vinitor* during the period from flowering until at least windrowing can rapidly reduce seed germination. Control strategies must therefore be applied in rapid response to infestation events. To facilitate the detection of *N. vinitor*

flights into carrot seed crops, sticky traps should be placed around the periphery of the crop at canopy height. These should be checked on a regular basis (twice weekly at a minimum) from 2 to 3 weeks prior to flowering until harvest. Particular emphasis should be given to the sides of fields facing into the prevailing winds.

3. Weedy host species such as capeweed (*A. calendula*) and wireweed (*P. aviculare*) in close proximity to carrot seed fields should be destroyed if possible, or regularly checked during their periods of flowering and seeding in spring and summer for the presence of *N. vinitor* populations. Where possible control measures should be applied directly to the developing populations when detected. If this is not possible, knowledge of the stage of development of nymphal populations on these hosts can be used as forewarning of a probable infestation of carrot seed fields when the nymphs mature to the adult stage.

Application of these guidelines has contributed to a marked improvement in the germination of the industry partner's carrot seed crops in Australia. In 2002 – 03, despite considerable Rutherglen bug pressure, all of their export carrot seed crop (approximately 300Ha) met or exceeded the required germination standard.

A number of factors were identified that combined to limit the hybrid seed yields in the varieties studied in this work. At this stage, further research is required to confirm that these problems are common to a range of varieties and to determine ways of addressing them. On the basis of the current understanding of seed yield limitations, some tentative recommendations can be made:

1. Pollinator populations should be maximised. This may involve greater stocking rates for honeybees, encouragement of native, or introduction of alternative pollinator populations into carrot seed crops, choice of production sites that offer few competing nectar sources, and careful balancing of the application of pesticides during flowering against the potential loss of native pollinators.

2. Carrot pollen longevity appears to be a limiting factor of hybrid seed yields. Production sites and management practices should be chosen to minimise relative humidity within the canopy during the flowering period. In particular, irrigation should be timed to minimise humidity within the crop during the daily period of pollen release (typically from 10am to 3pm).
3. There appears to be a significant genetic component to the occurrence of low pollen viability in carrot seed as evidenced by the inter- and intra-varietal differences observed in this study. Although some selection for pollen viability may occur where the outcrossing abilities of the male fertile lines are screened, seed production may be improved through a more direct screening of pollen viability within breeding programs. Of the currently available pollen viability tests, the FCR test appears to be most appropriate for use with carrot pollen.

### ***Recommendations for Further Research***

From an applied perspective, management of Rutherglen bug based on the guidelines developed in this research appears to be generally satisfactory for successful commercial production of carrot seed in Australia. There are, however, some aspects of Rutherglen bug management that would benefit from further research. Firstly, the current strategy of using pesticides just before or during the flowering period in response to infestation probably has a detrimental affect on natural pollinator populations. Alternative management strategies that delay the need to apply chemicals until after flowering, such as the use of trap crops, warrant further investigation. Although the current management practices provide an acceptable strategy for mainstream carrot seed production, they do not solve the issue of Rutherglen bug management in organic seed production. With the current trend of increasing demand for organic vegetable seed this area may warrant further investigation.

With improved management of Rutherglen bug there are now opportunities to address other comparatively minor germination problems in carrot seed produced in Australia. One such problem is the occurrence of relatively high levels of seeds with rudimentary embryos, which, on the basis of data collected in this thesis is likely to be a periodic cause of crop failure in both Australia and New Zealand. Rudimentary embryos have been reported from most other carrot producing regions around the world (Robinson, 1954; Dean *et al.*, 1989; Simon, 2000), but the reasons for their occurrence are unclear. It is interesting to note that during this study, rudimentary embryos were most common in seed lots from sites that had experienced cool and wet seasons, suggesting that the problem is linked to seed development rate and possibly maturity at harvest. As discussed in chapter 3, the results of some other studies of the effect of time of harvest (Gray and Steckel, 1982) and umbel order difference (Borthwick, 1931a; Gray, 1979; Jacobsohn and Globerson, 1980) could be interpreted to indicate a similar link, but there are conflicting studies (Gray, 1979; Sandin, 1980).

Unlike the issue of low germination of Australian carrot seed, which was largely due to a single factor, yield variability appears to be caused by a complex of factors that manifest through low pollen viability, inadequate pollination rates and, possibly post pollination limitation of seed set. The work in this study on yield limitation was restricted to two Nantes type hybrid varieties. Although it appears probable that low pollination rates and variable pollen viability affect the yields of other hybrid varieties, this should be confirmed. Within the issue of low pollen transfer there are many potential avenues for yield improvement including (amongst others) enhancement of pollinator populations in carrots, manipulation of flowering time to avoid competition from other nectar sources and the use of parent line arrangements that are more compatible with the foraging patterns of the predominant pollinator species. Such avenues for yield improvement should be investigated with guidance from the wide body of existing scientific literature on management of pollination in field crops.



The potential for management of the male fertile carrot lines for improved pollen viability and longevity in the field is currently unclear but warrants future investigation.

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