Improving yield in hybrid carrot seed crops

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Dr Cameron Spurr and Dr Phil Brown were project leaders. Ms Angela Geard, a PhD candidate at the University of Tasmania, undertook most of the studies described in the report and completed a thesis incorporating aspects of the project.

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Media Summary

Production of hybrid carrot seed is increasing in Southern Australia and New Zealand, and demand for hybrid seed is expected to increase in the future. Lack of consistency in yield between crops, and consistently low yields in crosses between certain parent lines, are challenges currently faced by the industry. In order to capitalise on future opportunities for expansion in competitive export markets, it is critical that Australasian growers are able to consistently produce economically viable yields of high quality seeds.

Low pollen viability was shown to be a limiting factor to achieving high seed yields. Trace element nutrition did not affect pollen viability and is unlikely to be a major contributor to poor seed yields in commercial production. Parent lines vary greatly in pollen viability at flowering, and screening of parent lines for pollen viability is recommended as crop management practices such as male to female line planting ratio's may be altered to reduce the impact of low pollen viability in poor lines. Exposure of pollen to high humidity causes rapid loss of viability, with some parent lines being more susceptible than others. For susceptible lines, site selection and management of crop flowering time through planting date selection and trimming treatments may be used to reduce the risk of flowering occurring during humid weather. Differences in the ability of the male sterile parent lines to retain pollen during pollination, and to support pollen germination, explain the low seed yields noted from some commercial crosses. The project results highlight the need for careful crop management to maximise percentage pollen viability and pollen transfer in the lower yielding parent lines to ensure sufficient viable pollen is deposited on the stigma within the narrow window of stigma receptivity.

Technical Summary

In this project, which was initiated by two major hybrid vegetable seed production companies in Australia and New Zealand, the factors affecting pollen viability before and after anthesis, and pollen pistil interactions after pollination, were investigated to identify key processes limiting seed yields. Pollen viability was found to vary highly on a daily basis, and to vary significantly between cultivars on any given day. Pollen viabilities between 28 and 48% at anthesis were observed in a trial screening 8 commercial pollinator lines over a period of six days. Pollen viability varied up to 13% in one given line over the six day sample period and the variation between lines was up 18% in any single sampling time.

As crop nutrition and canopy structure have been shown in other species to influence pollen viability, a number of agronomic practices including trace element fertilizer applications and treatments to reallocate carbohydrates within the plant were trialled. None of the treatments increased pollen viability at the time of anthesis, or subsequent seed set. It was therefore concluded that low pollen viability in hybrid carrot parent lines was not caused by deficiencies in boron, manganese, zinc or copper, or by the patterns of carbohydrate allocation within the plants.

Changes in pollen viability prior to and post anthesis were examined to determine if longevity of pollen contributed to low levels of seed set. Pollen viability was found to be up to 80% in anthers pre-anthesis but significantly decreased by anthesis and continued to decline after anthesis. Pollen viability at 10-11am, when pollinating insects would begin to work the commercial crops, was significantly lower than that at anthesis. The effects of ambient humidity both pre- and post anthesis on the percentage of viable grains was significant in all lines studied. Across the 8 lines screened, the effect of ambient humidity on the pollen viability at the time of collection was not uniform. In a study screening two lines for pollen viability hourly from pre-anthesis at 7am until 3pm, the effects of ambient humidity in the 24 hours prior to anthesis were also shown to vary between lines.

In south eastern Australia and particular southern Tasmania, the traditional time of flowering for most varieties falls in mid-late December coinciding with a high risk period for humid easterly weather patterns. This project confirms the need for careful management of flowering time, particularly in highly sensitive varieties to avoid these weather conditions. In addition, carrot seed production often involves overhead irrigation which can influence humidity around the flower heads. The effects of overhead irrigation as compared to dripper irrigation were investigated in one line, and overhead irrigation was shown to reduce the initial pollen viability by up to 8%; however when irrigation ceased at 6.30am it had no effect on the rate of decrease in pollen viability from 8am onward. This identifies the need for careful management of irrigation throughout the flowering period to avoid creating a high humidity environment around dehiscing flowers, ensuring maximum percentage pollen viability at anthesis for the given environmental conditions.

Industry observation and some preliminary research have also suggested that in some highly inbred male sterile lines there is a breakdown in the processes that occur between pollen deposition and early seed-set. In hand-pollination studies comparing one of these lines to a reliable yielding second cross male sterile line, the average number of pollen grains present on the stigma of the low yielding line was significantly less than the higher yielding line, and the total percentage pollen germination was also found to be significantly lower on stigmas of the lower yielding male sterile line. Stigmas of the reliable male sterile line supported a 60% germination of viable pollen grains for up to 3 days, while the lower yielding line supported germination of less than 60% viable grains and only supported more than 50% germination of viable grains for 12 hours or less. No structural differences were observed between stigmas of the two lines to account for this pollen germination response, so it was concluded that a chemical mechanism must be present. The project results highlight the need for careful crop management to maximise percentage pollen viability and pollen transfer in the lower yielding parent lines to ensure sufficient viable pollen is deposited on the stigma within the narrow window of stigma receptivity.

Technical Report

Introduction

Preliminary research undertaken by the Tasmanian Institute of Agricultural Research showed that in commercial conditions under the cool climate production environment in southern Australia, hybrid carrot seed crops generally achieve less than 70% of their full yield potential. The reasons for this difference between potential and actual yield are not known, but are likely to involve the pollination and fertilization processes at flowering. Very little research has focused on pollination, pollen-pistil interaction and their effects on carrot seed yield.

Under Australian production conditions hybrid carrot seed yields can be limited by low rates of transfer of viable pollen to the male sterile line during pollination (Spurr, 2003). Whilst environmental impacts on pollen longevity (post anthesis) have been demonstrated, pollen viability at the time of anthesis is also frequently poor. The reasons for this have not been studied.

Industry observation and some preliminary research have also suggested that in some highly inbred male sterile lines there is a breakdown in the processes that occur between pollen deposition and early seed-set. Lines have been identified that only set between 20-30% of the maximum potential seed set and that this seed set is only marginally increased by hand-pollination. This point of break-down is yet to be identified.

This project was initiated by two major stakeholders in the Australian and New Zealand carrot seed industries, Midland Seed Ltd and Bejo Seeds Pty Ltd, to address the deficiencies in knowledge associated with the seed formation processes in hybrid carrot seed crops. The objective was to contribute to an understanding of environmental and management practices affecting pollen viability and longevity, with a view to applying the knowledge through improved management for more reliable seed yields.

Section 1: Review of the literature

Taxonomy and Origins

The cultivated carrot, *Daucus carota* L. subsp. *Sativus* (Hoffm), is a biennial plant grown for the edible taproot produced during its first year of growth. It belongs to the family Apiaceae (formerly Umbelliferae), which includes other economically important species such as anise, caraway, celery, celeriac, coriander, dill, fennel, parsnip and parsley (Rubatzky et. al., 1999).

The cultivated carrot was domesticated from wild carrot (Daucus carota L.), commonly known as Queens Anne's Lace, and two origins are recognised: eastern Asiatic and western types. Eastern Asiatic types, originating in Afghanistan (Shinohara, 1984), are characterised by purple or yellow roots, pubescent grey green leaves and a tendency for early flowering. Western carrots have orange, yellow, red or white roots, less pubescent, green leaves and a vernalisation requirement for flowering (Rubatzky et. al., 1999).

Whilst wild carrots have a long history of presumed medicinal uses dating back as far as the 10th century (Rubatzky et al., 1999), carrots are primarily cultivated for consumption today. Early attempts to improve carrot root shape and colour characteristics occurred in Holland, where the present day western orange carotene carrot appeared in the 16th century. By the end of the 16th century, selective breeding of carrot had produced a crop that showed extreme variation in size, colour and shape. Today, carrots are grown worldwide and the crop retains much of the variation in characteristics introduced by the early breeding activities. Carrot cultivars are classified according to their root shape (length and width), maturity period, root tip and foliage (George, 1999). Temperate types include Nantes, Imperator, Danvers, and Chantenay, while sub-tropical types include Kuroda, Brasilia and Tropical Nantes (Rubatzky et. al., 1999). Genetic efforts to improve the carrot plant have been directed primarily toward improved root production, shape and quality. With the exception of the adoption of hybrid systems, the reproductive development of the domesticated carrot remains much the same as that of its undomesticated relatives (Braak and Kho, 1958; Steiner et al, 1990).

The modern carotene carrot is the most prolific of the umbelliferae cultivated worldwide. It was estimated that 23.6 million tonnes of carrot roots were produced from 1.1 million hectares of land worldwide in 2004 (FAO, 2005). In contrast to the three major food crops; wheat, rice and maize, carrots are not a major food crop. They are, however, second only to potatoes (328 million tonnes produced of 19.1 million hectares in 2004 (FAO, 2005) as the most popular vegetable consumed in the world (World Carrot Museum, 2005).

Carrots are primarily consumed as a fresh product however they can be prepared in a number of ways including baked, boiled, steamed or diced. Nutritionally, they are an excellent source of vitamin A and potassium; they also contain vitamin C, vitamin B6, thiamine, folic acid, and magnesium (World Carrot Museum, 2005).

Carrot Seed Production Worldwide

Carrot crops are grown from seed, and a commercial seed production industry exists to service this need. Worldwide, over 3000Ha of carrot seed is produced annually (Schrieber and Ritchie, 1995; Simon, 2000). It was estimated that in 2004 14,900 metric tonnes of carrot seed was harvested from this total area (FAO, 2005). A large proportion of this total production is contracted or 'in house' production of proprietary varieties for vegetable breeding companies (Spurr, 2003). The majority of carrot seed crops are grown in the United States and France, with other significant producers including Australia, New Zealand, Italy, Japan and Chile (Spurr, 2003).

Initiation of Flowering

The carrot cultivars originating in Asia tend to behave as annuals when grown in long days and do not have a requirement for vernalisation. The cultivars that have been developed in the temperate regions of Europe and North America, on the other hand, are biennials. Their developmental sequence is; 1) vegetative phase, 2) vernalisation phase, and 3) photoperiodic phase. The specific conditions required for each stage vary with each individual cultivar (George, 1999).

Carrots form a vegetative structure consisting of an above ground rosette of leaves, and a large, deep taproot. The leaves are double compound with the lower leaves of the rosette being more divided and larger than the upper leaves. Typically, juvenility ends after the plant has formed eight to 12 true leaves (Atherton et al, 1990), but appears to be independent of root size (George, 1999). After the completion of this juvenile phase, the plant requires a period of vernalisation to induce flowering (Atherton et al., 1990). The period of vernalisation required is cultivar dependent but generally ranges from 2-10 weeks exposure to temperatures of 0ºC to 10ºC (Dickson and Peterson, 1958; Atherton et al, 1990). Following vernalisation, flower stem elongation, flowering and seed development are promoted by long days (Atherton and Basher, 1984). The stem elongates to between 60cm and 120cm high, with branches that produce flowering heads, or umbels. Bolting in carrots is generally accompanied by lignification of the roots, especially the supporting tissues near the cambium cylinder, during the later stages of bolting.

Flowering and Pollination

The carrot inflorescence is a series of compound umbels borne terminally on the branches of the seedstalk (Borthwick, 1931). The appearance of the umbels is not uniform (Figure 1). The first and largest umbel to flower is the terminal umbel on the main flowering stalk and is known as the primary or king umbel. Secondary umbels form at the terminus of branches from the main flowering stem and flower in sequence from the top to the bottom of the inflorescence. Tertiary umbels originate on secondary umbel stems (McDonald, 1998). In many carrot seed plants only three to four umbel orders are observed, with generally over 90% of seed produced from the first three orders (Halevy, 1985). However, up to 6 umbel orders can be observed in some cultivars (Borthwick, 1931).

The primary compound umbel is typically 10-15cm in diameter, and the size of the umbel decreases as the order increases (Halevy, 1985).

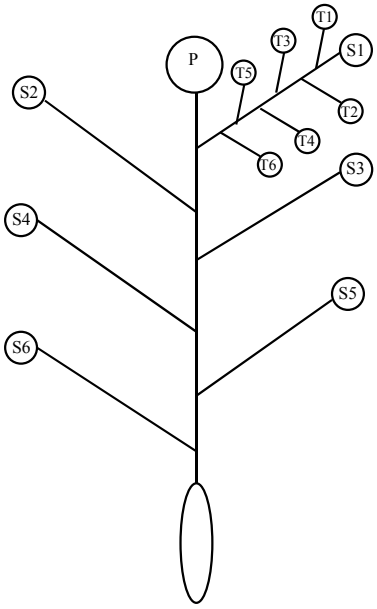


Figure 1 – Schematic diagram of a flowering carrot plant showing the general arrangement of umbels; P = Primary Order, S1- S6 = 1st to 6th secondary order umbels, and T1 to T6 = 1st to 6th tertiary order umbels on the first secondary branch. Fourth and higher order umbels may occur.

a)  b)

Figure 2 – a) View of a carrot umbel in the early stages of flowering. Approximate diameter of the umbel = 120mm, and b) a carrot seed crop at peak bloom.

As in other Apiaceae, carrot umbels are compound inflorescences, and each umbel consists of a number of umbellets. Carrot flowers are usually perfect, white or occasionally greenish white, pale yellow or purple/white, and approximately 3mm in diameter (Rubatzky et al, 1999). It has been reported that a tendency to produce only

male flowers occurs with increasing frequency in the higher umbel orders (Braak and Kho, 1958) leading to the classification of carrots as andromonoecious plants. Typical unmodified single flowers are composed of four concentric whorls of organs. The sepals of whorl one are always reduced to small green calyx teeth. The second whorl consists of five petals. Five stamens with pollen-producing anthers at the tip of the filaments make up whorl three. The fourth or central whorl consists of the gynoecium, a bicarpellary ovary with two styles (Linke et al., 1999). Each inferior two-celled ovary contains a single celled ovule, which ripen into two small, flattened, one-seeded fruits at maturity (Koul et al, 1993). Flowers open sequentially from the outer ring of umbellets to the central ring, however the development of seeds from different positions within the umbellet is not well understood (Halevy, 1999).

This growth habit of the carrot plant means that flowering is not a continual process in any one plant but proceeds in waves, with each wave corresponding to an umbel order. Flowering begins on the primary umbel, with the process beginning about a week later on successive umbel orders. Flowering lasts between 7-10 days on an individual umbel. This asynchronous pattern of flowering means that in commercial seed production, depending on variety, environmental conditions and cultural practices, a crop can flower for up to 4-6 weeks (Hiller and Kelly, 1985).

Carrot flowers are typically entomophilus, secreting nectar from the upper surface of the ovary (Erickson et al, 1982). Honeybees are efficient pollinators of carrots seed crops, but are often scarce in flowering carrot crops because other, more attractive, crop species may be flowering in the vicinity at the same time (Bohart and Nye, 1960). For this reason carrot seed producers often introduce bee-hives into carrot seed crops at the time of flowering. In Australia and New Zealand 6-8 hives per hectare, depending on the type of bee, are generally placed in the crop at flowering. Several other insect genera, including Hymenoptera, Diptera and Coleoptera have also been observed to be important pollinators of carrot flowers, particularly in the absence of honeybees (Bohart and Nye, 1960).

Figure 3 – A carrot umbel from a pollinator line being visited by a Honeybee (Apis mellifera) and a soldier beetle (Cantharidae)

Seed Development

The fruit that develops after fertilization is a shizocarp consisting of two indehiscent mericarps, each mericarp being a true seed (Rubatzky et al., 1999). At maturity the two mericarps readily separate (Halevy, 1985). The carrot seed consists of the testa or seed coat encasing the endosperm and embryo. At maturity, the embryo is equivalent of between 2 and 5% of the endosperm volume within the seed (Gray et al., 1984). The seed is a fawn to brown colour, oval in shape, 3-4mm long and 1-2mm wide. It has one flattened side and the other possessing 3-5 barbed ribs (McDonald, 1998) (See figure 1.3). Seed size variation is common within individual plants and between cultivars and can range from less than 0.5mg to greater than 2mg (Rubatzky et al., 1999). Mean seed weights at maturity from primary umbels have been found to be greater than those from the secondary umbels (Gray and Steckel, 1985), suggesting that the amount of reserve material per embryo is greater in seeds from primary than secondary an immature seeds have smaller embryos than mature seeds of the same weight. Therefore mature seed weight and seed quality is greatest from the primary umbel and decreases with increasing umbel order (Borthwick, 1931, Gray, 1981, Halevy, 1985).

a) b)

Figure 4 – Mature carrot seed a) with spines and b) after debearding

Cultural Practices of Seed Production

Carrots grown in a temperate environment generally have a biennial growth pattern, but carrot seed production takes place over a 12-month period (George, 1999). There is a high possibility of cross-pollination between carrot seed crops, and as such isolation distances between commercial crops should be at least 2 kilometers (McCartney, 1991, Fulton, 1999 cited in Spurr, 2003, Wilson, pers comm.). Cultivated carrots will readily cross-pollinate with wild carrot (Queen Anne's lace) and this must also be taken into account when choosing a location for carrot seed production (George, 1999). Contamination of seed crops with pollen from wild carrot is a major reason for the deterioration of genetic stocks in some parts of the world (George, 1999). Good friable soils that are well worked prior to drilling, free draining and with an optimum pH of between 6.5-7.5 are ideal for carrot seed production (McCartney, 1991).

There are two distinct methods of carrot seed production; the root to seed and seed to seed methods (George, 1999). In the seed to seed method, the seed is planted directly into the ground in late summer, is vernalised in situ in winter, flowers in late spring to early summer and the seed is harvested in late summer or early autumn of the following year. In the root to seed method, stock seed is planted in summer and raised in seedling (or steckling) beds before being transplanted into the field in the following spring. Depending on the local practices and winter conditions, the stecklings can either be vernalised in situ, or can be lifted, examined for trueness to type, and vernalised in cool

stores at 1°C and a relative humidity of no less than 95%, before being replanted in spring. The seed to seed method is preferred among seed companies for large-scale commercial seed production (George, 1999). This method is cheaper than the root to seed method and allows for higher planting densities, but means that roots cannot be inspected or rouged. Therefore this system relies on a high quality of stock seed, and appropriate isolation of crops. Successful seed to seed production relies on an environment in which carrot seeds can overcome juvenility before a period of cold of sufficient low temperature and duration to satisfy the requirement of the cultivar for vernalisation. This is particularly crucial when choosing a production location for a cultivar with a high vernalisation requirement or where the environment of two different hybrid parent lines must be considered to enable synchronization of flowering (Spurr, 2003). An insufficient vernalisation can lead an increase in variation of flowering times or at worst, a plant that does not bolt. This problem can be partly overcome in some areas with the use of growth regulators, such as GA_3, to induce bolting and flowering in an environment where there is insufficient natural vernalisation (George, 1999).

In small-scale carrot seed production where hand labor is cheap and/or plentiful, the umbels are cut by hand as they mature (Rubatzky et al., 1999). This system can also be useful in small plots where high value seed is being produced. On a larger scale, a single mechanized harvest is performed. The timing of cutting is a balance between percentage germination and yield loss through shattering (Sandin, 1980; Gray, 1979; George, 1999). Currently, typical commercial practice is to cut the crop when the seed on the primary umbel begins to shatter (Sandin, 1980; George, 1999). It is preferable that combining also be done either early in the morning or late at night, to prevent loss of seed through shattering (George, 1999).

Prior to any further cleaning or grading operations, the carrot seed spines are removed in a process known as debearding, to improve both seed flow and reduce seed volume. To achieve a marketable standard, further cleaning is achieved using aspirated screens, indent cylinders and gravity separators (Spurr, 2003). Industry standards for purity and germination of carrot seeds lots vary between varieties produced and with the target

markets demands. For export seed grown within Australia and New Zealand minimum standards of 99.9% purity and 85% germination generally apply (Spurr, 2003).

Hybrid Production Systems

Hybrid carrot seed production is used by most major seed companies. The main method used in carrot hybrid breeding is based on the application of cytoplasmic male sterility (CMS). CMS plants are incapable of normal pollen development, preventing maturation of viable pollen. Two forms of CMS exist for practical breeding, with different morphological expressions. In the first, referred to as "brown anther form", where stamens are affected during anthesis, pollen ceases any development beyond the microspore stage. Filaments remain unrolled and anthers are brown and shriveled (Welch and Grimball, 1947, cited in Linke et al, 1999). This type was identified to varying degrees in many cultivars of cultivated carrot and also in wild carrot germplasm (Morelock, 1974, cited in Nothnagel et al., 2000). The other type of CMS is the petaloid from, where the five anthers are transformed into petaloid structures during early development and do not produce any pollen. This form was first observed by Thompson (1961, cited in Nothnagel, 2000) in wild carrot populations. Extensive genetic studies have demonstrated a nuclear-cytoplasmic interaction for both CMS types. It is believed that the maternally inherited trait of cytoplasmic male sterility results from incompatibility between the nucleus and the cytoplasm (Kaul, 1988, cited in Linke et al., 1999). Most commercial hybrid crops utilize the petaloid form of CMS (Riggs, 1987, cited in George, 1999).

The hybrid cultivars have the advantage of high vigor and production of uniform roots, both traits desirable for the production of table carrots. Carrot hybrids are usually three way crosses, (A x B) x C, because the hybrid vigor in a single cross F1 male sterile parent line generally results in a higher seed yield than that of an inbred male sterile parent. Single-cross hybrids, A x B, however result in more uniform progeny than three way crosses and they do not require an extra year to produce the F1 parent stock. Therefore, if the seed yield of the inbred male sterile line is adequate, they are often used in preference

to F1 male sterile parent lines (George, 1999). The ratio of female to pollinator rows is generally 2:1 or 4:1 (Takahashi, 1987, cited in George, 1999).

Globally, there is a trend towards greater use of hybrid carrot varieties, with these accounting for around 60% of the total value of carrot seed production (Simon, 2000). This trend is set to continue, as vegetable growers increasingly demand the benefits of vigor and uniformity that hybrid varieties deliver. The production of parent lines for hybrid seed production requires the intensive selective inbreeding of carrots for several generations resulting in inbreeds with reduced plant vigor and challenging seed production characteristic. The production of economically viable hybrid seed crops requires intensive management and innovative seed production techniques to ensure reliable, economical seed yields. Hybrid seed production is therefore more expensive than open pollinated carrot seed production because the parent lines generally yield less seed and only seed from the male sterile line is harvested. In contrast to this, all seed from an open pollinated carrot seed crop is harvested.

Previous Research into Improving Carrot Seed Yield

Crop yield can be divided into a number of components, each of which is determined at different stages in the crops development. In carrot seed crops total yield (grams of seed/plant) is the product of the number of umbels, the number of flowers per umbel, the proportion of flowers that set seed and the size (weight) of the seeds (Gray and Steckel, 1985). Umbel number is determined by the time macroscopic seedstalk elongation is evident (Borthwick, 1931, Tsukamota, et al, cited in Elbella and Cantliffe, 1997) while flower number is also determined at this stage and in stages of bolting when flower abortion may occur. The proportion of flowers that set seed is determined by variables including pollination rate, pollen viability and vigor and abortion of seed. The number of seeds that are set and the capacity of the mother plant to provide resources determine seed size.

Carrot seed yields vary widely between varieties, production locations and with different cultural practices. For the United States it is estimated that most open pollinated crops

yield 800-900kg/ha of seed with hybrid crops typically producing 200-700kg/ha (Rubatzky, 1999). Low yields and/or variation in crop yield from season to season and site–to–site are problems for carrot seed producers, particularly in the production of hybrid seed (Spurr, 2003). While previous research aimed at improving carrot seed yield has been published, most of it has focused on the effect of plant spacing and on seed yield per unit area. In a two-year trial undertaken in India, stecklings of two carrot cultivars were planted with spacings of 40 x 30, 50 x 30 and 60 x 45 cm. Plants with the widest spacing produced the highest number of umbels and while these umbels produced a higher seed yield per umbel, the seed yield per unit area rose with planting density (Gray, 1981). This finding is further supported by research undertaken in the UK, the United States and Chile (Krarup et al., 1976; Gray, 1981; Oliva, et al, 1988), where seed yield per unit area was also found to rise, to a point, with closer plant spacing. The responses of carrot seed yield to plant densities ranging from 110 000 to 2 560 000 plants/ha were examined in the United Kingdom. Yields from the seed-to-seed method of production were found to increase from 700 to 2400kg/ha over the densities used. The contribution of the primary umbels to total seed yield was found to increase from 20% at a plant spacing of 80cm to between 60-80% with a plant spacing of 5 cm (Gray 1981, Oliva, et al, 1988). It was also reported that no significant effect of plant density on plant height, time of flowering or crop maturity was observed (Gray, 1981). Later research by Gray and Steckel (1985) contradicted this by suggesting that increased plant densities increased the spread of flowering time of the primary umbels and variation of the subsequent seedling weight and quality.

The removal of the third and higher order umbels at the flower bud stage in the carrot cultivar Chantenay resulted in higher seed yields from the primary and secondary umbels. Pruned plants yielded 15.43g per plant from the primary and secondary umbels only compared to 10.74 grams from untreated plants. Removal of the tertiary and higher order umbels also resulted in a shorter seed production cycle, greater seed and embryo length and a higher average seed weight, yet did not affect germination percentage significantly (Krarup and Duran, 1982). In contrast to this, research undertaken by Gray and Steckel

(1983) suggested that manual pruning of the tertiary and higher order umbels greatly decreased the yield of the crop, and was labour intensive.

Section 2: Pollen Viability in Hybrid Carrot Seed Production

Introduction

Pollen Viability and Crop Production

Information about the ability of pollen grains to germinate when they reach the stigmas of a compatible flower is valuable both for horticultural purposes and general botanical research (Firmage and Daphni, 2001). The role of the pollen grain is to deliver the two sperm cells, via a pollen tube, to the embryo sac to bring about the double fertilisation required to initiate seed development. Studies of plant reproduction prior to the 1970's focussed little attention on paternal contributions to plant reproduction. However in hybrid crop production systems and many natural heterozygous plant reproduction systems, high seed yields rely upon adequate male fitness (Bertin, 1990, in Doust and Doust, 1990). Male success can be affected by pollen viability, a measure of the number of pollen grains with the ability to germinate on a compatible stigma. Pollen limitation is defined as occurring when plants produce fewer fruits and/or seeds than they would with adequate pollen receipt. To test for pollen limitation researchers often conduct supplementation experiments that compare reproductive success of control plants versus those given supplemental pollen (Doust and Doust, 1990). The presence of pollen limitation reduces seed production and therefore has the potential to significantly impact on hybrid seed crop yields.

Pollen Development and Anthesis

Pollen development takes place within the anthers of all angiosperm plants. Meiosis in these anthers gives rise to haploid microsporocytes that develop into mature pollen grains in the anther locule and the diploid sporophytic tissues of the anther. The highly resistant outer wall (exine) of the pollen grain, forms around the haploid cell but includes material derived from the sporophytic tapetum (the tissue surrounding the locule) (Blackmore et al, 2007). Beneath this extremely hardy exine lays the intine or cell wall of the pollen grains and the cellular material. An additional extracellular lipidic matrix, the pollen coat,

covers the interstices of the exine and has many important functions, particularly in pollen dispersal and pollen-stigma interactions.

Most plants shed their pollen at a binucleate stage prior to sperm formation; however some plants, including carrots, shed the pollen as trinucleate cells following sperm formation (Brewbaker, 1967). The phylogenetically advanced trinucleate pollen species generally respire at a much higher rate than binucleate species and contains fully developed mitochondria at dehiscence that allows for much faster germination (Hoekstra and Bruinsma, 1979).

Pollen Viability in Hybrid Carrot Lines

Previous research conducted by Spurr (2003) has shown that under Australian production conditions hybrid carrot seed yield can be limited by poor pollen viability. Research on hybrid carrot seed yields by the Tasmanian Institute of Agricultural Research over the three years from 2001 to 2003 established that in southern Australia and New Zealand, European (Nantes and Amsterdam) hybrid carrot varieties typically achieved less the 26 to 70% of their yield potential (Spurr, 2003). Poor pollen viability was identified as a key factor contributing to low seed yields. Under field conditions, typically less than 50% of pollen grains from recently dehisced anthers were viable, with pollen viability fluctuating widely on a daily basis. High humidity, particularly in combination with high temperatures was demonstrated to have a damaging effect on pollen longevity (Spurr, 2003) but other studies of the impact of climatic variables and cultural practices on pollen viability at anthesis and pollen longevity have not been made. Investigations of pollen viability and the effects of environmental influences and management practices on this is a key component of research within this chapter.

While genetic factors determine the ultimate potential of the pollinizer (Hill et al., 1985, cited in Marschner, 1995), environmental variables, including mineral nutrition, temperature, humidity and carbohydrate partitioning within the plant, can influence the quantity and quality of the pollen produced and its subsequent performance. The following section is a summary of research undertaken into management factors affecting

pollen viability in other crops. Many of these factors have been chosen as a basis for treatments within field trials in this study.

The Effects of Temperature and Humidity

The combined effects of high temperature and high humidity have been shown to have adverse effects on the viability and longevity of carrot pollen shortly after anthesis (Spurr, 2003). Some studies have been undertaken in other agriculturally significant crops to determine the effects of temperature during floral development and subsequent pollen viability and seed/fruit set. In kiwi fruit, pollen viability was found to be higher when vines were exposed to warmer temperature regimes from bud-break through to flowering. Pollen viability was maximized when temperatures were 21^{o}C maximum and 17^{o}C minimum, and was the lowest at 13^{o}C maximum and 7^{o}C minimum (Jansson and Warrington, 1998). These results were similar to a study investigating the effects of low night temperatures on developing pollen grains in pepper (*Capsicum annuum* L.). In this study, low night temperatures caused a reduction in pollen production and viability in all four pepper cultivars studied (Shaked, et al., 2004). This was found to be due to a reduction in the pollen starch content and it was suggested that the low night temperatures interfered with starch accumulation and therefore decreased the concentration of soluble sugars in the mature pollen grains. High temperatures before anthesis have also been found to impair pollen viability in peppers. While pollen production was found to be maintained at both high (32/26^{o}C) and normal (28/22^{o}C) temperatures pre-anthesis, in vitro germination of pollen subjected to high temperatures during development was greatly reduced (Aloni, et al., 2001). This effect was reversed by exposing the plants held at higher temperatures to increased atmospheric CO_2, which restored pollen germination levels in high temperature treatments and did not affect the germination percentage of pollen developed at normal temperatures. Concentrations of starch in pollen from high temperature treatments was found to be high immediately prior to anthesis as opposed to the lower starch concentration and higher sucrose concentration found in pollen developed at normal temperatures.

Plant Nutrition and Pollen Viability

Research on basic carrot seed crop nutrition has been published (eg Krarup et al., 1982), but none of this previous research has focused on the influence of soil and plant nutrition on pollen production and viability at the time of flowering. Most of the carrot seed nutrition research so far has focused on the plants requirements for the four macronutrients Nitrogen, Phosphorus, Potassium and Sulphur. The macronutrient calcium and potassium and the micronutrients boron, manganese, copper and zinc have all been associated with pollen viability in other agriculturally significant crops and may influence pollen viability and/or longevity in carrot flowers.

Application of boron fertilisers has been found to increase the viability of pollen and the final yield in a number of agriculturally significant crops. A number of extensive studies on the effect of boron on pollen quality have been undertaken in wheat (Longbin et al., 1999, Subedi, et al, 1998), rice (Yu and Bell, 1998), and almond trees (Nyomora et al., 2000). Boron plays an essential role in the structure and functioning of cell wall and cell membranes. While the direct roles of boron in sporogenesis, pollen germination and pollen tube growth are yet to be confirmed, a role for boron in pollen cell walls may be expected (Longbin et al., 1999). The critical period for supply of adequate boron in wheat has been found to be the short period of microsporagenisis (Longbin et al., 1999). If adequate boron is not supplied during this time anther development and pollen viability will be reduced. Foliar application of boron to almond trees in spring and autumn is widely practiced in California (Nyomora et al., 2000), to maximise pollen germination and tube growth. The particular role of boron in pollen tube growth and fertilization is a major factor responsible for the usually higher demand of boron supply for seed and grain production than that needed for vegetative growth alone.

Copper deficiency can also have a negative effect on a plants reproductive phase. The critical stage for copper deficiency in plants has been found to be at the onset of pollen formation (microsporogenesis). When the copper deficiency is severe in wheat, no grains were produced even though the straw yield remains quite high. As the copper supply increases, grain yield rose sharply, whereas straw yield was only slightly enhanced. The

primary cause of failure of grain-set in copper deficient plants is believed to be the inhibition of anther formation an the production of a much smaller number of pollen grains per anther, as well as particularly the non-viability of pollen grains (Graham, 1976). This may also be in part due to a lack of supply of carbohydrates to the developing pollen grains, as the huge starch reserves found in copper sufficient plants were lacking in the copper-deficient plants (Jewell, et al, 1988).

Similar results to those obtained from copper deficiency have been obtained from plants grown under zinc or manganese deficient treatments. In maize, zinc deficiency prior to microsporogenesis (~35 days after germination), was found not to have any adverse effects on vegetative growth and ovule fertility but decreased pollen viability and dry weight by about 75%.

Calcium plays significant roles in the development of viable pollen as well as pollen tube growth and fertilization. (Tian et al., 1998, Okada, et al., 1999) and as such several calcium ion-binding proteins are expressed in anthers (Okada, et al., 1999) Calcium provides cell-wall rigidity by providing a cross-link between the pectin chains of the middle lamella. For cell extension, as occurs as when the pollen tube begins to grow down the style, cell wall loosening is required. The mechanism behind cell wall loosening leads to activation of calcium channels in the plasma membrane, leading to a transient increase in cytosolic free calcium ion concentrations. Therefore pollen tube growth is dependent on the presence of calcium in the substrate, and the direction of the pollen tube growth is likely to be controlled chemotropically by the extracellular calcium gradient.

While the adverse effects of deficiencies of micronutrients such as copper, zinc and boron on development and viability of pollen grains have been widely studied, the effects of manganese deficiency on grain and seed yield is less well known. One study has been undertaken to investigate the effects of manganese deficiency on the dry matter, development and fertility of pollen grains and seed yield of maize. In this study maize was grown to maturity in sand with two treatments, 0.55mg L-1 (sufficient) and 0.0055

mg L-1 (deficient) manganese in a glasshouse. A number of effects of manganese deficiency on the fertility and yield of the plants were observed (Sharma et al., 1991). While the time at which plants changed from vegetative growth to reproductive growth was the same in both treatments, the development of tassels was retarded in the manganese deficient treatments. Anthesis in the manganese deficient plants was delayed by 8 days; the tassels were short and also developed female flowers. The anthers of the manganese deficient plants were small and a large percentage appeared shriveled and failed to dehisce. Manganese deficiency also decreased the pollen producing capacity of the anthers; the pollen produced was smaller and lacked dense cytoplasm contents. The manganese deficient pollen grains showed loss of viability when stained with I2-KI solution and in vitro germination was shown to be less than 10% that off the manganese sufficient plants (Sharma et al., 1991). Manganese deficiency caused a 90% or more decrease in seed formation, with manganese deficient plants producing only 23 seeds per cob compared to 353 on the manganese sufficient plants. In contrast to this, ovule fertility was unaffected by manganese deficiency (Sharma et al, 1991).

In this section of the report, a range of nutritional treatments have been tested to determine any influence they may have on pollen viability in commercial carrot seed production. In addition, the effects of manipulation of carbohydrate availability were investigated as carbon supply was identified in the literature review as a factor affecting pollen production and viability. During pollen ripening the tapetum disappears and the vegetative cell plastids always store starch. Depending on the type of pollen this starch may then be converted to simple sugars or polysaccharides. Pollen draws on substances derived from one of four sources 1) photosynthesis of the mother plant, especially in plants flowering with the presence of leaves; 2) Photosynthesis by the anther, especially when the calyx and corolla are reduced or absent; 3) Materials stored in other parts of the flower or plant especially in plants that flower before they develop leaves or 4) Utilization of transient cells and material of the anther, such as the tapetum, the connective tissue, the middle layer and the callosic walls. The amount and type of carbohydrate stored in the ripening pollen grain affects the ability of the grain to maintain viability in time (Pacini, et al., 2006).

Materials and Methods

Plant Material and Environmental Conditions

An experiment screening the range of pollen viabilities and the effects of temperature and humidity on the pollen viability of each line was undertaken at Tinwald in Canterbury, Zealand (43^o 53' S, 171^o, 44' E). Eight pollinator lines common to Bejo Zaaden's commercial hybrid seed production were chosen. The eight lines chosen represented a mixture of lines that industry had observed to be good, average and poorer pollinator lines in commercial hybrid crosses. The plants used in this experiment were grown using the seed-to-seed method, at a density of 12 plants/m^2. The trial site consisted of 4 blocks with 8 x 10m rows within each block. Each row contained a different variety and varieties were arranged randomly within each of the four blocks. A summary average daily temperature and humidity for the month leading up to and including the trial sampling dates is given in figure 6.

The effects of foliar nutrition, carbohydrate partitioning and shading on the viability of carrot pollen were investigated in trials in a commercial crop at Richmond, Tasmania (42^o 43', 147^o 27' E) and in Ouse, Tasmania (42^o 31'S, 146^o 43' E). A summary of the average daily temperature and humidity for both Tasmanian sites is given in Figure 7. In both treatment experiments at Ouse and Richmond, the treatments were arranged in 4 replicate plots in a randomized complete block design. Treatments were applied to at least 20 plants per treatments per plot.

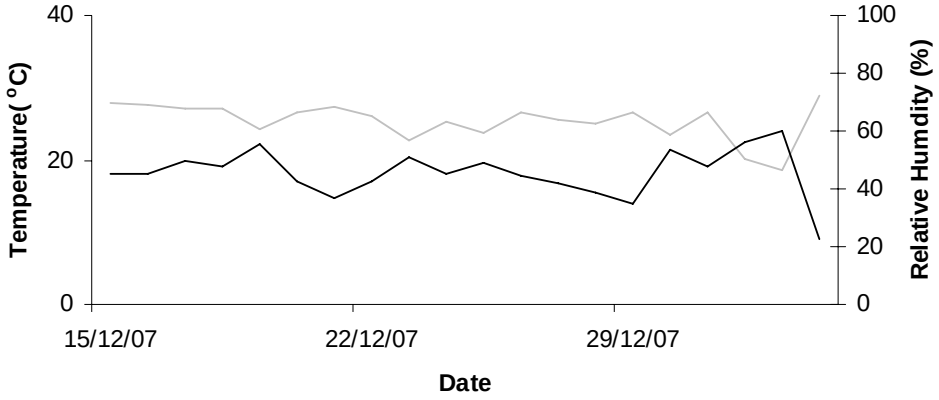


Figure 6 - Average daily temperature (ºC) (grey) and percentage relative humidity (black) for one month, up until and including the period of pollen sampling from the pollinator screening trial site at Tinwald in Canterbury, New Zealand.

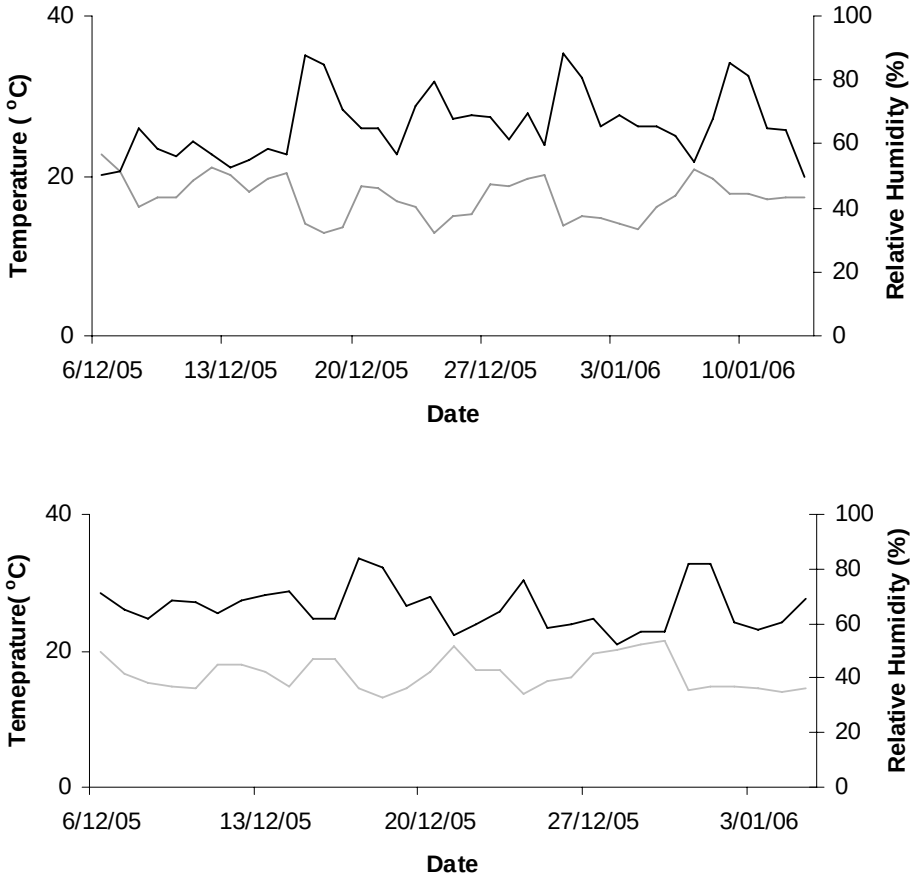


Figure 7 - Average daily temperature (ºC) (grey) and average daily humidity (%) (black) for approximately one month, up until and including, the period of pollen sampling from the nutrition and resource allocation trial at a) Ouse and b) Richmond in Tasmania.

The Effects of Nutrition on Pollen Viability

Nutrition treatments were applied approximately two weeks before the secondary umbels began to flower. The nutrition treatments used and the rates of application are summarised in Table 1. All nutrition treatments were applied as a foliar spray except for Potassium which was side-dressed. Foliar sprays were applied using a standard 5 Litre knapsack sprayer (CRT, Australia) calibrated to 240L/ha. All fertilisers were sourced from Impact Tasmania (Bridgewater, Tasmania, Australia)

Table 1 - A summary of the foliar fertiliser treatments used at each trial site and the rates of application.

Treatment	Description	Application	Ouse Trial	Richmond Trial
Foliar Boron	Solubor	Foliar 2kg/ha	√	√
Managanese	Manganese Sulfate	Foliar 2kg/ha	√	√
Potassium	Muriate of Potash	Side-dressed 60kg/Ha	√	√
Zinc	Zinc Hepta-Sulfate	Foliar 2kg/Ha	√	
Copper	Copper Sulfate	Foliar 2kg/Ha		√
Combined Treatments	All of above	Foliar and side dressed	√	√
Leaf Removal	Every second leaf removed from the stem base upward		√	√
Umbel Removal	All umbels removed bar the four highest secondary umbels		√	√
1/2 Umbel Removal	Half the umbellets removed from the top 4 secondary umbels		√	√
Shading	70% shade cloth. 20 plants shaded 9am-3pm AEST			√

Effect of modifying resource availability

Along with nutrition treatments, both Tasmanian trials included a range of treatments aimed at modifying the plants resource allocation. Umbel removal, ½ umbel removal and leaf removal treatments were implemented at both trial sites. These treatments were

implemented at the time of fertiliser application, approximately two weeks before the secondary umbels began to flower. Umbel removal treatment was applied so that the primary umbel and every second secondary umbel from the top of the plant down were removed. The half umbel removal treatment involved cutting off umbellet buds from one whole side of the developing umbel. The leaf removal treatment was applied so that every second leaf from the base of the plant to the top of the canopy was removed. Weekly checks were made to remove any newly forming umbel buds or leaves in both the umbel and leaf removal treatments.

Shading

Shading treatments were applied using a 70% shade rated shadecloth® (30% transmission of light), that was positioned horizontally above each plot in such a way that at least 20 plants would be shaded from 9am to 3pm Eastern Australian Standard Time (EAST). Shading was only applied at the Richmond trial.

a) b)

Figure 8 – a) Shading and b) ½ umbel removal treatments at the Richmond trial site.

Pollen Collection, Storage, Handling and Testing

At all trial sites, including Tinwald, Ouse and Richmond, pollen sampling began once the secondary umbels began to flower. Sampling was undertaken between 10am and 2pm

(EAST in Tasmania, EAST + 2 in Tinwald) on between 5 - 8 days within the period that the secondary umbels were in bloom. The beginning of sampling was 12th December, 28th December and the 2nd January 2005 for Richmond, Tinwald and Ouse respectively. Samples for pollen viability assessment consisted of ten individual flowers per treatment plot, with care taken to ensure that the pollen collected had been released that morning. The ten flowers were sourced from ten different plants within the plot where possible.

Pollen Production Assessment

A further 5 samples of exactly 10 individual flowers was collected from each line screened on 3 of the 6 sampling days. Each pollen sampled had a small amount of sucrose solution (20% w:v) added to it at the time of assessment, and the pollen grains were counted in a haemocytometer.

Results

Screening Pollen Viability in a Range of Commercial Pollinator Lines

There was significant variation between the average pollen viability of each pollinator line over the five sample days. The lines C680019 and C680459 had the highest average viabilities over the sample period with 45.16% and 40.59% respectively and were the only two lines that achieved an average viability above 40%. Most other lines had an average viability in the range of 33-35%, except for C680209, which had the lowest average viability at 30.32% over the five day sampling period (Figure 9).

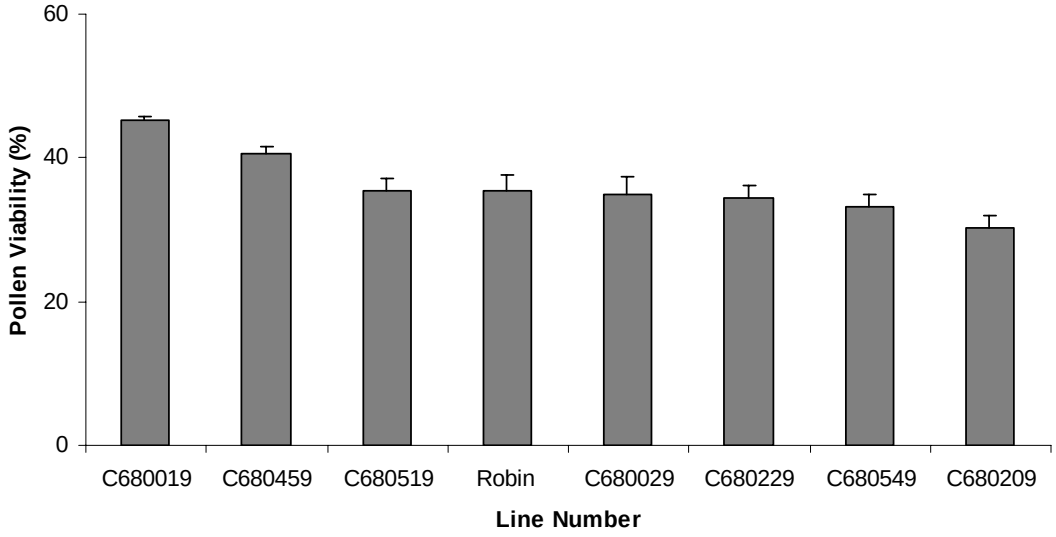
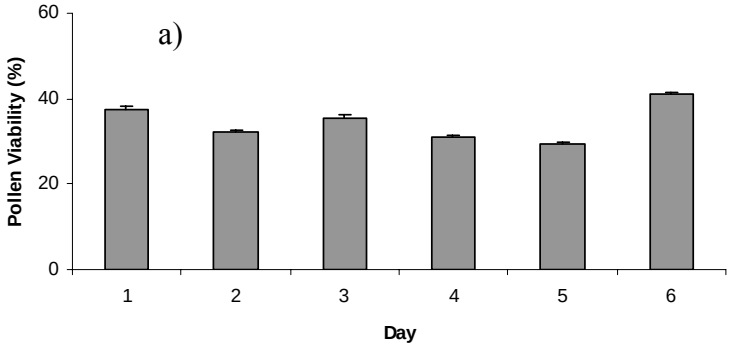
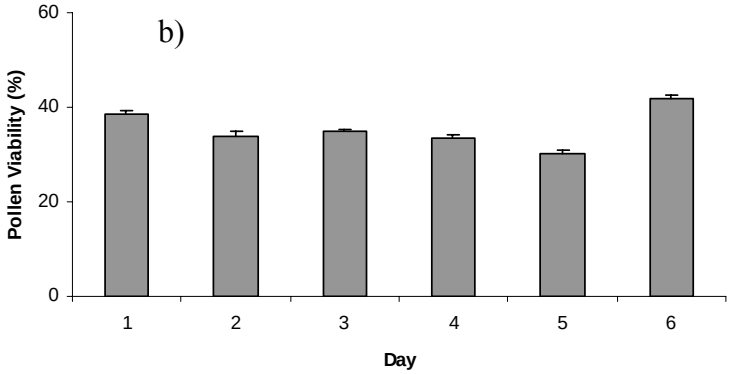


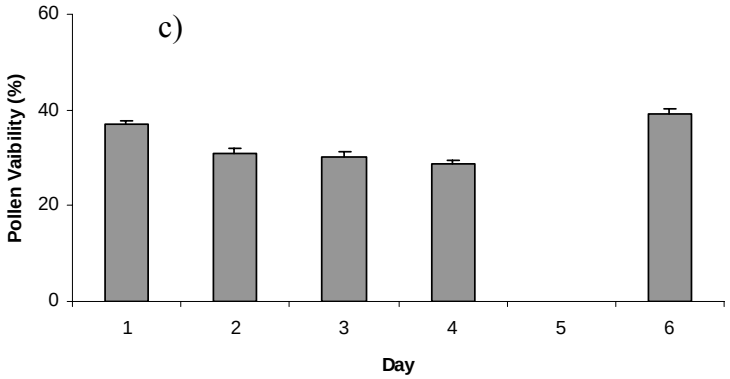
Figure 9 – Average pollen viability for each of 8 pollinator lines examined over a six consecutive day sample period, beginning 28th December 2005. Error bars indicate standard errors (N=10).

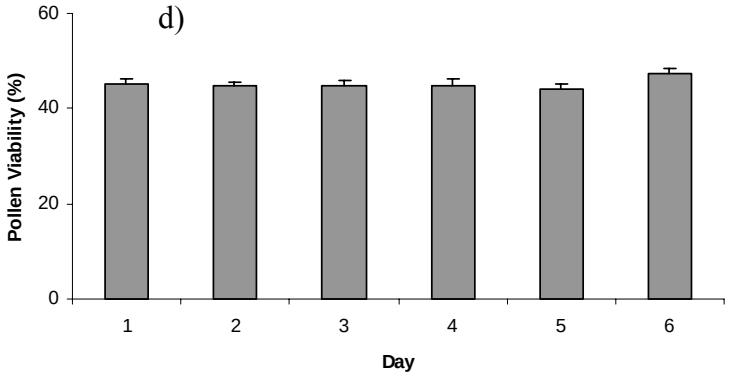
The pollen viability also varied on a daily basis within each line. The overall range of viabilities was between 28 - 48%, shortly after anthesis (10am – 2pm EAST + 2). The two lines with the highest average pollen viabilities over the sample period also had the least variation within the 6 day period. Line C680019 had the least variation between days, at only 3.5%. The pollen viability of line C680459 varied by 7% over the five day period, this still being lower than most other lines, with variations in percentage viabilities ranging from 11-13%. However the line C680209, having the lowest viability

over the sample period, also had a relatively low variation of 8% viability over the 6 days (Figure 10).









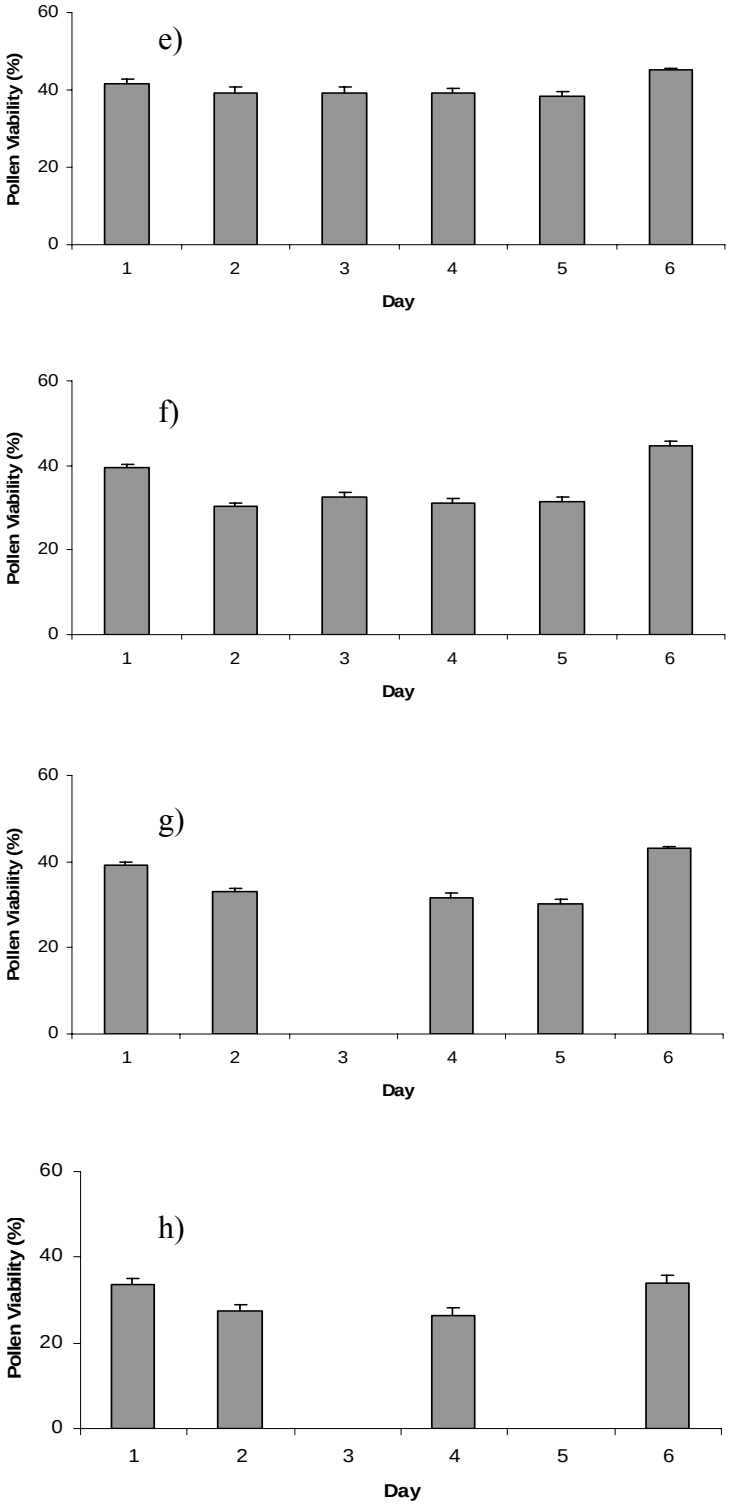


Figure 10 - Pollen viabilities of 8 pollinator lines; a) C680229, b) C680519, c) C680549, d) C680019, e) C680459, f) 680029, g) C680639, h) C680209 on each of 6 consecutive sampling days, beginning December 28, 2005. Error bars indicate standard errors (N=10)

Temperature and Humidity Effects on Pollen Viability

The effect of humidity on pollen viability collected shortly after anthesis (10am-12pm EST) was significant ($P<0.001$). The average viability of the 8 lines tested decreased from 42% at 22.7% relative humidity to 34% viability at 54% relative humidity (Figure 11). The effect of temperature on percentage pollen viability ($R^2= 0.7545$) at the time if sampling, was not as strong as the effect of humidity ($R^2 = 0.9702$) (Figure 12).

However the effect of ambient humidity at the time of collection was not uniform across the lines. There is a significant difference in the regressions equations for each line when the relationships between relative humidity and pollen viability are compared between lines (Table 2). This was also reflected in differences in variation between days of pollen viabilities (%) across pollinator lines in Figure 10.

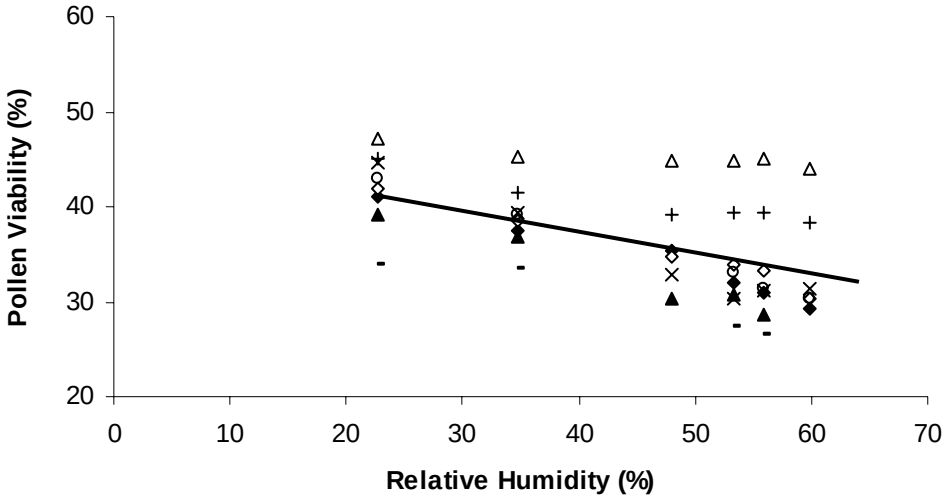


Figure 11 – The effect of varying relative humidity (%) on pollen viability shortly after anthesis in the field. The lines represented are C680229 (♦), C680519 (◊), C680549, (▲), C680019 (Δ), C680459 (+), C680029(x), C680639 (o) and C680209 (-). The relationship between relative humidity (%) and pollen viability is significant ($P<0.001$) and is described by the equation $y = -0.2396x + 47.344$ ($R2 = 0.9702$), where y = pollen viability and x = relative humidity.

Table 2 – The relationships between relative humidity (%) and pollen viability (%) for each line tested are represented in this table, where y = pollen viability (%) and x = relative humidity.

Line Number	Equation	R^2 Value
C680229	y = - 0.3061x + 48.342	0.9631
C680519	y = - 0.2866x + 48.591	0.9716
C680549	y = - 0.3195x + 46.857	0.9536
C680019	y = - 0.071x + 48.409	0.8187
C680459	y = - 0.1693x + 48.234	0.9282
C680029	y = - 0.3968x +53.095	0.9487
C680639	y = - 0.3414x + 50.819	0.9987
C680209	y = -0.242x + 40.406	0.9336

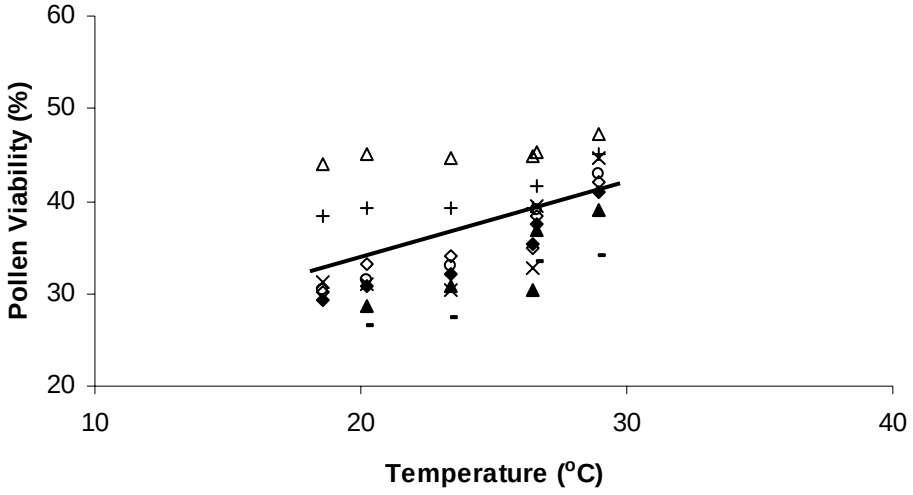


Figure 12 – The effect of temperature (oC) on pollen viability shortly after anthesis (10am-2pm) in the field. The lines represented are C680229 (♦), C680519 (◊), C680549, (▲), C680019 (Δ), C680459 (+), C680029(x), C680639 (o) and C680209 (-). The relationship between temperature (oC) and the average pollen viability of the 8 lines is described by the equation y = 0.746x + 18.464 (R2 = 0.7545), where y = average pollen viability of the 8 lines and x = temperature.

The relationship between pollen viability (%) and pollen production (grains/10 flowers) was not significant (P > 0.1, R2 = 0.307) and varied highly between lines. The line with the strongest average pollen viability across sampling days of 45.4%, C680019, also has the highest average pollen production of 53.92 x 104 grains/flower over the three consecutive testing days. However the line with the lowest pollen production of 9.33 x 104 grains/ flower, C680519, has close to average pollen viability of 35.18% (average of 8 lines = 36.24%).

Table 3 – The mean pollen production (grains/flower) x 104 and mean pollen viability (%) of each line over three consecutive sampling days is summarised in this table.

Line Number	Pollen Production (grains/flowers) x 10^4	Pollen Viability
C680019	53.92	45.40
C680459	22.33	40.92
C680029	31.50	35.71
C680519	9.33	35.18
C680639	43.33	34.90
C680549	18.25	33.89
C680229	41.00	33.70
C680209	11.50	30.24

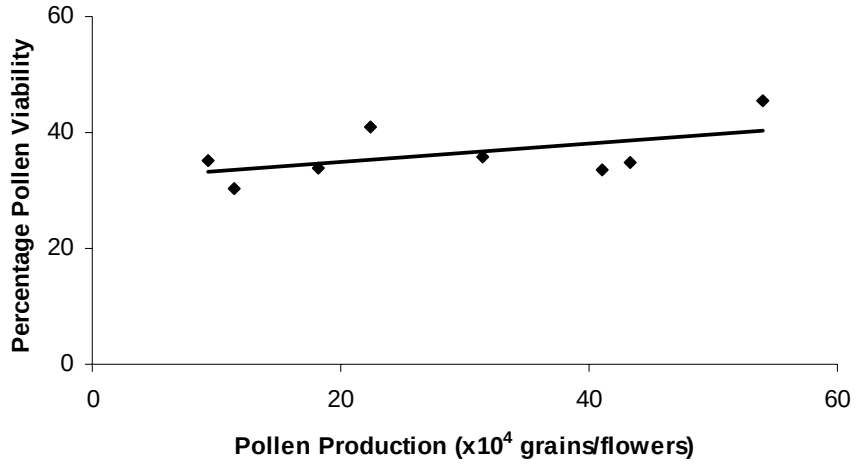


Figure 13 – The relationship between the average pollen production and average pollen viability shortly after anthesis of each of the 8 lines over three consecutive sampling days. This relationship is not significant and is described by the equation; y = 0.162x + 31.55 (R2 = 0.307), where y = percentage pollen viability and x = pollen production (x 104 grains/flower).

Trials undertaken at Ouse and Richmond looked at the effects of a range of nutritional treatments, including the addition of zinc, boron, copper and manganese above normal commercial applications. There was no significant increase in pollen viability due to applications of any of these nutrients just prior to flowering. The effects of shading, umbel removal, ½ umbel removal and leaf removal were also insignificant in increasing and/or reducing pollen viability post-anthesis.

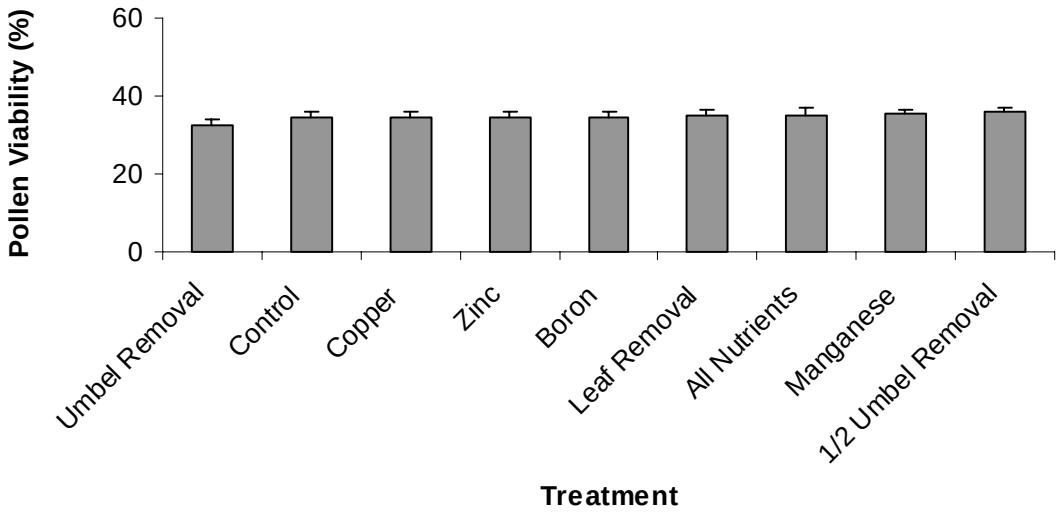


Figure 14 - The effects of nutritional and carbohydrate partitioning treatments on average pollen viability in trials in commercial crops at Ouse. There is no significant treatment effect ($P > 0.1$).

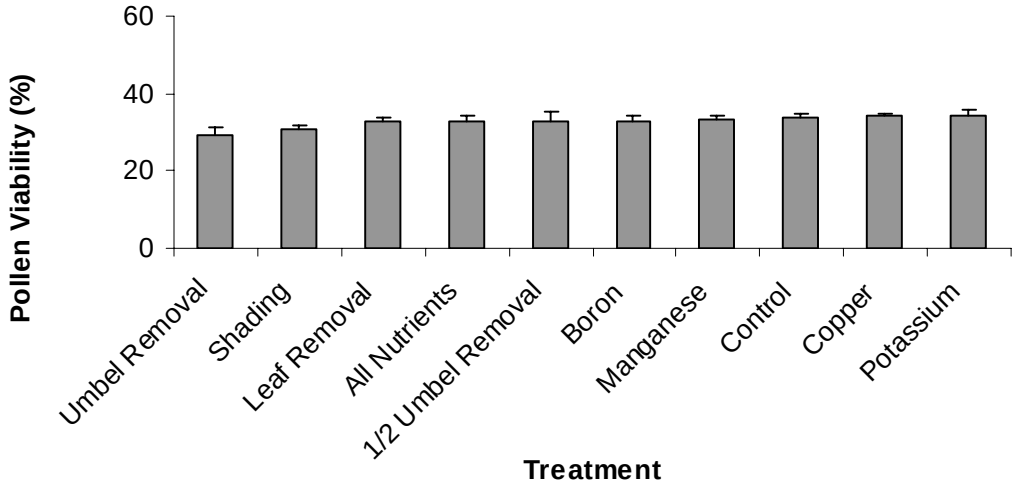


Figure 15 - The effects of nutritional and carbohydrate partitioning treatments on average pollen viability in trials in commercial crops at Richmond. There is no significant treatment effect ($P > 0.1$).

Conclusions

The plant materials used in the pollen screening trial were chosen by production specialists as a representative range of lines that ranged from male fertile that appeared to be good pollinators through to lines that appeared to be consistently poor pollinators. The data collected from these lines confirms low pollen viability as a potential limiting factor in hybrid carrot seed crops in cool climate production system such as those in southern Tasmania and the Canterbury Plains of New Zealand. Samples were taken between 11am and 1pm, as this was estimated to be the time that the pollinating insects would begin working commercial crop. The average pollen viability of these lines at this time over the 6 day sampling period ranged from a minimum of 26.48 for C 680209 on day 4 to a maximum of 47.26% in pollen collected from C680019 on day six of sampling.

There is a lot of variation in pollen viability between lines and also between days of sampling for any given line. Out of the 8 lines chosen, only two lines had average pollen viability above 40% across the 6 sample days. These two lines, C 680 019 and C680 459, also had the highest pollen production for the same sampling period. Five of the six lines studied had average pollen viabilities between 33 and 36%. Four out of these 5 lines fell between third and sixth positions when ranked in descending order on pollen production, however within this range there is no significant correlation between pollen production (grains/flower) and pollen viability (%). The line with the lowest pollen viability, C 680209 had low pollen production of 11.50 x 104 grains per flower, second only to C 680519 with a pollen production of 9.33 x 104 grain/flower.

It appears from this data that the major factor influencing pollen viability post-anthesis is relative humidity on the day of anthesis. It appears that the combined effects of high temperatures and high humidity is particularly detrimental to pollen viability. There was a strong relationship between pollen viability and relative humidity. As relative humidity increased the pollen viability at the time of sampling was reduced. It appears that though relative humidity is the major factor influencing pollen viability post anthesis, high humidity and high temperatures in combination were particularly detrimental to pollen viability. The relationship between pollen viability and relative humidity was strong in all

lines, with R2 values ranging from 0.08187 in C680019 to 0.9987 in C680639. Interestingly the two lines that had the highest pollen viability (%) and pollen production (grains/flower) showed less of a decrease in percentage pollen viability under conditions of increased humidity. The equations for these two lines were;

$y = - 0.071x + 48.409$, with a R2 value of 0.8187 for C 680019 and

$y = - 0.1693x + 48.234$, with and R2 value of 0.9282 for C 680459.

This shows that pollen from these two lines decreased less with increasing humidity than pollen from the other 6 lines studied. Though the two strongest lines showed the smallest reduction in the potential viability, this was not a trend amongst the other 6 lines studied, as average viability and reductions in viability due to increased humidity varied independently amongst the remainder of lines studied. This has consequences for management of commercial crops, in terms of flowering time, with manipulation of flowering time to coincide with periods of low likelihood of high humidity likely to increase pollen viability and therefore yield.

A range of treatments were applied to plants in two commercial crops in Tasmania, to determine the effects of a range of nutrients on pollen viability. Nutrients were chosen that had a beneficial impact on pollen viability in other crops. However no treatments effects were observed in plants treated with foliar application of boron, zinc or manganese just prior to flowering, or side-dressed with potassium. Shading was applied as a treatment to decrease the exposure of the umbels to irradiance from the sun, in order to determine any effects of sunlight on pollen viability. However, no treatment effects were observed. A number of treatments were designed to alter the plants carbohydrate partitioning immediately prior to flowering to observe any influence this may have on pollen viability (%) post-anthesis. Once again, no treatment effect was observed.

It appears that the dominant factor influencing pollen viability post-anthesis in cool climate hybrid carrot seed production is the ambient humidity. There are a number of

factors that may contribute to loss of viability under high humidity conditions, and particularly under conditions of combined high temperatures and high humidity. The major cause of loss of viability in pollen post-anthesis is the deficiency of metabolites. This is particularly relevant in trinucleate pollen which respires at a higher rate than binucleate pollen. It may be expected that high humidity conditions particularly combined with high temperatures may increase this respiration and break-down of metabolites. It is often thought that once desiccated, pollen grains enter a phase of stasis. In contrast there is recent evidence to suggest that rather than entering a phase of stasis many pollen grains particularly trinucleate pollen grains simply enter a phase of reduced metabolism rather than stasis. The survival of pollen during this phase may then be affected by environmental conditions and the length of time from anthesis to pollen deposition.

Though humidity reduces pollen viability significantly by the time of sampling (11am-1pm EAST), it is unclear what initial pollen viability may have been at anthesis or immediately before anthesis. The mechanism behind the reduction in pollen viability, and the increased effects of high humidity conditions on this process, is also unclear at this stage.

The type of carbohydrate reserves present in the mature pollen grains may also affect the ability of the pollen grains to regulate water uptake and loss post anthesis and the consequent survival of pollen grains under differing environmental conditions. In some species such as *Cucurbita* and *Zea mays* whose pollen is not partially or fully dehydrated upon release, a high concentration of starch grains are found in the cytoplasm and polysacharrides are absent. Pollen of this type loses water quickly and viability is short-lived. However in other pollen types a gradient exists between pollen where the starch is partially hydrolysed before anther opening and that where starch reserves are totally hydrolysed before this point. While these pollen grains remain viable in time, the level of dehydration of the grain and the presence and absence of furrows differs between groups of species and this may affect the longevity of the pollen post-anthesis.

There are some current commercial practices undertaken that may affect the longevity of carrot pollen post-anthesis. There is an increasing use of overhead irrigation through centre pivot style irrigators in hybrid carrot production in south eastern Tasmanian and the Canterbury Plains. It is recommended that the effects of this overhead irrigation on canopy humidity and pollen viability be investigated.

Section 3: Pollen Development and Release

Introduction

It is generally accepted that mature pollen grains become dessicated and enter a state of metabolic stasis upon maturation. An investigation into the behaviour of vacuoles during pollen development and maturation in *Arabidopsis* found evidence to the contrary in this trinucleate pollen species (Yamamoto, et al., 2003). In this study autolysis of mature pollen grains by lysosomal structures with acid phosphatases was observed. This was the first species in which this has been observed. A number of similarities between the pollen biology of *Arabidopsis* and *Daucus carota* exist. *Arabidopsis* has short-lived trinucleate type pollen, with longevity of less than 3 days at room temperature. There are also a number of differences between the two species. In mature flowers of *Arabidopsis* the stamens are the same height as the pistil before anthesis; in contrast the pistil becomes higher than the stamens after anthesis. In *Daucus carota*, this is reversed. The anthers develop and release pollen prior to the extension of the pistil and the separation of the two stigmas, a point that is thought to mark the onset of stigma receptivity (Spurr, 2003). *Arabidopsis* will often self-pollinate under natural environmental conditions. In *Arabidopsis* the pollen viability is found to be highest shortly before anthesis and declines from anthesis onward. It is unclear as to whether this is the case in *Daucus carota* or not.

It is not known as to whether the viability is higher in carrot pollen collected from flowers shortly before anthesis, than has been observed in pollen from flowers collected shortly after anthesis. There is also very little published research on the pollen biology of carrot. Is it presumed that carrot pollen is partially dehydrated at presentation (Spurr, 2003), but the presence or absence of furrows has not been confirmed. The type and amounts of carbohydrates stored in carrot pollen is unspecified in the current literature as are any possible differences in carbohydrates storages between lines that may be

responsible for the significant differences in pollen viabilities observed across a range of lines in Section 2.

Materials and Methods

Pollen Viability up to and shortly after anthesis.

At approximately 8.30am on any given sampling day, 100 flowers were tagged across a commercial field of pollinator line C68019 in a completely randomised design. These flowers were chosen on the basis that they would be expected to release pollen on the day of sampling. At the time of tagging flowers were collected, twenty flowers were collected from the 100 tagged, and the anthers were burst with the fine point of a needle. Twenty flowers were randomly collected on an hourly basis, and placed in an eppendorf tube to form four replicate samples each of five flowers. The anthers were burst with a pin up until the point in the day where anther dehiscence occurred and the pollen was released. This point differed depending on the environmental conditions present on the day. Collected flowers were then place immediately into a desiccator, and pollen was stored and tested according to the methods described in Section 2.

Overhead Irrigation vs Dripper Irrigation

This trial was undertaken with carrots grown in a controlled environment glasshouse at the University of Tasmania. Irrigation was provided by means of over overhead sprinklers until approximately 2 weeks prior to first flower opening. Half the plants were then transferred to a dripper irrigation system, with the remaining half continuing to be irrigated using the overhead sprinkler system. The rate of irrigation was equal for both groups of plants.

Sampling began when the second whorls of secondary umbels began to flower. Sampling was undertaken on an hourly basis from 7am until 3pm, as per the previous experiment. Five repetitions of 10 flowers were collected from each group of plants. Samples were collected on a completely random basis, based on the allocation of a number to each flowering umbel and each floret within the second whorl of each umbel.

Results

Pollen Viability up to and shortly after anthesis

The average pollen viability of C680019 at 7am was found to be 5% higher than C680029, and consistent difference between the lines were found from that point onwards (Figure 16).

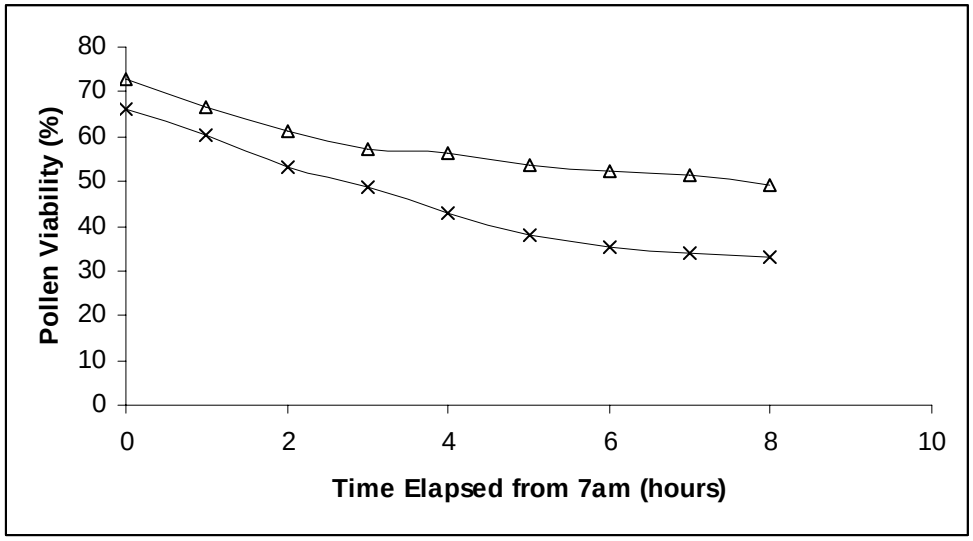


Figure 16. Average change in pollen viability in samples collected every hour from 7am until 3pm in two lines, C680019 (Δ) and C680029 (+). Averages are determined from 5 non-consecutive sampling days.

Pollen viability decreased continually from 7am until 3pm in both lines studied. The decrease in pollen viability in this time period was significantly higher in C680029 than C680019. The greatest decrease in viability was observed in the first four hours from 7am until 11am. This coincided with the beginnings of visual anthesis in all sampling days. In this period the average pollen viability of C680019 decreased 23% from 67% to 44%. In the following 4 samples, taken at 12pm – 3pm, the pollen viability only decreased 10% from 44% to 34%. A similar trend was observed in C680029, where pollen viability decreased 14% from 72 to 58% in the period from 7am to 11am, and then continued to decrease only a further 7% from 11am until 3pm.

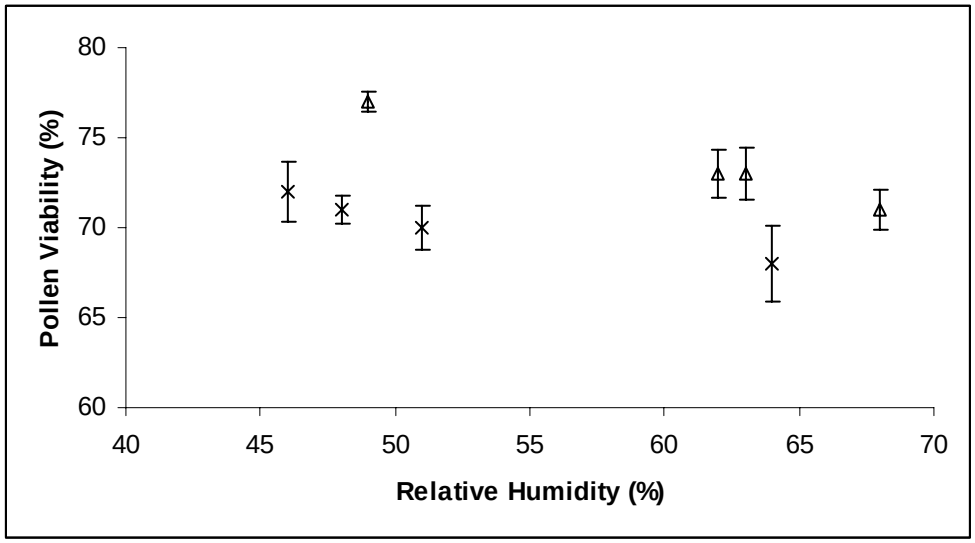


Figure 17. Average hourly relative humidity for the 24 hours prior to sampling and the viability of the initial daily sample collected at 7am, for two lines, C680019 (Δ) and C680029 (+). Error bars indicated standard errors (n=5).

A significant reduction in pollen viability with an increase in the average hourly relative humidity (%) for the 24 hours prior to sampling was recorded. This is consistent across both lines. Initial pollen viabilities observed for C 680029 were lower than those observed for C680019, and pollen viability remained lower throughout the sampling period. This is consistent with results observed from these lines presented in Section 3. In C680019, initial (7am) pollen viability was observed to decrease 5% from 77% to 72% with an increase in humidity of 19%. The reduction in pollen viability was greater in C680029; a 7% decrease in viability was observed when the average hourly relative humidity increased 18% (Figure 17).

Overhead vs Dripper Irrigation

A reduction in initial pollen viability was observed when plants of C680019 were grown under overhead irrigation as opposed to dripper irrigation. An average pollen viability of 78% was recorded at 7am in dripper irrigated plants, 7% higher than those grown under overhead irrigation conditions (Figure 18).

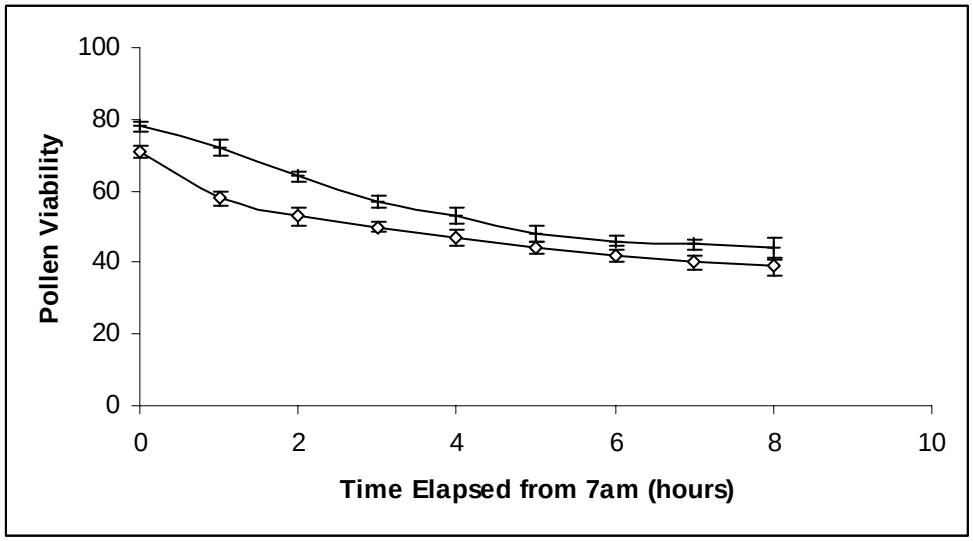


Figure 18. Changes in pollen viability on an hourly basis between 7am and 3pm from plants irrigated by overhead irrigation (o) as compared to those irrigated with dripper irrigation (-). Error bars show standard errors (n=10).

The reduction in pollen viability in the first hour was also significantly greater in plants grown under overhead irrigation, with a 13% decrease in average pollen viability between 7am and 8am. In the same time period the average pollen viability in C680019 decreased by only 6%. If an average reduction in pollen viability is calculated from 7am until 10pm there is no difference between the two methods of irrigation in rate of decrease of pollen viability (%) with a reduction in percentage viability of 21% from 71 to 50% in C680029 and in the same time period the pollen viability of the plants irrigated by drippers, decreased from 78 to 57%. Therefore at 11am the differences in pollen viabilities between the two lines directly reflect the initial difference in pollen viability from the initial sample at 7am.

Discussion

Based on the results of the experiments presented above, the differences in pollen viability recorded in the experiments presented in Section 3 could be attributed both to differences in pollen viability prior to anthesis and the rate of decline in pollen viability after anthesis. Pollen viability of up to 80% was observed in anthers pre-anthesis, but significantly decreased in the 2 to 3 hours leading up to anthesis and continued to decline after anthesis. When pollinating insects commence working in commercial crops at approximately 10-11am, pollen viability was found to be significantly lower than that at anthesis. Line C 680029 was found to be more sensitive to humidity than line C680019. Differences in the rate of decrease of pollen viability with increasing humidity may explain the effects of ambient humidity treatments both pre- and post anthesis on the percentage of viable grains in all lines studied in Section 3. Across the 8 lines screened, the effect of ambient humidity on the pollen viability at the time of collection was not uniform.

As some lines are more sensitive to the effects of high humidity, differences in the reliability of seed yields from different lines in commercial production may be at least partially attributed to the interaction between environmental conditions and response pattern of the lines. In southern Tasmania, the traditional time of flowering for most varieties falls in mid-late December, coinciding with a high risk period for humid easterly weather patterns. Careful management of flowering time, particularly in highly sensitive varieties, is required to avoid these weather conditions. Overhead irrigation was shown to reduce the initial pollen viability by up to 8%, demonstrating that irrigation management may also affect seed yield through an effect on pollen viability. Careful management of irrigation throughout the crop flowering period is recommended to avoid creating a high humidity environment around dehiscing flowers, thus ensuring maximum percentage pollen viability at anthesis for the given environmental conditions.

Section 4: Pollen Pistil Interactions

Introduction

Stigma Structure and Function

Pollen is designed to deliver haploid sperm cells to the ovules to bring about fertilisation. There is a highly specialized target for the pollen to land on; in the case of angiosperms this is the stigma. Whilst angiosperm pollen can be dispersed by a number of methods including wind, insects or vertebrates, carrot pollen is dispersed only by insects, predominantly from the genera in the Hymenoptra (bees and wasps), Diptera (Flies) and Coleoptra (Beetles) visiting the carrot flowers (Bohart and Nye, 1960). Once the pollen meets the stigma, the pollen grain hydrates, releasing stored RNA, proteins and bioactive small molecules that allow rapid germination and outgrowth of a tube (Taylor, 1997), which penetrates the stigmatic cell wall and continues to grow through the transmitting tissue of the style to reach the ovary where fertilisation occurs. Pollen tube elongation is the most rapid growth of any known cell, this growth being restricted to the tip of the tube only (Taylor, 1997).

The receptive structure of the female tissue, the stigma, binds the pollen and mediates tube growth down the style. The stigma can be classed as either wet or dry. Wet stigmas are covered with cells that lyse to release a nutrient-rich secretion containing proteins, lipids, polysaccharides and pigments. Examples of plant species with wet stigmas are most Rosaceae, Apiaceae and Solanaceae (Heslop-Harrison and Shivanna, 1977). Wet stigmas may or may not have surface papillae, but if present they are generally low to medium length papillae. The cells on the receptive surface of wet stigmas are generally necrotic at maturity. Dry stigma on the other hand, have intact surface cells that generally extrude as papillae, and are covered by a primary cell wall, a waxy cuticle and a proteinaceous pellicle. Examples of plant species with dry stigmas are Cruciferae, Cannabaceae, and Papaveraceae.

Fertilisation is essential for carrot seed production. The effective pollination period is one of the most important factors in determining the likelihood of successful fertilisation, and subsequent seed set. The effective pollination period (EPP) is defined as the longevity of the ovule of the flower minus the lag time between pollination and fertilisation. This definition holds providing the EPP does not exceed the period of stigma receptivity. The EPP is generally limited by one of three factors: The length of stigma receptivity, pollen tube kinetics and ovule longevity. Stigma receptivity refers to the ability of the stigma to support the germination of viable, compatible pollen grains (Weiguang, et al., 2006). Stigma receptivity to pollen can persist for a short period of time (<24 hours) or for several days. Stigma receptivity is influenced by a number of factors. Whether it is a wet or dry stigma, receptivity is defined as the ability to capture pollen by adhesion, to let it hydrate and develop a pollen tube. The appropriate stage of stigma receptivity is crucial for successful pollen rehydration and pollen tube development. On a degenerating stigma, pollen was found to adhere, hydrate and germinate but pollen tube growth was quickly arrested. Optimal stigma receptivity can vary between species, from a few hours after flower opening to a few days after anthesis (Weiguang, et al., 2006). Stigma receptivity has been found to improve with increased flower age in almond (genus, species). Flowers that had opened to the flat petal stage or petal fall showed a higher percentage of pollen germination and more extensive pollen tube growth than less mature flowers. This improved stigma receptivity was attributed to enhance elongation of stigma papillae and increased amounts of stigma exudate covering the papillae (Weiguang, et al., 2006).

Pollen Deposition, Germination and Pollen Tube Growth

The adhesion of pollen onto a wet stigma is facilitated by the presence of an exudate, which can be aqueous, as in lily, or lipidic, as in tobacco and petunia. Proteins and sugars are present in both these types of exudates (Sancenon, et al., 2004). In tobacco and other solanaceous species, the lipidic exudate is produced in the cells of the secretory zone of the stigma and is secreted at pistil maturity. Beneath this exudate lies a layer of water

surrounding the secretory zone. After pollen deposition the pollen grains sink through the exudate and establish direct contact with the stigma or with each other.

If the pollen on the stigma surface is successfully hydrated then it must germinate. Within minutes of hydration the pollen grain organises its cytoplasm and cytoskeleton to support the extension of a single tube. It is not clear how the pollen selects a point for tube emergence but it is suggested that lipids, ions and water may a role to play in this polarisation. In other species with wet stigmas it has been proposed that lipids present on the stigma surface also have a role in the guidance of the pollen tube by controlling water flow to the pollen (Wolters-Arts et al., 1998). A major component in the stigma of Solanacea is triglycerides (fatty acid chains) and it has been suggested that the hydrobobicity of the exudate is a major factor in directing the pollen tube and in penetration of the stigma. Pollen tubes enter the stigma through the intercellular spaces and their growth along the surface of the stigma is likely to provide a physical cue (rather than a chemical cue) that increases the chance of penetration of the stigma surface (Lush, et al., cited in Wolters-Arts et al., 1998).

Following penetration of the stigma surface the pollen tubes must grow down the style to penetrate the ovary for successful fertilisation. Pollen germinated in vitro does not show any inherent directionality and does not appear to grow as fast as pollen germinated in vivo. The method by which the pollen tube is guided down the style is generally accepted to be by physical, rather than chemical cues. In maize and Solanaceae sp. (Heslop-Harrison and Shivanna, 1977) it is proposed that the directionality of tube growth is due to mechanical influences within the style, with the cellular architecture of the transmitting tissue provides topography to guide the pollen tube to its destination.

A small number of studies have suggested that chemical cues are involved in directionality of pollen tube growth. Localised apical changes in Ca 2+ also appear to play a role in pollen tube guidance (Malho, 2006). It has been reported in *Nicotiana tabacum* (Monteiro et al, 2005) that a transmitting-tissue specific (TTS) protein, a member of the arabinogalactan family, adhered to the surface of and the tips of the

growing tube, and was involved in the attraction and stimulation of pollen tubes. This is in contrast to the traditional beliefs of physical pollen tube guidance.

Incompatibility Systems in Flowering Plants

An incompatibility system allows the plant to discriminate between the genetically diverse pollen that land on the stigma surface, so that only compatible and not incompatible pollen germinates. There are a number of ways in which pollen tubes may be prevented from germinating on the stigma or pollen tube growth may be terminated before reaching the ovule for successful fertilisation. Incompatibility or lack of fertilisation from pollen landing on the stigma can begin at the site where the pollen adheres to the stigma. Control of pollen acceptance at the site of adhesion is seen as particularly important in plant species, such as Brassicaceae, that have a dry stigma, where surface molecules provide contact and initiate the signalling necessary for successful adhesion of compatible pollen (Taylor, 1997). In contrast, Zinkl and Preuss (2000) observed that in Solanaceae and Leguminosae species that have wet stigma, many species of pollen were able to adhere to the stigmas. As carrot stigmas produce an exudate from the surface of the stigma, carrot flowers are believed to be classified as wet stigmas, and therefore adhesion of pollen to the stigma surface should not be a yield limiting factor in carrot seed production. This theory is supported by preliminary observations under a florescence microscope of numerous pollen grains on the stigma surface, many of which do not germinate or only produce small pollen tubes in the suspected incompatible crosses.

Self-incompatibility is by far the most studied and best understood mechanism of pollen recognition and rejection (Taylor, 1997). It is estimated that between 30-50% of flowering plant species have the ability to discriminate between self (incompatible) pollen and non-self (compatible) pollen. Self or incompatible pollen is rejected at some point in the pollination process between pollen deposition and early seed set. In different species this may happen in the hydration and/or germination of the pollen grain, during growth down the style, in the ovule or even soon after fertilisation. While there are a

large number of mechanisms by which self pollen is rejected, most are under the control of a single multi-allelic S-locus, which contains the genes encoding for at least one stylar component and one pollen component. The exception to this occurs in the grasses, where genetic control of self-incompatibility is more complex. There are two predominant types of self-incompatibility systems. In sporophytic incompatibility the S-phenotype is determine by the diploid S-genotype of the parent plant, in contrast to gametophytic incompatibility, where the pollen S-phenotype is determined by its own haploid S-genotype (Taylor, 1997).

Although there is no documented evidence that either self- or cross-incompatibility reactions occur in carrot seed production or in pollination of other apiaceae species, preliminary investigations into the low yields of an inbred female line may prove this to be untrue.

a)  b)

Figure 19 - typical seed set for a) A680 029 and b) a higher yielding line

As hand-pollinations only marginally increase yields in some crosses, it is likely that there is a point of break-down somewhere between pollen deposition and early seed set. The focus of the research in this section was to identify the point of breakdown in pollination and/or fertilisation in the consistently low yielding hybrid carrot line crosses.

Materials and Methods

Plant Material and Cultural Practices

From industry observations, it has become apparent that the inbred male sterile carrot line A680029 has a history of low and inconsistent yields, regardless of the male (pollen parent) line used in the hybrid cross. This male sterile line was included in both field and glasshouse trials in an attempt to determine the point of breakdown in pollination, fertilisation or seed set that has resulted in these inconsistent and often low yields.

Table 4 - A summary of lines used in experiments undertaken described in this Section.

Line Number	Root Type		Comment
A 680 029	Amsterdam	Male Sterile	Low Unreliable Yields
A 680 209	Amsterdam	Male Sterile	Vigorous, Reliable Yields
C 680 209	Chantenay	Male Fertile	Low pollen Production/ Viability
C 680 019	Amsterdam	Male Fertile	High Pollen Production/ Viability
Yellow	Flakkee	Open Pollinated	High Pollen Production/ Viability

Plants utilised to determine the point of breakdown in fertilisation and/or seed development were grown under glasshouse conditions at the University of Tasmania, Sandy Bay. Seeds were planted in 35L easy-carry bags, filled with a standard potting mix, at a density of six seeds per bag. The planter bags were placed in a temperature controlled glasshouse (15-25^{o}C), under natural light and day-length conditions. Irrigation was provided on a daily basis via overhead sprinklers. The stecklings were allowed to grow for 8 weeks in these bags, to develop a root and pass the juvenile phase of growth (approx 8-12 leaves). After 8 weeks the stecklings were lifted, the tops of the stecklings trimmed

to 30mm above the shoot apex, dipped in a fungicide mix consisting of Benlate (a.i. benomyl), Kocide (a.i. copper hydroxide) and Manncozeb and packed into wet sand in polystyrene boxes. These boxes were placed in a cool store set at around 4^{o}C to vernalise. After 8 weeks a selection of visibly disease free stecklings required for trials was removed from the box, dipped in another Benlate, Kocide and Manncozeb fungicide mixture and replanted in 35L easy-carry bags filled with standard potting mix, at a density of four plants per bag. To avoid devernalisation, the temperature was set at 25^{o}C. Transplanted stecklings were irrigated with daily overhead irrigation. Irrigation rates were set to maintain non-stressed growing conditions until the point of flowering. Flowering commenced approximately 8 weeks after transplanting. Once flowers become receptive, cross-pollination and sampling commenced. Irrigation at this time was provided by hand watering and supplied sufficient moisture to avoid stressing the plants.

Each trial included thirty plants of each of the nominated lines. In each trial a series of reliable and unreliable crosses was performed to identify the point of system breakdown in the unreliable crosses. Crosses have been determined to be reliable or unreliable based on industry observations.

Hand Pollinations

When the flowers became receptive on the male sterile carrot lines, controlled pollinations based on the above crosses were undertaken. Stigmatic receptivity was considered to have commenced with the separation of the stigmas, which coincided with the onset of nectar secretion (Spurr, 2003). Pollen was sourced from recently dehisced anthers on the male lines included in each trial. Controlled pollinations were performed twice a week. Thirty male sterile umbellets per male sterile line were randomly selected on the basis of being receptive, at the time of hand-pollination. Of these flowers, for each male sterile line the flowers on 10 umbellets were hand pollinated with pollen from selected anthers of each of the male lines, making a total of 30 pollinated umbellets per male sterile line, 10 umbellets per cross. Hand pollinations were undertaken using pollen transferred directly from the anthers of male flowers to the stigma of the female flowers.

This involved cutting a male umbellet with freshly dehisced pollen and brushing it directly onto the chosen male sterile umbellet. Hand-pollinated umbellets were tagged with cotton tags and the male line from which the pollen for that umbellet was sourced was noted on the tag. The following tests were performed on the pollen collected from flowers of the male lines used in each cross. These tests were performed at each date of pollination.

Determination of Pollen Viability

Pollen collected from male lines used in the crosses was used to determine pollen viability for that line at the time of pollination. 10 flowers were collected from each male umbellet used in hand pollination, and these were placed into 1.5mm Eppendorf tubes. Immediately after collection, pollen samples were macerated in a solution of FDA (2mg/ml in 20% sucrose solution) on a microscope slide and assessed for viability using the methods described by Spurr, 2003.

Pollen Germination on the Stigma

At 2, 6, 12, 24 and 48 hours after hand-pollination 20 flowers were randomly selected from tagged umbellets. This sample was then split into two. One of the subsamples was halved again, with half of the sample placed into an FAA solution in a small glass phial for tissue clearing later. The other half of the sample was used to observe pollen germination on the stigma.

The remaining 10 pollinated flowers were removed from each plant, and the stylar and stigmatic tissue excised. The excised tissue was mounted on a microscope slide in aniline blue stain (0.05% water-soluble aniline blue in 0.1M K_3PO_4). 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. 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.

Determining the Period of Stigma Receptivity

Two lines, the low yielding A680029 and the reliable yielding male sterile line A680209 were used in this investigation. Pollen was sourced from the open pollinated line Yellow (Mok C), as reliable pollen production and viability were ensured from this line. The Mok C and SW A4 cross is commonly used for hybrid seed production at Bejo. For each line, 80 umbellets of uniform development were tagged from 20 plants. An umbellet was considered appropriate for tagging if the outside whorl of flowers were open with the stigmas extended but still joined together.

At 3pm on the same day, ten of the umbellets from each line were hand pollinated with fresh pollen. This was repeated at 11am and 3pm for several days until a total of 8 hand-pollinations had been undertaken on each line. Pollen germination was scored 24 hours after hand-pollination, using the methods described above.

Results

Pollen Germination on the Stigma

Pollen germination percentages of 96, 95 and 98% from lines C 680 209, C680 019 and Yellow respectively were recorded in crosses involving Yellow as the female parent (Table 5.2).

On stigmas of the first cross female, A 680 209, similar germination rates of viable pollen grains were observed. Germination of viable grains of 93, 91 and 94% were observed from the pollen of C 680 209, C680 019 and the open pollinated line Yellow, respectively. The line A 680 029 had significantly lower germination of viable grains regardless of the pollen source. The average germinations on the stigma of A 680 209 was 34, 28 and 36% of pollen grains from C 680 209, C680 019 and Yellow, respectively.

Table 5 – Average germination of viable pollen grains on the stigmas of three male fertile lines, the low yielding A 680 029, the consistently higher yielding A 680 209 and the open-pollinated line Yellow.

Male Sterile	Male Fertile	Pollen Germination (%)
Yellow OP	C 680 209	96
	C 680 019	95
	Yellow OP	98
A 680 209	C 680 209	93
	C 680 019	91
	Yellow OP	94
A 680 029	C 680 209	34
	C 680 019	28
	Yellow OP	36

Pollen Deposition on the stigma of A 680 029 compared to A 680 209

In experiments looking at the number and germination percentage of pollen grains deposited on the stigma of A 680 209 and A 680 029, pollen was found to be deposited and adhered to the stigmas of both A 680 209 and A 680 029 from the second time of hand-pollination (Day 1; 11am) through until the final hand-pollination (Day 7; 3pm Sample). Where pollen was present, the average number of grains deposited on the stigmas of A 680 209 was significantly higher than that of A 680 029. The number of grains present on the stigma surface on A 680 209 peaked on flowers hand pollinated between day 3 and day 4 with an average of 20 grains present per stigma after hand pollination from 11am on Day 3 to 11am on day 4. In the same time period, only an average of 8 pollen grains were present per stigma on A 680 029.

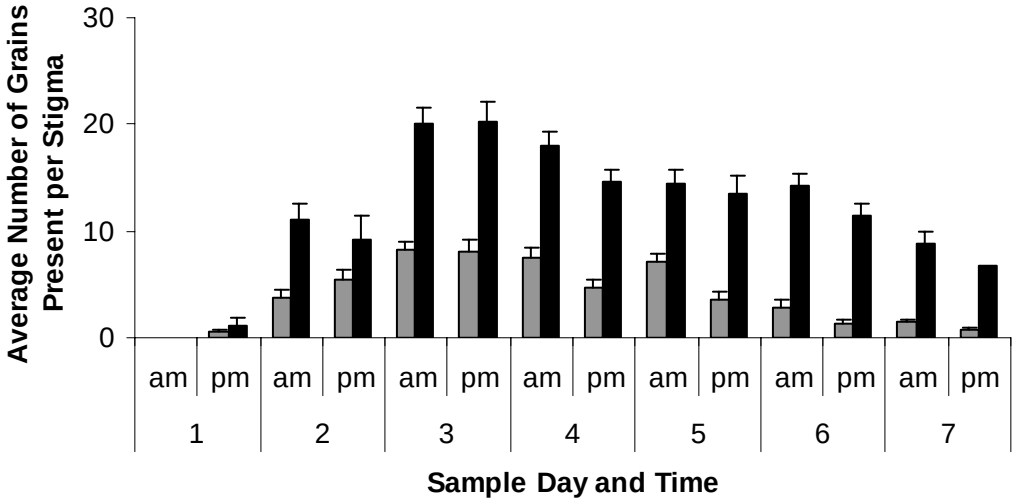


Figure 20. Average number of pollen grains deposited on individual stigmas of high yielding A 680 209, (black) and the lower yielding A 680 029 (grey).

Stigma Development and Receptivity of A 680 029 compared to A 680 209

Initial hand pollination was performed on Day One at 3 pm. No pollen germination was observed from flowers hand-pollinated on this day. Pollen germination on the stigma was first observed on stigmas hand-pollinated at 3pm on the second day of the trial for both lines. A significantly higher pollen germination of 62% was observed on A 680 209 flowers, than flowers of A 680 029 where 20% of viable grains germinated. Pollen germination increased on day 3 of the trial where the maximum pollen germination for A680 029, of 53% was observed on flowers hand-pollinated at 11am. At this time 87% of viable grains germinated on stigmas of A 680 209. Pollen germination was reduced on stigmas of A680 029 at 3pm on day three and 11am on day 4, with germinations of 34 and 32% of viable grains observed. In contrast, pollen germination on A 680 209 flowers was at it highest in this time. At 3pm on day 3, 96% of viable grains germinated, and similar germination percentage of 94% was observed at 11am on Day 4. From 3pm onward on day 4 a decreasing trend in germination of viable grains on A 680 209 grains was observed. No pollen germination was observed on stigmas of A 680 029 after from 11am on day 6 onward, and from 11am on day 7 onward in A 680 209.

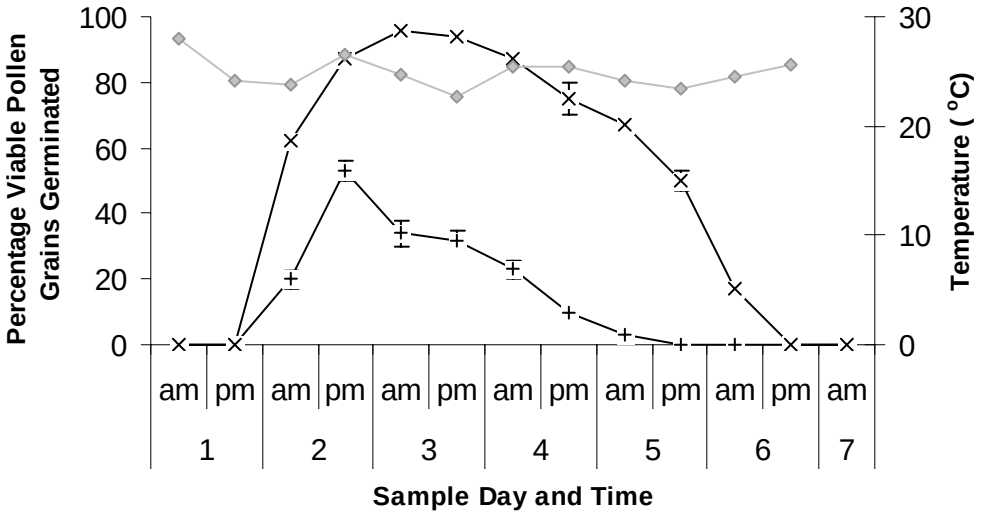
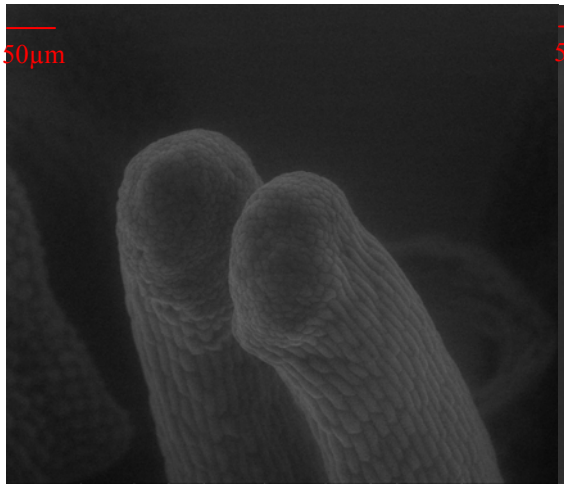


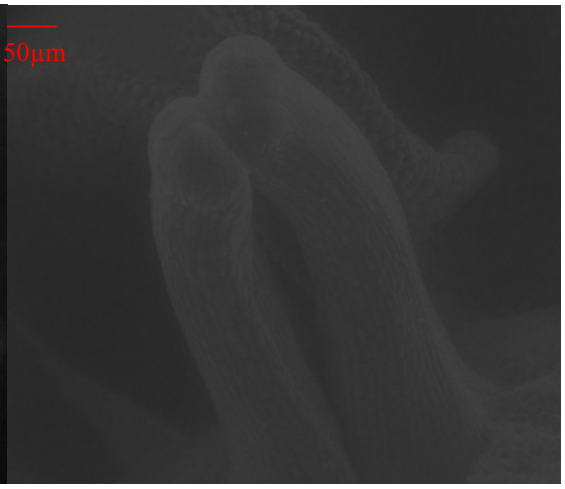
Figure 21. Percentage of viable pollen grains germinated on the stigmas of high yielding A 680 209, (x) and the lower yielding A 680 029 (+). The grey line shows temperature.

The physical development of the stigmas was documented using environmental scanning electron microscopy. Stigma development for both lines A680 029 and A 680 209 can be tracked in the images below. No obvious physical differences between the two lines were detected over an 8 day period.

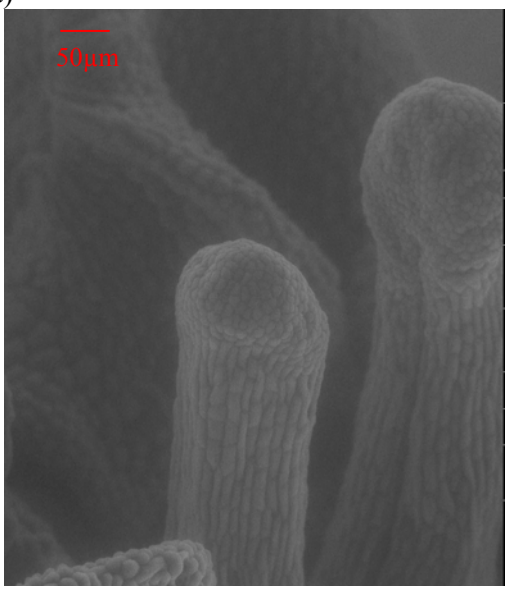
1 a)



2 a)

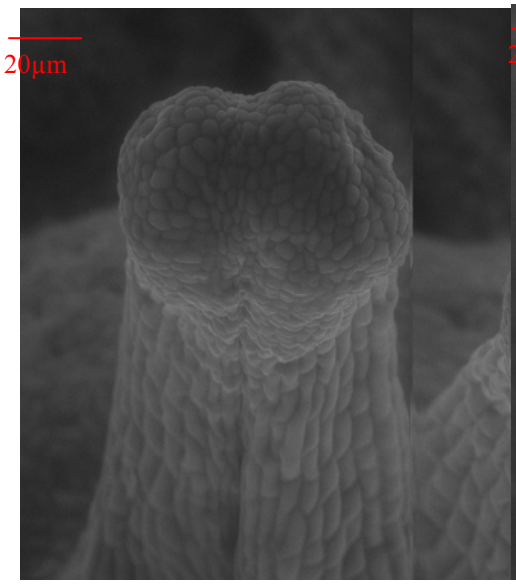


2 a)

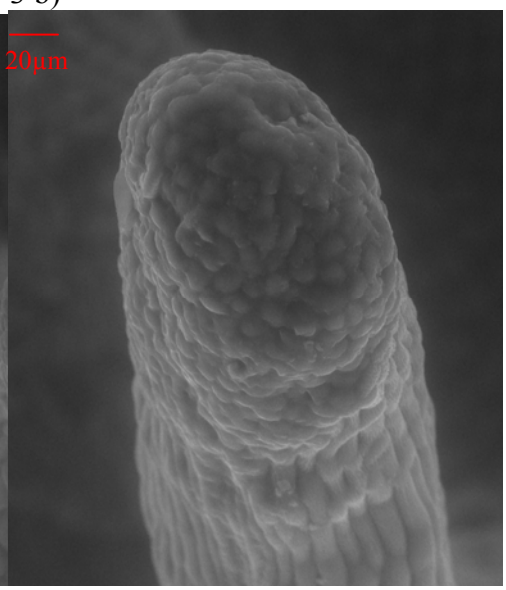


2 b)

3 a)



3 b)



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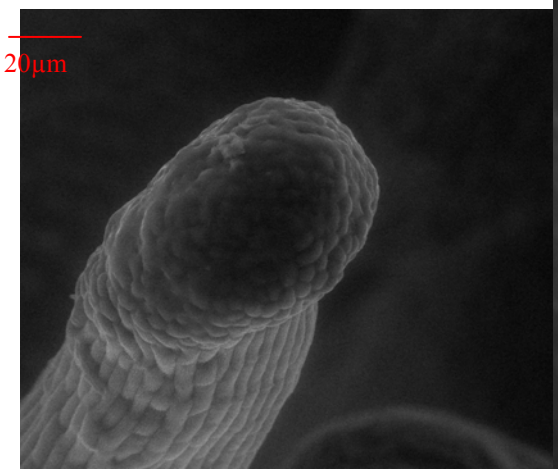

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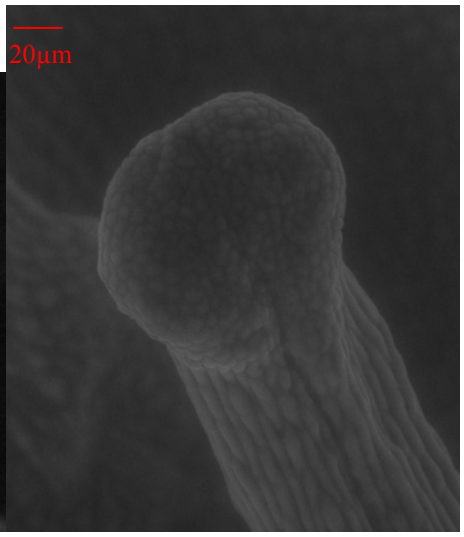
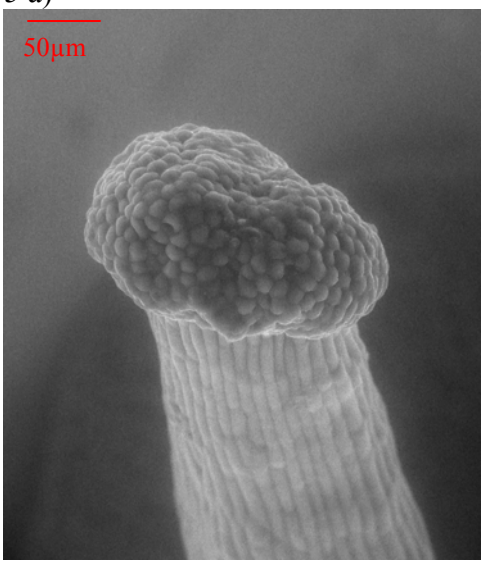
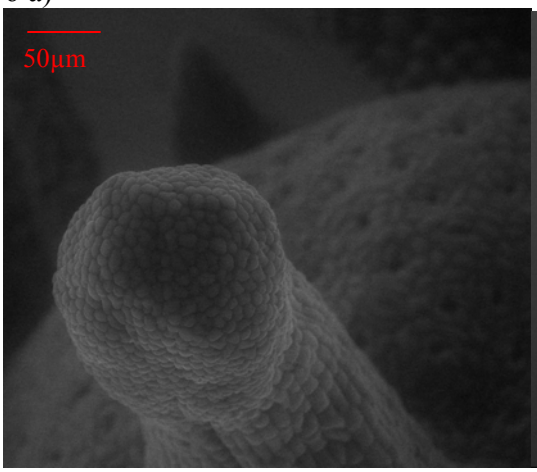


5 a)



6 a)



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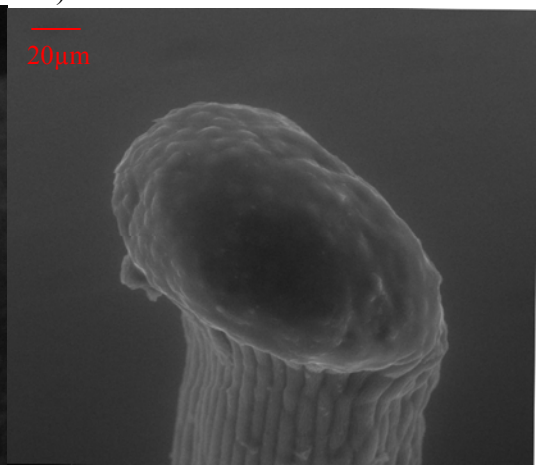

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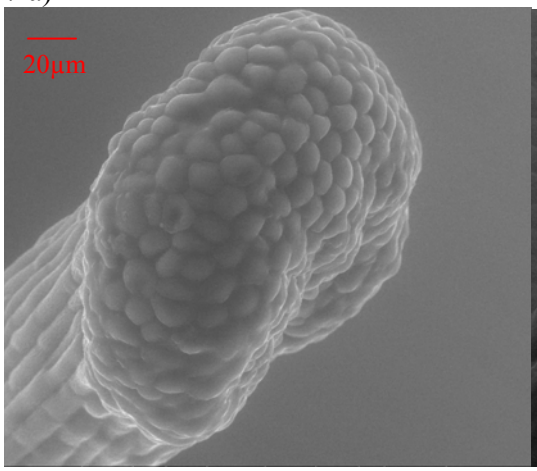
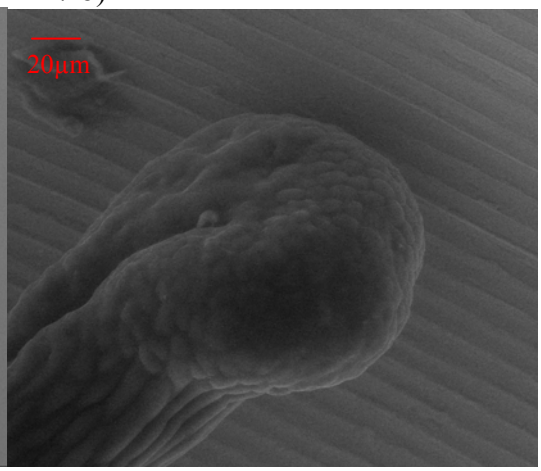


7 b)



8 a) 8 b)

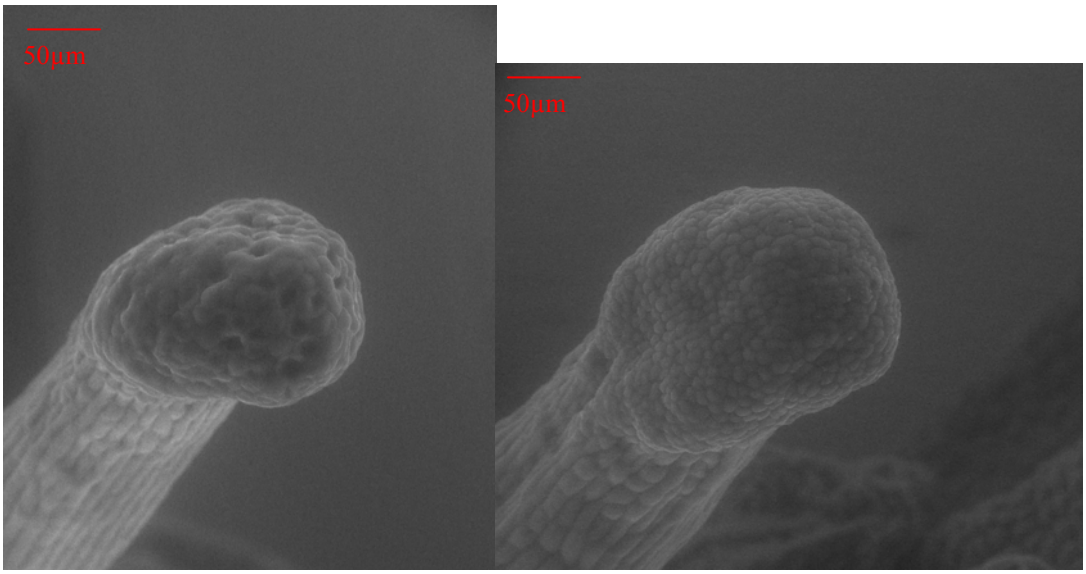


Figure 21 – Stigma morphology using environmental scanning electron micrography for Lines A680 029 (a) and A 680 209 (b) over an 8 day period commencing 1 day prior to flower opening.

Discussion

Incompatibility responses between carrot varieties have not previously been documented, but in the experiments described in this section a significant difference between stigmas of different parent lines in capacity to support pollen germination were documented. This apparent incompatibility response may explain some of the differences in yield reliability observed between different crosses under commercial seed production conditions. Consistent high yielding male sterile parent lines were documented to support higher pollen germination percentages, and greater numbers of pollen grains on the stigma surface, following hand pollination.

The duration of stigma receptivity was also noted to vary between lines, with the lower yielding A 680 029 line having a reduced duration of stigma receptivity and low pollen germination percentage on the stigma surface throughout its receptive period. A study of stigma morphology did not reveal any obvious physical differences between the high and low yielding lines, suggesting that differences in the capacity of the stigmas to retain pollen after hand pollination and support pollen germination were related to chemical rather than physical attributes. Attempts to characterize chemical differences were undertaken but the methodology employed was not sufficiently sensitive to detect any differences. Given the significance of the differences in compatibility between parent lines, further investigations into the nature of the pollen pistil interactions is recommended.

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