Characterisation and Management of Flower Abortion in Inbred Male Sterile Onion Parental Line ON019A

By

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Declaration

I declare that this thesis contains no materials which has been accepted for the reward of any other degree diploma in any other tertiary institution and, to the best of my knowledge and belief, contains no copy or paraphrase previously published or written by any other person except where due reference is made in the text of the thesis.

Padmanaban Sriram

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Abbreviations

% = Percentage

® = Registered Trade Mark

Cm = Centimetre

Cv = Cultivar

G = Gram

GA_3 = Gibberellic acid

Hrs = Hours

Km = Kilometre

L = litre

LSD = Least Significant Difference

M = Metre

Ml = millilitre

MM= millimetre

NR = Not Recorded

NS = Not Significant

°C = Degree Celcius

P/L = Proprietary Limited

PC = Personal Computer

SEM = Standard Error Mean

W/W = Weight / Weight

Abstract

Hybrid onion seed production is popular throughout the world. Production in Australia is small by world standards but with significant potential for expansion due to availability of suitable production sites and the counter-seasonal production to Northern Hemisphere producers. Variable and often very low seed yield from inbred parent lines has been identified as a significant problem limiting expansion of hybrid onion seed production. This thesis investigated the issue using the weak inbred male sterile onion parental line ON019A used in hybrid seed production by Enza Zaden Australia Pty Ltd as a model system. ON019A is prone to poor seed yield, typically less than 0.5 g/plant but has produced yields up to 8 g / plant at some sites. The typical low yields of this line coupled with potential for much higher yields under certain circumstances make this a useful line for identifying and studying the processes contributing to low yield in inbred male sterile onion parent lines. The project had two broad objectives

i) to identify the cause of the variability in seed yield between onion crops and

ii) to improve the seed yield by developing commercially applicable management practices.

The research initially focussed on pollination as seed industry representatives had suggested that insufficient pollination may be the primary cause of low yields; failure of hand pollinated flowers to set seeds confirmed that pollination was not a significant contributor to low seed yield. Observation of flowers during the supplemental hand pollination experiments identified two classes of flowers; a) flowers with fully extended stigma (>4 mm long) and open petals; and b) flowers in which the stigma extended less (<2 mm long) and petals did not fully open. Whilst flowers with fully extended stigmas set seed after hand pollination, flowers in which the stigma only extended to

2 mm did not. This observation led to the hypothesis that abortion of the flower before anthesis was the primary cause of poor seed set in ON019A.

Evidence supporting the flower abortion hypothesis was gained in experiments comparing flower development in ON019A with other higher yielding inbred lines ON013A and ON0138A together with Kingswood an open pollinated pollinator line. Experiments then focussed on crop management practices to manipulate flower development of ON019A; these included mother bulb characteristics (timing of lifting, bulb size, bulb storage temperature), planting date, crop nutrient status, irrigation management, application of plant hormone and anti-transpirant sprays.

The results suggested that the size of bulb had a significant effect on flower development with large sized bulbs having a reduced flower abortion percentage and increased seed yield. A single late application (at the 60% piping stage) of gibberellic acid (GA_3) at 450 ppm also improved the seed yield and reduced the flower abortion percentage. While improvements in seed yield through reduced rates of flower abortion occurred in the large bulb size and GA_3 application treatments in most trials, significant variability existed between production locations in the size of the response. It was concluded that site specific environmental conditions could interact with bulb size and GA_3 application treatments and affect the seed yield.

Subsequent experiments on irrigation and protection of plants from conditions that favoured high transpiration rates under glasshouse conditions revealed a reduction in flower abortion and an increase in the seed yield following the application of an anti-transpirant together with frequent irrigation. The percentage of flower abortion was low and seed yield per plant high in all treatments under glasshouse conditions, again suggesting an environmental influence on flower abortion.

Under field conditions protecting umbels with wind breaks resulted in a low percentage of flower abortion and comparatively high seed yields (up to 5g/ plant) in the low vigour inbred ON019A line.

Improvement in seed yield from the inbred male sterile line ON019A can be made through careful production site selection, use of wind breaks during flowering, use of large mother bulbs, strategic application of gibberellic acid and anti-transpirants. There is a good opportunity to increase the onion seed production in Australia particularly with further research focussing on understanding the effects of environmental conditions at different locations and refinement of management techniques to overcome the flower abortion problem and to improve reliability of seed yields.

Chapter 1

General Introduction

1.1 Introduction

Hybrid onion seed production is an increasingly important component of the vegetable seed industry throughout the world. As yields can be quite variable, there is a significant potential for increases in size of the industry through improvement in reliability of seed yield. Hybrid seed production requires crossing between inbred parent lines, the use of some lines with low vigour and narrow range of cultural and environmental requirements for adequate crop performance have led to problems in consistently achieving high seed yields. The continuing development of new parental lines presents challenges to seed producers as the effect of environmental factors and management practises on crop growth and development must be assessed in order to develop production guidelines for each line. Seed producers who are able to gain an understanding of the factors affecting crop development and therefore quickly develop and refine crop management strategies are able to take advantage of emerging hybrid seed production markets.

Australia has a small but expanding hybrid onion seed production industry, and there is a possibility to increase the seed production. The Southern Hemisphere location allows counter season production to major Northern Hemisphere markets and a range of climatic zones available for production. The challenge facing Australian producers is to identify major limiting factors in production of specific lines and develop strategies to overcome these limiting factors. An understanding of onion plant physiology and agronomy is a necessary component of a research program identifying limiting factors and developing management strategies.

1.2 Onion History

The earliest record of onion cultivation comes from Egypt, as carvings on pyramid walls and in tombs from the third and fourth dynasties (2700 BC), onions were also cultivated by the ancient civilisations of Greeks and Romans (Hanelt, 1990). Its use in embalming ceremonies is depicted in various necropolis wall murals, Dioscorodes gave an account of the medicinal properties and the culinary value of the plant. The use of onion in medicines is recorded in Indian script dated 6th century BC (National Onion Association www.onions-usa.org), onions were cultivated by the ancient Egyptians as an important food item and it was also considered to have religious associations. It is thought that the Romans took the onion north of the Alps and onions were among the first cultivated plants taken to the Americas from Europe. Europeans took the species to East Asia during the last century (Hanelt, 1990).

1.3 Taxonomic Position

The onion, *Allium cepa* is cultivated mainly as a biennial, but some varieties are treated as perennials propagated from seeds, bulbs or sets (small bulbs). The bulb shows a wide variation in shape, size and colour; intensive selection during domestication and natural hybridisation may have created this variability. The taxonomic position of *Allium* and related genera is still a matter of controversy. In early classification of the angiosperms Engler, Bentham and Hooker (1862) placed this genus in the family Liliaceae, however, for the past 50 years British and American botanists like Hutchinson (1973) and Traub (1972) have included it in the Amaryllidaceae, on the basis of inflorescence structure. More recently, prominent taxonomists such as Cronquist (1968) and Takhtajan (1973) have favoured a very wide concept of Liliaceae that includes the former Amaryllidaceae. *Allium* and its close relatives are recognized as the distinct family Alliaceae, closely related to Amaryllidaceae and the following hierarchy has been adopted;

1. Class: Monocotlyedons
2. Sub-class: Liliflorae
3. Order: Asparagales
4. Family: Alliaceae
5. Tribe: Allieae
6. Genus: *Allium*
7. Species: *cepa*

There are more than 700 species in the genus *Allium* originating in both the Old and New World, they are classified in to six subgenera (Hanelt *et al.*, 1992); this polymorphic and taxonomically complicated genus has attracted the attention of botanical investigators for many years so changes in this classification system may occur in the future. The genus *Allium* is widely distributed over the sub-tropical and temperate zones of the Northern Hemisphere. The genus is of a great economic significance because it incorporates several important vegetable crop and ornamental species, including onion and shallot (*A. cepa*), garlic (*A. sativum*), leek and elephant garlic (*A. ampeloparasum*), Japanese bunching onion (*A. fistulosum*) and chives (*A. tuberosam*). Many *Allium* species are also grown as ornamentals including *A. giganetum*, *A. christophii*, *A. karataviense*, *A. aflatunense*, and *A. caeruleum.*

1.4 The Australian Onion Seed Industry

Onion seed production in Australia commenced in the early 1940's. Primarily the industry concentrated on the open-pollinated varieties such as Spanish types and brown onions originating from Pukekohe Longkeeper varieties from New Zealand. These were produced in areas around Victoria, South Australia and Tasmania to supply the local bulb production industry (www.mkseeds.com.au). The major seed companies involved in onion seed production in Australia are South Pacific Seeds, Henderson, Bejo Seeds and Enza Zaden, who manage their own breeding programme in Australia as well

as producing seed. Production statistics for the onion seed industry are not publicly available in Australia but have been estimated at $3-5 million annually (Richard Jones, *personal communication*). As much of the seed produced in Australia is used for the domestic bulb production, the size of the bulb industry is indicative of the size of the seed industry. Approximately 250,000 tonnes of onion bulbs are produced with a farm gate value of $A140 million annually (HAL, 2004). South Australia and Tasmania are the major production locations, producing approximately 65% of the national crop (HAL, 2004).

In Australia the predominant open-pollinated varieties of onion are Kingswood, Creamgold, Early Lockyer White and Lockyer Brown. While hybrid cultivars are not widely grown, they have been assessed in industry trials and hybrids including Gladiator, HA890, Predator, Omega and SPS 846 were reported to be outstanding (New South Wales Department of Primary Industries, 2004).

1.5 Cultural Practices for Onion Seed Production

Two different strategies are commonly used for onion seed production, the methods are referred to as bulb to seed and seed to seed respectively. The bulb to seeds strategy requires a bulb crop to be grown, harvested and stored and the seed crop is then produced from the planted bulbs. The seed to seed strategy involves establishing the crop from seed, allowing the plant to be vernalised to induce flowering and subsequently produce seeds (Brewster, 1994).

The advantage of the bulb to seed method is that it allows selection of the mother bulbs for seed stock and this permits easy identification of off types (Peters, 1990). The bulb to seed method does, however, require an extended time for seed production and generally requires storage facilities for bulbs. In hot regions it is essential that mother bulbs be vernalised at 8 – 10 °C for at

least 90 days before planting (Currah *et al.*, 1990). In seed to seed production the plants require sufficient vernalisation to induce 100 percent flowering; seed is therefore sown several weeks earlier than bulb crops to ensure plants are large enough (no longer juvenile) to receive the vernalisation stimulus commencing in autumn (Jones & Mann, 1963). The juvenile phase includes germination and the vegetative growth phase prior to attaining competence to initiate flowering. Growth during this phase is promoted by temperature around 25 °C, good water supply, root zone aeration and adequate nutrition especially nitrogen (Brewster, 1994).

During the post juvenile phase (also referred to as the thermo-phase) flowering may be induced by exposure to low temperatures (7-12 °C), this phase has also been termed the competition phase because of the apparent competition between the inflorescence development and bulb development (Brewster, 1994). The inflorescence rate of initiation is also dependant on the bulb size, in the life cycle of onion and sometimes there is a chance the progression to flowering can be reversed, for example by devernalisation.

This makes progress in the flowering phase difficult to manage and assess as (i) different phases need different environments in the sequence, (ii) cultivar and bulb size affect duration in each phase, and (iii) the possibility exists for deviation or reversion as a result of environmental factors (Brewster, 1994). In hybrid seed production the most important thing to be followed is isolation distance, since onions are cross-pollinated and it is easy to get varietal mixture from the foreign source. The hybrid seed production plot should have an isolation distance of 2-5 km between seed fields (Jones & Mann, 1963; Pathak, 2000), in Australia generally 2 km isolation distance for onion hybrid seed production is adopted.

1.6 Reproductive Biology

The flowering stages have been broadly classified into four different phases: (i) juvenile phase, (ii) thermo-phase, (iii) competition phase and (iv) completion phase (Kampen, 1970). During the juvenile stage the onions cannot be induced to flower and must reach certain critical weight or leaf number to enter into the second stage, the thermo-stage, where induced by low temperature the flower development sequence starts. For each stage the plants require specific temperatures; for the juvenile phase requiring 5-25 °C and the thermo phase requiring low temperatures of 7-12 °C. Once the spathe starts breaking the plant enters into the competition phase of flowering, the completion phase involves the spathe opening and expansion of the approximately 50 – 2000 flowers (Brewester, 1982).

1.6.1 Individual Onion Flower Development

The individual onion flowers are perfect; the floral organs are three outer petals, three inner petals, and six stamens arranged in two whorls consisting of three outer stamens, three inner stamens and a trilocular ovary with two ovules inside the carpel. The styles arise from the apex of the three fused carpels and elongate when the flowers open (Jones & Emsweller, 1937) (Figure 1.1). The individual onion flower developmental stages have been studied and divided into six main stages (Lesley Currah & Ockendon, 1978):

Stage 0	Opening of flower bud.
Stage 1	Start of anther dehiscence.
Stage 2	End of anther dehiscence.
Stage 3	Appearance of stigmatic knob.
Stage 4	Withering of filaments.
Stage 5	Start of withering of style.
Stage 6	Completes withering of all floral organs except ovary.

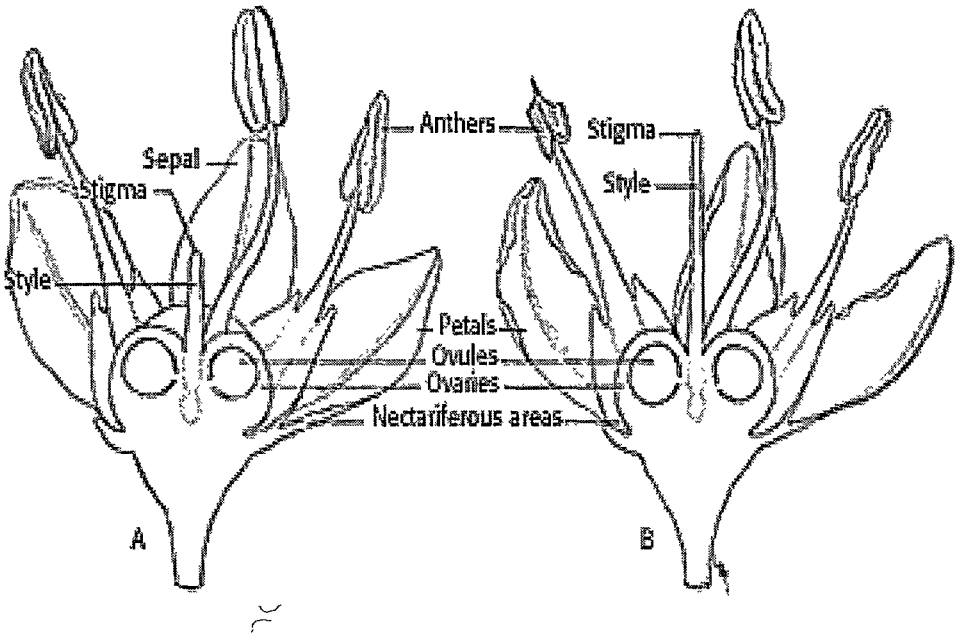


Figure 1.1 – Dissected onion flower with all the floral organs. A) Flower with the short style prior to anthesis, B) Flowers with extended style after anthesis, Figure source from http://gears.tucson.ars.ag.gov/book/chap6/onion .html.

Onion flowers are protandrous in nature and most of the onion flowers open during the warm part of the day between 9 am and 5 pm (Agati, 1952). The three anthers of the inner whorl shed their pollen first followed by the anthers of the outer whorl up to a day later; the pollen is usually shed in the 2 days after flower opening (Agati, 1952). Nectar is secreted from nectaries on the ovary walls opposite to the broad bases of the inner stamens (Brewster, 1994).

Stigma receptivity commences about 84 hrs after the beginning of flower opening and lasts for about a further 108 hrs. The stigma does not attain a fully receptive state until the stigmatic knob has developed and produced a sticky exudate (Lesley Currah & Ockendon, 1978). At the time of flower opening the style is approximately 1 mm long, and it rapidly elongates to a final length of 4–7 mm; variations in style length occur between cultivars and to a lesser extent between individual plants within cultivars (Currah, 1981).

1.6.2 Pollination in Onion

Onion flowers are entomophilous (insect pollinated) and many different species are attracted to full bloom onion flowers, although honey bees are the most important insects that pollinate onion flowers (Pathak, 2000). Other insects including syrphids and blowflies such as *Syrphus vitripennis*, *Syrphus ribesii* and *Thricops rostratus* are common visitors of onion flowers (Williams, 1973). Optimal rates of pollination occur when the air temperature is above 16 °C as pollinators move frequently from umbel to umbel increasing the chance of cross pollination (Brewster, 1994). Successful pollination of onion flowers is highly dependant on the nectar production (Silva & Dean 2000) In order to attract bees in hybrid seed production the male sterile flowers should produce and retain sufficient nectar while pollinators are active, honey bees are not interested in visiting certain varieties of onion because they produce little or no nectar (Silva & Dean, 2000).

High seed set and good embryo development have been recorded in plants held at 35/18 °C day/night temperatures. Relative air humidity below 70% has been shown to improve the pollen shedding from the anthers (Chang & Struckmeyer, 1976b).

1.6.3 Pollen Viability

Viability of pollen is also one of the factors to be considered for successful seed production. Viability of the pollen also varies with temperature, thus temperature effects on seed production can be a direct effect of temperature on pollen viability and an indirect effect through the behavioural aspect of the insects. Pollen viability has been shown to vary in duration, with rapid reduction in viability immediately after anther dehiscence under some conditions and significantly higher percentages of viable pollen several days after anthesis under other conditions (Lesley Currah & Ockendon, 1978). The

viability of pollen was high at a temperature of 23 °C and reduced by exposure to higher temperatures, pollen viability in this study was assessed *in-vitro* with the aniline blue method (Kho and Baer, 1968).

1.6.4 Pollen Germination and Fertilization

Fertilisation may occur as early as 12 h after pollination and is complete within 3-4 days. The embryo was reported to be visible under light microscope after 6 days (Brewster, 1994). At 24 days after flowering the outer coat of seeds turns black and the seeds are fresh (Brewster, 1994), the endosperm matures, solidifies and turns black 45 days after flowering (Gray & Ward, 1987) and at this stage the capsules shed their seeds.

1.6.5 The Onion Seeds

Onion seeds are black in colour, oval and flattened. At high temperature and humidity, onion seeds lose their viability quickly, so commercially harvested seeds are dried properly until the moisture content comes below 6.3%, then seeds are sealed in moisture proof containers permitting a storage life of more than three years (Ellis & Robert, 1981). The embryo in onion seeds is curled within the seed, the cotyledon forms the bulk of the embryo and consists of mostly small cells densely packed with reserves of globular fatty protein and phytin sugar phosphate (Demason, 1990). At the tip of the cotyledon, embedded in the surrounding endosperm, is a swelling termed the haustorium.

During germination the lower portion of the cotyledon elongates first and root extension occurs later in germination. Following sowing, the primary root grows downwards at seed sowing depth (5 cm) while the cotyledon forms a U shaped bend described as a sharp knee and pushes upward to the soil surface

by the elongation of cotyledon on either side of the knee, thus the cotyledon emerges from the soil as a characteristic loop shape (Brewster, 1994).

1.7 Hybrid Seed Production System in Onion

Hybrid onions have been able to be produced commercially since the discovery of a male sterility system in an Italian red variety (Jones & Emsweller, 1936). The popularity of hybrid onion is still growing. The advantages of hybrids are that they show more vigour then normal cultivated varieties, more uniformity and improved yield. Onion breeders have recognised the potential value of the hybrid cultivars and there have been many hybrids introduced into commercial seed production (Dowker & Gordon, 1983). Hybrid seed production in onion uses cytoplasmic male sterility systems in parent lines. A hybrid programme often involves three parents; a male sterile line referred to as the A line, a B line (Maintainer line) and C line (Male fertile). The hybrid seeds are collected from the A line following pollination from the C line (Pathak, 2000), while the B line in this hybrid system is used for the maintenance of the inbred parental lines (Figure 1.2).

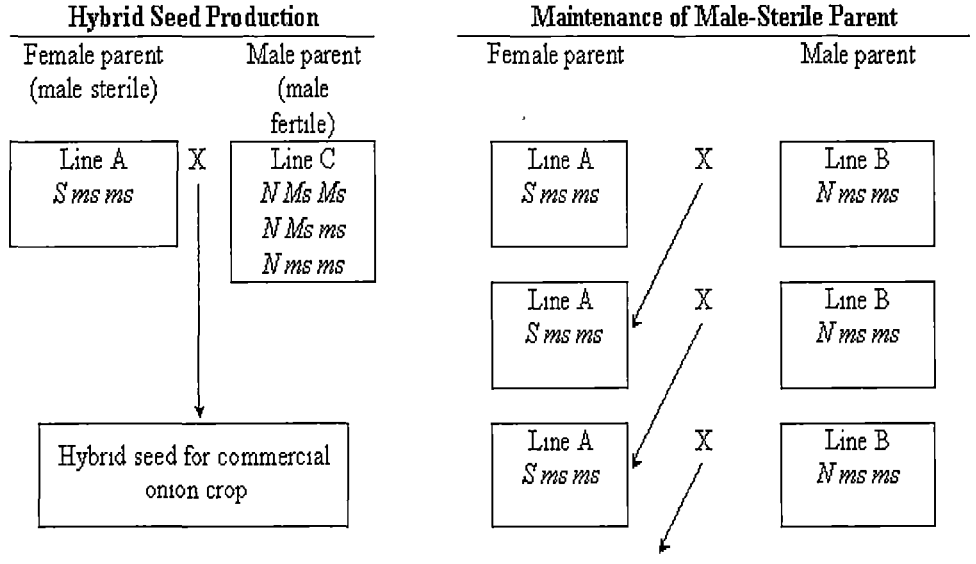


Figure 1.2 – Schematic representation of hybrid seed production in onion and maintenance of male sterile parent.

1.8 Importance of Male Sterile Line in Hybrid Breeding

According to Erickson & Gabelman (1954) male sterile parents tend to be more susceptible to crop failure because of their inbreeding depression and for various reasons they lose their vigour and eventually fail to set seeds. The low yield in a male sterile line compared to an open pollinated variety was shown to be linked to development of abnormal florets, which were present at a rate 20 - 48 % greater in an inbred parent line than in the open pollinated variety 'Ebenezer'. Generally the problem with hybrid seed production is the failure of parental line to set enough seeds and the difficulties in maintaining these lines.

1.9 Background of ON019A

The male sterile parent ON019A was bred and released by Enza Zaden B.V Netherlands, this line has quality attributes including partial resistance to downy mildew, good general combining ability with different male parents and produces hybrids of intermediate day length requirement with good skin colour and skin strength.

The industry partner has stated that the line has a major weakness in that it is not a reliable producer of acceptable seed yields. While it has potential to produce yields as high as other inbred male sterile lines an unacceptably high proportion of crop yields can be very low. Crop failures in other male sterile lines, have also been observed but at a lower frequency than in ON019A and therefore the line is a useful model system for examining mechanisms of low seed yield in hybrid onion parent lines.

1.10 Research Impetus and Project Focus

This research project was developed by Enza Zaden B.V Netherlands in collaboration with South Pacific Seeds and was initiated primarily to address unreliable seed yield in male sterile onion parent lines used in the hybrid breeding programme. The project focused on the weak inbred male sterile onion parent ON019A. The main objective of the present project was to identify the problem behind the poor seed yield and to develop feasible crop management techniques to mitigate the problem in order to achieve more reliable seed yield.

1.11 Outline of Thesis

Chapter 1 Provides a general background to the Australian onion seed industry, introduces aspects of reproductive development and seed production in onions that are relevant to the research undertaken in the project and outlines the project focus and thesis structure.

Chapter 2 Describes the general materials and methods used in the project. The plant material used in all experiments, details of field sites, controlled environment facilities and experimental protocols are outlined.

Chapter 3 Describes preliminary research aimed at identifying the nature of yield limitation in ON019A hybrid seed crops. Data collected in these trials were used to identify flower abortion as the major yield limiting factor in ON019A.

Chapter 4 Details the effects of a range of agronomic treatments aimed at ameliorating flower abortion in ON019A. The purpose of these treatments was twofold; firstly to provide possible management strategies to improve hybrid seed production from ON019A and secondly to identify possible mechanisms by which flower abortion is induced.

Chapter 5 Outlines experiments designed to refine promising agronomic treatment and initial investigations into the physiology of flower abortion in ON019A with particular references to the role of water relations.

Chapter 6 Draws together the major conclusions from the research chapters and outlines a series of recommendations for the production of ON019A crops and ongoing research into flower abortion.

Chapter 2

General Materials and Methods

2.1 Plant Materials

The onion cultivars used in the project were inbred male sterile lines produced by the Enza Zaden BV Netherlands breeding programme. The main focus of the project was the line designated ON019A, in addition to the inbred lines. The open-pollinated cultivars Kingswood and Creamgold were used in plant development comparisons with the inbred lines and as pollen parents in crosses with the male sterile lines. The inbred lines, cultivars ON019A, ON013A and ON0138A, were intermediate day length brown bulbing onion types, these cultivars were selected based on their reliability in seed yield. ON019A was unreliable in seed set with crops often producing low seed yields, while ON013A was a slightly more reliable seed producer and ON138A consistently produced good seed yields.

The open-pollinated varieties were long day length, brown, bulbing onions; the cultivar Kingswood was used in the 2005-06 growing season, and the cultivar Creamgold was used in the 2006-07 growing season. All trials utilized bulbs grown by commercial suppliers involved in onion seed production in Australia; bulbs were produced using standard commercial practice and unless stated otherwise in the methods for specific experiments were stored at 4 °C prior to use.

2.2 Field Trial Sites

The project involved experiments conducted under both field and controlled environment conditions. Field experiments were conducted in commercial seed crops using standard commercial production practices or in research plots where a range of treatments could be imposed under standard field environmental conditions. The field experiments were conducted in several seed production regions in Australia; Swan Hill in Victoria (35.34°S, 143.5°E), Mt Gambier in South Australia (35.34°S, 143.5°E) and at Cambridge (42.5°S, 147.3°E), Campania (42.87°S, 147.3°E) and Old Beach (42.5°S, 147.3°E) in Tasmania (Figure 2.1). The prevailing climatic conditions are shown in the (Figure 2.2 and 2.3).

All field trial sites in Swan Hill and Mt Gambier were commercial crops. The Campania site in Tasmania was a commercial seed crop, while the remaining sites in Tasmania were experimental plots at the University of Tasmania farm (referred to as University Farm) 15 km North of Cambridge, the South Pacific Seeds research farm (referred to as SPS Farm) 12 km North of Cambridge, and a leased area of land at Old Beach 10 km south west of Cambridge.

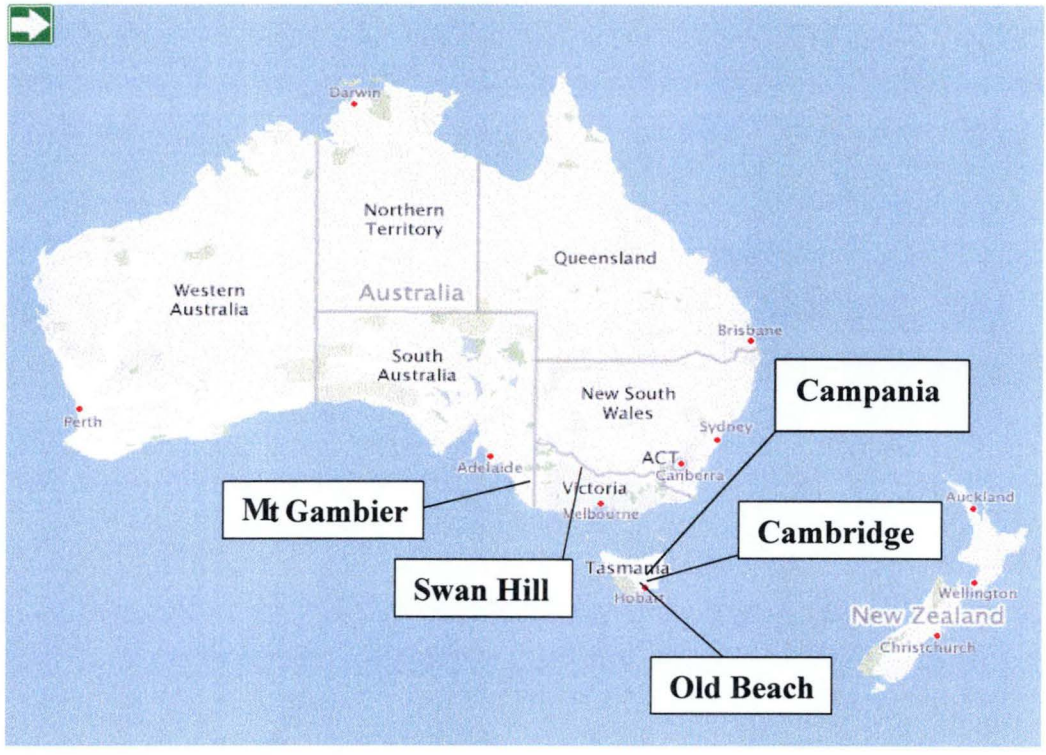


Figure 2.1 – Australian field sites were located in Cambridge (42.5°S. 147.3°E) and Old beach (42.5°S, 147.3°E) and Campania (42.87°S, 147.3°E) in Tasmania (42.5°S, 147.3°E), Swan Hill, Victoria and Mt Gambier (35.34°S, 143.5°E) in South Australia. Map source: www.streetdirectory.com.au

Old Beach Months	Minimum (° Celsius)	Maximum (° Celsius)	Day length (Hours)
July	6.65 °C	17.88 °C	8.9
August	4.82 °C	16.82 °C	10.2
September	4.50 °C	18.39 °C	10.6
October	8.08 °C	21.18 °C	12.9
November	9.12 °C	24.75 °C	13
December	10.76 °C	26.47 °C	13.5
January	11.66 °C	29.76 °C	13
February	12.26 °C	27.27 °C	12.8
Uni Farm Months	**Minimum (° Celsius)**	**Maximum (° Celsius)**	**Day length (hours)**
July	9.66 °C	24.48 °C	8.9
August	5.21 °C	16.16 °C	10.2
September	5.00 °C	17.36 °C	10.6
October	7.98 °C	19.97 °C	12.9
November	9.26 °C	22.04 °C	13
December	10.61 °C	23.17 °C	13.5
January	11.24 °C	25.80 °C	13
February	10.98 °C	24.54 °C	12.8

Table 2.1 Mean temperature data from the Old Beach and University Farm from the Tasmanian sites. Soil type Old Beach Red Soil (vertosol) Cambridge (black clay)

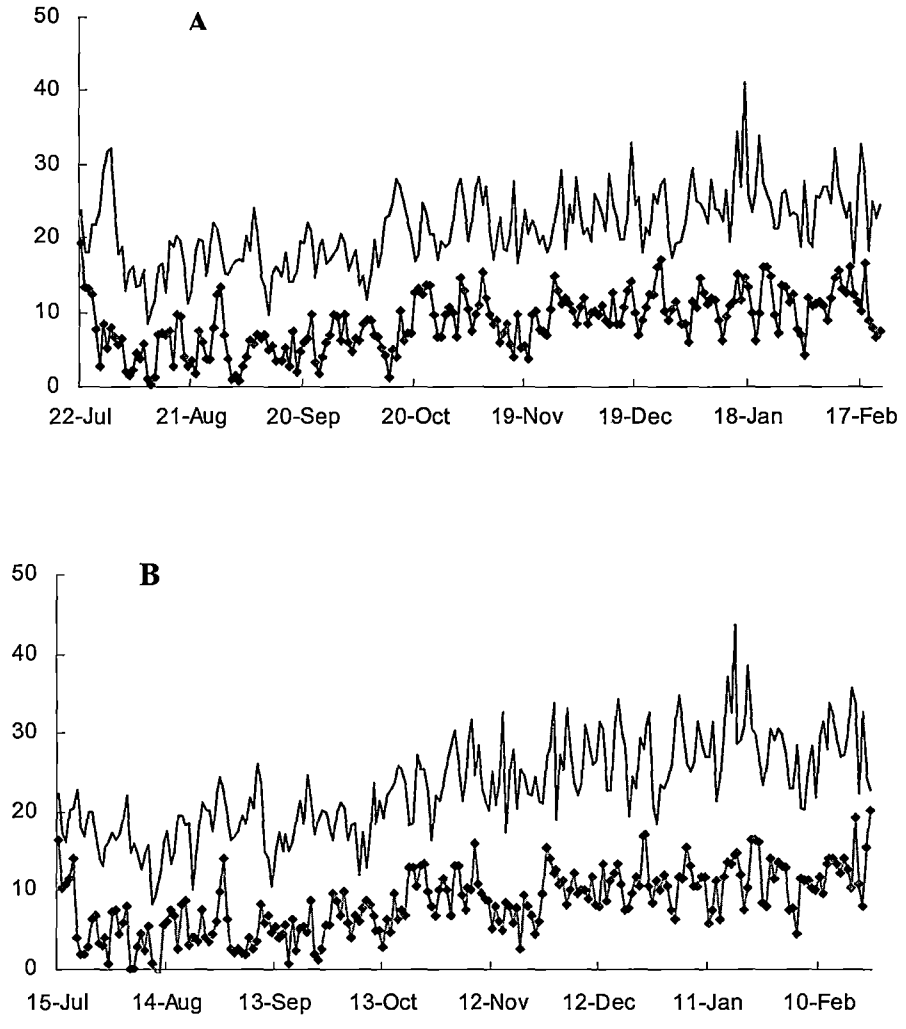


Figure 2.2 – Long term minimum (♦) and maximum (straight line) temperature conditions prevailing at the experimental site in Tasmania at two locations A) University Farm (Cambridge) and B) Old Beach 2006

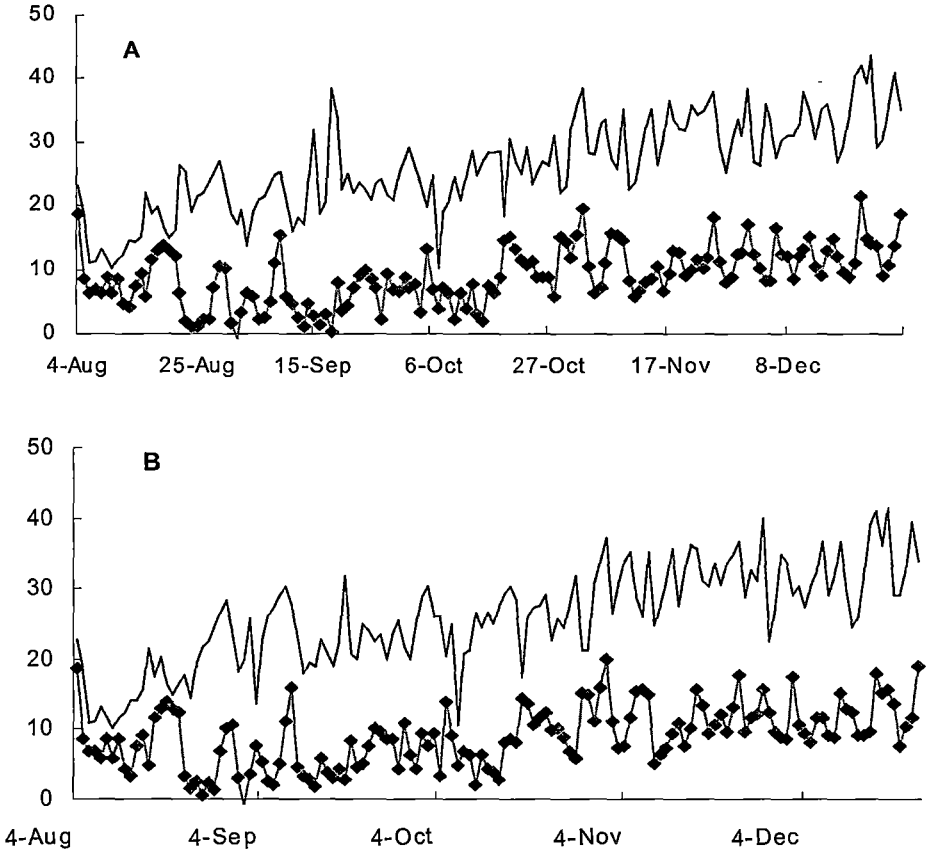


Figure 2.3 – Long term minimum (♦) and maximum (straight line) temperature of monthly mean at the two experimental sites at Swan Hill, A) Grivell and B) Hardy 2006.

Hardy Months	Minimum (° Celsius)	Maximum (° Celsius)	Day length (hours)
August	7.314286	17.50714	10
September	6.110714	22.99643	10.5
October	8.903226	25.60968	12.8
November	11.36	31.71333	13
December	12.19583	32.67083	13.5
Grivell			
Months	Minimum (° Celsius)	Maximum (° Celsius)	Day length (hours)
August	7.428571	18.06786	10
September	5.33	22.49333	10.5
October	8.425806	25.03548	12.8
November	10.92	31.49	13
December	12.49 °C	33.88 °C	13.5

Table 2.2 Mean temperature data from the Hardy and Grivell sites Swan Hill

Experimental plots at the two Cambridge sites (University Farm and SPS Farm) were on duplex soils with grey sandy loam soil and a clay sub soil. Field trials at Old Beach were on deep, red sandy loam soils. Water status at the University Farm and SPS Farm field trials was monitored with gypsum blocks (MEA, GBReader®) positioned in the root zone. Temperature data for all trials were collected using Tiny Tag® (Hasting Data Loggers P/L) temperature loggers placed in Stevenson's screens located 1 m above ground level. Where needed, other data was collected onsite or where appropriate from the nearest Australian Bureau of Meteorology weather station. All field trials were managed using current commercial crop management practises for the production of bulb to seeds in onion bulb, except where specific treatments varying from commercial practice were imposed. Nutritional, pest, weed and disease management was done following the current commercially recommended cultural practises and techniques.

2.3 Controlled Environment Trials

The controlled environment trials were conducted in the University of Tasmania, Hobart campus, School of Agricultural Science glasshouse complex. The specific glasshouse and shade house used were glasshouse number 8, and shade house number 12. The glasshouse had thermostatically controlled heating and cooling systems, while the shade house was constructed with wooden slatted walls and whitewashed glass roof and did not have heating and cooling systems. The temperature in the glasshouses and shade houses was monitored for the duration of the project with Tiny Tag® temperature loggers placed in Stevenson screens located 1 m above ground level. In addition, temperature and relative humidity measurements were made in some experiments using Tiny Tag® temperature and humidity loggers located in Stephenson screens placed on the benches on which the plants were growing.

The bulbs used in controlled environment studies were planted in 4.5 L capacity plastic pots; standard potting mixture was supplied by Horticultural Supplies Hobart Tasmania (details of potting mixture are provided in Appendix 1). Irrigation was maintained by automatic overhead sprinkler systems in the glasshouse and shade house. Plants received a weekly application of Hoaglands complete fertilizer mixture (see Appendix 2); fungicide and insecticide treatments were applied where necessary to maintain plant health.

2.4 Seed Harvesting, Preparation and Storage

Umbels were harvested by hand and placed in hessian bags with tags indicating number of plants, number of umbels, treatment and replicate number. Umbels were dried for two weeks at 25 °C in a commercial gas heated, forced air dryer. Once the umbels were dried, seeds were removed using a modified garden mulcher (Stihl, Virginia Beach, USA) in which the cutting blades were replaced with strips of hardened rubber. Hand sieves were used to remove the coarse trash and fine dust, finally seeds were cleaned with help of seed blower (Seedburo, Chicago, USA). Clean and dry seeds were stored in labelled, sealed bags at 4 °C.

2.5 Design of Experiments

The experimental work described in this thesis was undertaken between September 2004 and January 2007 (Table 2.3). At the commencement of the project, the major factors responsible for low seed yield in some crops of ON019A were not known; the initial trials therefore focussed on identifying the stage(s) in flower and seed development where normal development breaks down to prevent seed development. Flower abortion was identified as the likely cause of low seed yield and subsequent experimental work was

undertaken to confirm the occurrence of flower abortion, identify factors contributing to flower abortion and assess treatments designed to reduce the occurrence of flower abortion.

Season	Location	Trial Summary
2004	Swan Hill, Campania, Old Beach	Supplementary hand pollination
2004	Swan Hill, Mt Gambier, University Farm	Characterisation of flower abortion using electron microscopy and light microscopy. Effect of single application of GA_3 at 30% piping, effect of mother bulb size, effect of bulb source, bulb maturity at lifting towards flower abortion.
2005	University Farm, Old Beach, Swan Hill	Timing of flower abortion, and relationship to plant growth patterns
2005	University Farm, Old Beach	Effect of mother bulb size, bulb storage, different time of planting, nutritional survey.
2005	University Farm, Old Beach, Swan Hill	Effect of GA_3 at various concentrations and time of application, split application, on flower abortion and seed yield.
2006	University Glass house and shade house	Effect of irrigation, shading, air blowing over surface of umbel on flower abortion.
2006	University Farm, SPS Farm, Swan Hill	Effect of GA_3, anti-transpirant (vapour guard, Envy), anti stress, wind shelter, irrigation in relation to flower abortion and seed yield.

Table 2.3 – The sequence of trials followed during the study.

2.6 Statistical Analysis

The results were analysed with ANOVA using general linear model package of SPSS (12.0.1, 1989-2003) and SPSS (V14.01) for comparison of means. Fishers (Steel, 1981) least significant difference (LSD) was calculated at $P< 0.05$ level of probability unless otherwise specified. Error bars shown in the graphs were standard errors of mean (SEM).

Chapter 3

Characterisation of Flower Abortion in Inbred Male Sterile Onion Parent Line

3.1 Introduction

The production of hybrid onion seed commenced in the early 1930's after the identification of a male-sterile source from the Italian red variety (Jones & Emsweller, 1937) and Scott Country Globe (Peterson & Foskett, 1953). These male-sterile sources allowed field scale production of hybrid seed in onion replacing the traditional laborious hand emasculation and pollination techniques that had been used on a small scale previously. Hybrid seed production in onion was introduced commercially notably in Japan and Europe (Dowker & Gordon, 1983) and has since become a significant component of global onion seed production (Pathak, 2000).

3.1.1 Advantage of Hybrids

Hybrid seeds have increased in popularity as they offer several advantages over seed produced from open-pollinated seed crops. The major advantage of hybrids is associated with hybrid vigour where heterosis confers improvements in many traits including yield (Aghora & Panthak, 1991; Dowker & Gordon, 1983; Hosfield *et al.*, 1977; Jones & Davis, 1944; Joshi & Tandon, 1976). Another important attribute of hybrids is genetic uniformity providing advantages in synchronicity of growth and development events within crops and uniformity in the final harvested product. The advantages associated with genetic uniformity are also present in the hybrid parent lines resulting in synchronisation of flowering within lines in the seed crop (Jones & Davis, 1944).

3.1.2 Inbred Parents in Hybrid Breeding Programme

Inbreeding in the male-sterile and pollinator lines is used to maintain the homozygous level allowing the parental lines to be used in the hybrid breeding programme for the production of hybrid seeds with high levels of hybrid vigour. The inbred male-sterile and inbred pollinator lines are susceptible to low yield (Ali *et al.*, 1984; Campbell *et al.*, 1968; Erickson & Gabelman, 1954; Franklin, 1970) due to inbreeding depression resulting in reduced vigour especially during the flowering stage and seed set.

3.1.3 Problems in Hybrid Seed Production

The transition in the onion seed industry from open pollinated seed production to hybrid seed production driven by the demand for improved attributes offered by hybrid seed has seen a number of production issues identified. The hybrid seed production systems require pollen transfer from the pollen donor line to the male-sterile line, so synchronicity of flowering between the lines and effective transfer of pollen is needed.

The use of inbred parent lines has also introduced problems associated with low vigour, poor disease resistance and narrow windows of environmental conditions for optimal crop growth. Specific crop management strategies have been developed to address most of the problems that have emerged in hybrid onion seed production.Hybrid seed production requires synchronised flowering time in the male-sterile and male fertile parent lines. Synchronisation in flowering may be achieved by staggering the planting dates of the male fertile line to ensure pollen availability when the male-sterile line flowers.

Flowering time may also be adjusted by manipulating storage conditions of bulbs before planting (Brewster, 1994) and using growth hormones such as GA_3 (Bhople *et al.*, 1999; Looper & Waller, 1982 ; Naamni *et al.*, 1980),

Ethephon (Corgan & Montano, 1972) or NAA (Naphthalene Acetic Acid) (Bhople *et al.*, 1999).

Pollination is necessary for production of hybrid onion seeds and nearly all hybrid seed production systems depend on honey bees (*Apis mellifera*) and blowflies for cross-pollination. Seed production without specific management of pollination is highly inconsistent and may fail due to lack of pollen transfer (Shaw & Bourne, 1936). The introduction of 12 to 37 honey bee hives per hectare has been recommended in the US for hybrid onion seed production (Brewster & Rabinowitch, 1990), the introduction of bee hives should be staggered in time starting when about 50 % of inflorescences show some open florets as bees may be tempted to move towards other crops and staggering the introduction of hives will help to keep the bees in the onion crop. Pollination requires the presence of viable pollen as well as pollinator insects.

Temperature has been shown to have an effect on pollen fertility; the viability of pollen is low at 14 °C but increases at 23 °C, and at 40 °C pollen tube growth and seed set are adversely affected (Chang & Struckmeyer, 1976a, 1976b). Pollen viability (Ali *et al.*, 1984; Ockendon & Gates, 1976) and stigma receptivity (Ali *et al.*, 1984) are adversely affected by exposure of onion flowers to high temperatures leading to poor fertilisation. Management of pollination in hybrid onion seed crops therefore usually involves avoiding production regions or seasons where the risk of exposure to damaging temperatures is high. Management practices used to address poor growth associated with use of inbred parent lines in hybrid seed production vary between lines, between production regions and seasons.

While management of environmental conditions, crop nutrition and irrigation, is generally site and crop-dependent a consistent response of onion lines to exposure to unfavourable conditions during or shortly after flowering is abortion of inflorescences or developing seeds. Exposure of onion ovary wall to temperatures above 20 °C leads to seed abortion, with 100% seed abortion

if the temperature reaches 50°C leading to crop seed failure (Peterson & Trammell, 1976).

Exposure to high light levels and/or high temperatures can result in formation of a disorder known as "dollar spot"; a circular area of aborted flowers forms on the side of the inflorescence. The condition resembles sunscald, with desiccated and aborted flowers on the sunlit side of the inflorescence and a huge reduction in number of viable seeds present in the affected area (Tanner & Goltz, 1972). Several authors (Chang & Struckmeyer, 1976a; Halevy, 1988) concluded that light directly affected the mobilisation of reserve from the mother bulb to the developing flower bud. Abortion of developing flowers just prior to anthesis is thought to occur because the mother bulb is not able to supply sufficient reserves over the whole developmental stage of the flower bud (Vonk & Ribot, 1982).

3.1.4 Flower Abortion

Onion umbels are capable of producing around 50-2000 individual flowers depending on variety and size of bulb, but only 200 – 600 normal flowers are present at anthesis with the remaining flowers aborting at earlier developmental stages (Campbell *et al.*, 1968). Abnormal flower development, descriptions of which are consistent with flower abortion at late stages of flower development, have been reported in production regions in the US and linked to inconsistent yields between hybrid onion seed crops in these regions (Campbell *et al.*, 1968). Studies on causal factors of flower abortion were not undertaken in inbred onion lines despite the evidence suggesting a link between abnormal flower development and seed yield. Many studies report that failure of pollination was the major factor affecting seed production in onion (Shaw & Bourne, 1936; Silva *et al.*, 2003).

In Australia, major seed companies have reported failure of onion seed crops because of poor pollination and have also noted abnormal flowers in low

yielding crops. Variability in yield exists between inbred lines suggesting either a difference in pollination efficiency between lines or a difference in % of flower abortion. Most attention has been given to managing pollination in these lines with flower abortion being neglected as a potential cause of low seed yield in hybrid onion lines.

Many studies of flower abortion in ornamental bulbous species have been published. Flower formation in bulbous plants is favoured by warm temperatures as indicated by the optima recorded for various species: 25 °C for hyacinths, 17 - 20 °C for tulip, 23 °C for *Lilium* (Kamenetsky & Japarova, 1997). In other bulbous species, flowers formation has been reported at a lower optimal temperature; 9 °C for irises, and 13 °C for onion and shallots. Exposure to temperatures outside the optimal range results in delayed flowering or prevention of flowering through either inhibition of flower initiation or abortion of developing flowers. High temperature exposure results in floral abortion in many species including *Lycopersicum esculentum* (Abdul-Baki, 1991), *Capsicum annuum* (Erickson & Markhart, 2002), *Phaseolus vulgaris* (Konsens *et al.*, 1991), *Vigna unguiculata* (Craufurd *et al.*, 1998), *Persea americanum* (Sedgley *et al.*, 1985), *Gossypium hirsutum* (Reddy *et al.*, 1992), and *Arabidopsis thaliana* (Ryan M. Warner & John E. Erwin, 2005). Low relative humidity and high radiation also cause damage to the umbel surface and lead to aborted florets (Tanner & Goltz, 1972).

While anecdotal evidence from seed companies indicates that increasing levels of inbreeding predisposes lines to increased likelihood of low seed set the effect on specific aspects of reproductive biology such as pollen viability, stigma receptivity, seed set and flower abortion have not been well studied. Identification of the key stages in seed formation that are prone to failure in the inbred lines displaying significant variability in seed yield may permit development of strategies to reduce the risk of low yields in these lines. The aim of the research described in this chapter was to identify the stage of flower development and seed formation in the inbred line ON019A preventing seed set in crops with low seed yield.

3.2 Materials and Methods

3.2.1 Plant Materials

The inbred male-sterile intermediate day length brown onion lines ON019A, ON013A, and ON138A were used to investigate flower development and seed formation processes. The open pollinated brown cultivar Creamgold was used in a study comparing inbred and non-inbred lines. All plants used in experiments were grown under field conditions during the 2004 and 2005 seasons either in commercial crops or in trial plots. Trial locations were Campania (42.87°S, 147.3°E), University Farm (Cambridge) (42.5°S, 147.3°E), Swan Hill (35.34°S, 143.5°E) and Mt Gambier (37.7°S, 140.7°E) Australia. All trials were grown using current commercial practises for bulb to seed production.

3.2.2 Supplemental Pollination Experiment

Supplemental hand pollination experiments were undertaken to determine if pre- or post-pollen transfer processes were contributing to poor seed yields. Trials were conducted in three commercial hybrid seed crops with male-sterile line ON019A, two sites located near Swan Hill, Victoria and one site at Campania, Tasmania. At each site 12 flowering male-sterile plants were selected during peak bloom for hand pollination between 12 noon to 2 P.M, on three consecutive days all receptive flowers were hand pollinated using pollen collected from the corresponding pollinator line. An additional twelve male- sterile plants matched by size, stage of development and position, to that receiving hand pollination were used as controls for the experiment. On each day of hand pollination, a sample of pollen was collected and tested *in-vitro* for viability using Brewbacker and Kwack medium (Brewbacker and Kwack, 1963).

After hand pollination samples of receptive stigmas were removed from the hand pollinated and control treatments for comparison of pollen deposition levels. Stigmas were mounted in basic fuschin stain (Kearns & Inouye, 1993)

and pollen grains counted under a light microscope. Stigmas of hand pollinated flowers were also collected 24 hrs after pollination for assessment of pollen germination *in-vivo* using fluorescence microscopy (Spurr, 2003). At maturity the umbels of the hand pollinated and control plants were collected individually and dried, umbels were threshed by hand and the seed cleaned in an air column for determination of seed yield.

At anthesis, two classes of flowers were identified. These were; a) flowers with fully extended stigmas greater than 4 mm long and open petals and b) flowers in which the stigmas did not extended more than 2 mm and petals that did not fully open. At the Campania site in 2004 one hundred flowers of each type were hand pollinated between 12 noon and 2 P.M and tagged; half of each flower type was recovered 24 hrs after pollination for assessment of *in-vivo* pollen germination using aniline blue fluorescence microscopy (Spurr, 2003) and the remainder recovered at maturity to compare seed set rates. During the 2004, 2005 and 2006 seasons 14 commercial and trial crops of ON019A grown in southern Tasmania, Mt Gambier and Swan Hill were surveyed for incidence of each flower type at anthesis and seed yield to establish if a relationship existed between the two variables.

3.2.3 Characterisation of Flower Abortion

In 2004 inflorescence development in ON019A was compared with other inbred lines with more reliable yield characteristics (lines ON013A and ON0138A) in commercial crops located in Tasmania, Mt Gambier and Swan Hill. For each line representative plots 15 m long by 4 rows of plants were marked for sampling, nine randomly selected plants from each plot were destructively sampled at 14 day intervals from prior to inflorescence initiation until seed maturation.

The primary inflorescence of each plant was removed and scored for diameter and percentage of flowers that had aborted. Flower abortion was scored on the basis of visual symptoms (desiccated and/or necrotic tissue) under a dissecting microscope.

After scoring, sections of representative inflorescences were fixed in formalin acetic alcohol (50% ethanol; 6.5% formalin and 2.5% glacial acetic acid) for anatomical studies with an environmental scanning electron microscope (FEI, Quanta 600, USA). Individual flower samples were examined without further preparation in low vacuum environmental mode.

3.2.4 Flower Abortion and Plant Growth Patterns

Relationships between incidence of flower abortion and plant growth characteristics were examined using plant samples collected from trial plots of ON019A grown at University Farm (Cambridge) and Old Beach in Tasmania. At peak bloom in 2005, 120 randomly selected plants from each trial site were measured for height, and number of vegetative and reproductive bulb divisions. Plants were destructively sampled for assessment of flower abortion rate and determination of dry weight of individual plant components (roots, bulbs, leaves, scapes and umbels). Correlations between plant growth characteristics and flower abortion were identified using the regression function of SPSS V14.0 (SPSS Inc., 2005).

A relationship between flower abortion and biomass allocation to the bulb suggested in initial correlations was investigated in a comparative study of growth patterns of ON019A and cv Kingswood in 2006. Samples of ten randomly selected plants were collected from commercial plantings of ON019A grown at Swan Hill and trial plots grown at Old Beach and University Farm (Cambridge) at 14 day intervals from transplanting to peak bloom. Samples were divided into root, bulb, scape / leaf and inflorescence components.

Inflorescence diameter data was collected and the components then dried and weighed. Correlations between dry matter percentages of different plant parts and flower abortion percentages were identified using the regression function of SPSS V14.0 (SPSS Inc., 2005).

3.3 Results

3.3.1 Supplemental Pollination Result

Viability of pollen used in the supplemental hand pollination experiments ranged from 24% at Swan Hill to 35% at Campania. Although 95 to 98% of all flowers within the supplemental pollination treatments were pollinated with on average 34 (Swan Hill Grivell), 45 (Swan Hill Hardy) and 102 (Campania) pollen grains per stigma, this treatment failed to significantly increase seed set at any site despite the wide variation observed in seed yield between sites (Figure 3.1).

At the Campania field site, 64% of flowers with normally extended stigmas (Plate 3.1) at anthesis set seed, but flowers with abnormally short stigmas at anthesis did not set seed and senesced soon afterwards (such flowers hereafter referred to as aborted at anthesis). *In-vivo* pollen germination data were consistent with the seed set data with more then 50% pollen germination on normally extended stigmas and no germination of pollen on flowers with short stigmas.

Seed production survey of ON019A

For ON019A, seed yield was closely correlated to percentage of flower abortion at anthesis ($P<0.001$; $r^2 = 0.89$) at 14 sites surveyed in southern Tasmania, Swan Hill and Mt Gambier between 2004 and 2006 (Figure 3.2).

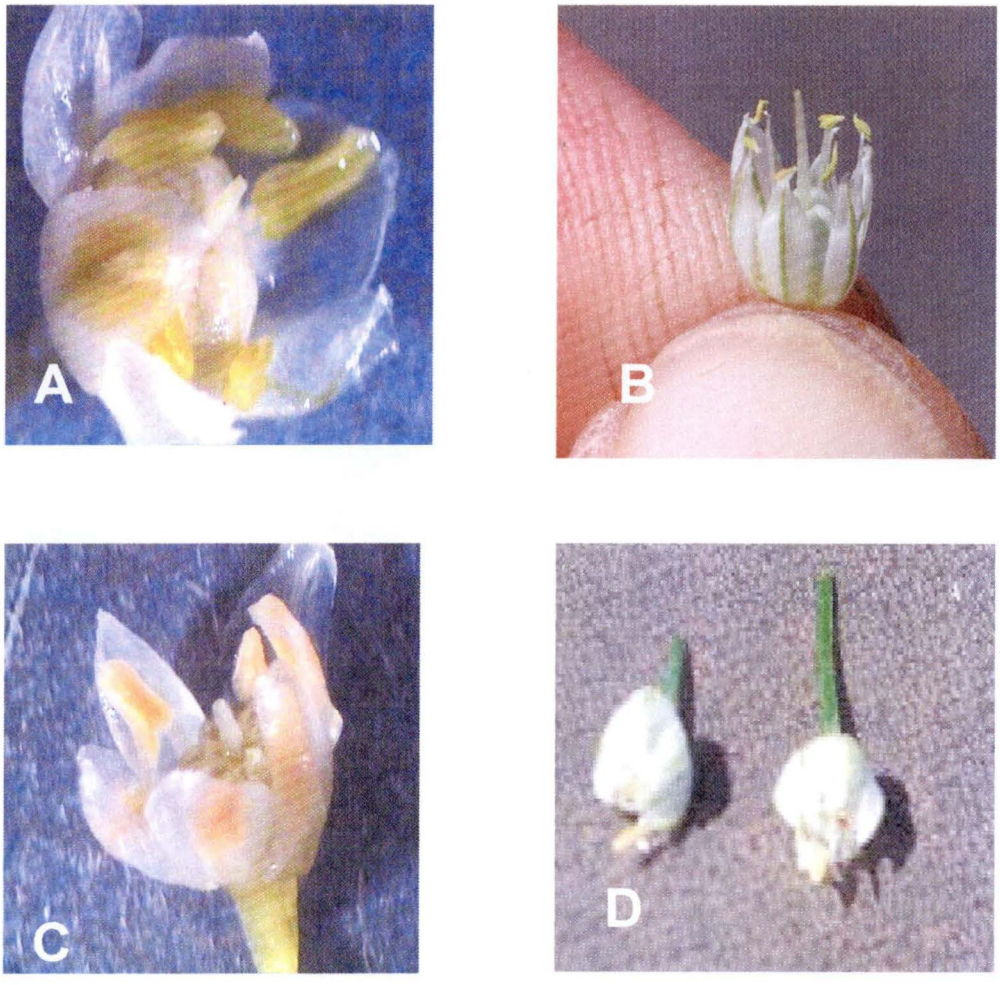


Plate 3.1 – Flowers of onion seed parent line ON019A showing a) a stigma of normal length at anthesis (stigma approximately 4 mm long); and c) shortened stigma (~2 mm long) and unopened petals at anthesis. A flower of the pollinator line Creamgold is shown at anthesis in b) and d) for comparison.

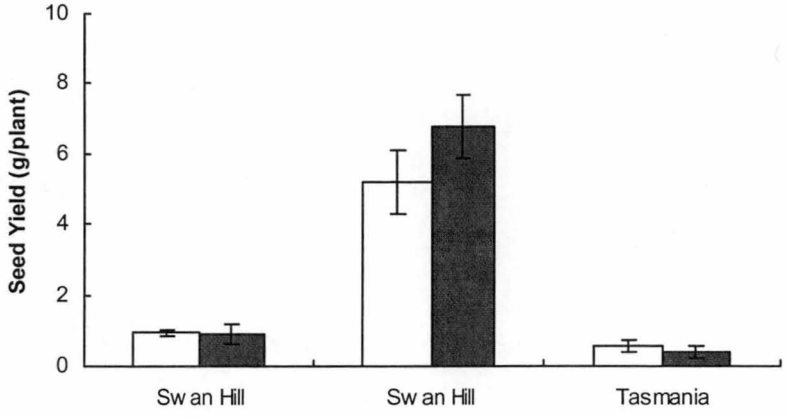


Figure 3.1 – Seed yield of hand pollinated (□) and control (■) plants. were located at Swan Hill, Victoria and was located at University Farm (Cambridge), Tasmania. Data are means of 12 replicates, with standard error bars.

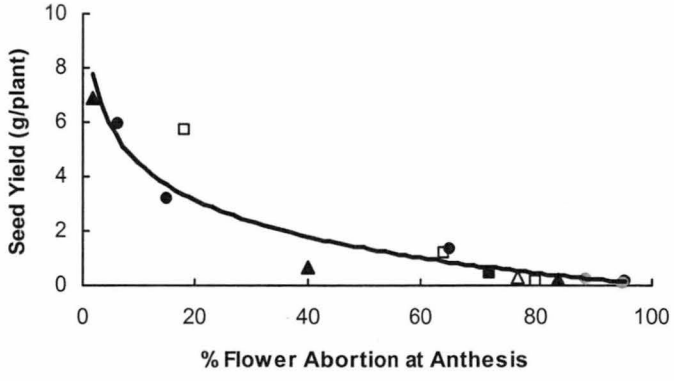
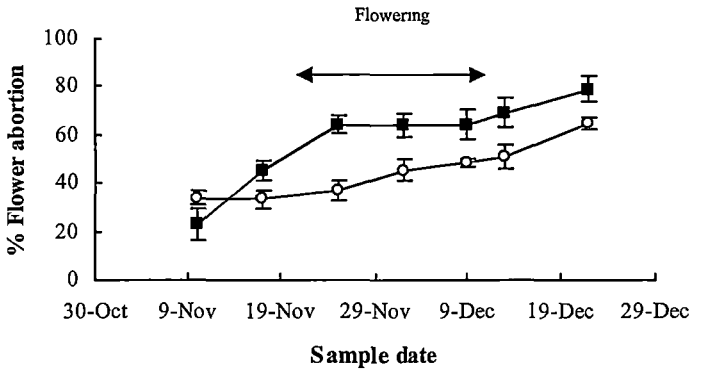


Figure 3. 2 – Relationship between seed yield and flower abortion at anthesis from ON019A crops grown in Swan Hill (open symbols), Mt Gambier (filled grey symbols) and Southern Tasmania (filled black symbols) in 2004 (■), 2005 (●) and 2006 (▲). Hand pollination were not used in the trial. The relationship is described by the equation $y=-1.93\ln(x) + 8.8$ is significant ($P<0.001$; $r^2 = 0.89$).

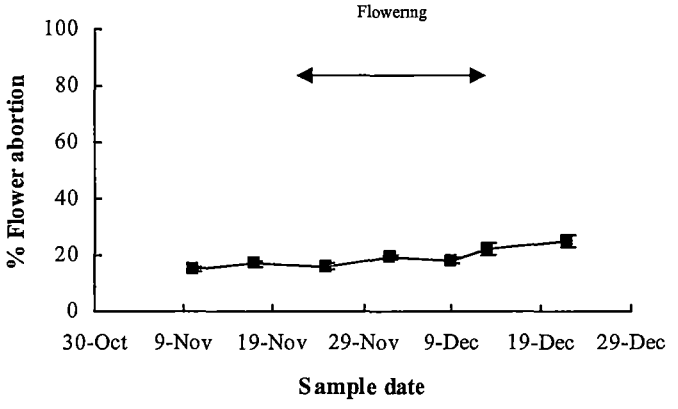
3.3.2 Characterisation of Flower Abortion in ON019A

Comparative studies of inflorescence development in ON019A and other more reliable seed yielding lines were made using light and scanning electron microscopy. All inbred male-sterile lines observed in this study aborted a significant proportion (more than 50%) of flowers at late stages of development; high rates of flower abortion in susceptible lines were recorded commencing one week prior to crop flowering and throughout the crop flowering period (Figure 3.3). The timing of abortion of individual flowers appeared to be during the phase of rapid elongation of floral organs prior to flower opening and therefore resulted in the observed patterns at the crop level due to the spread of time of flower opening of individual flowers within umbels in each crop, markedly lower levels than in ON019A (58% for Creamgold compared with 84% for ON019A). Although flower abortion was most apparent in ON019A lower frequent occurrences were observed in other inbred male-sterile lines including ON013A and ON138A (Figure 3.3).

a)



b)



c)

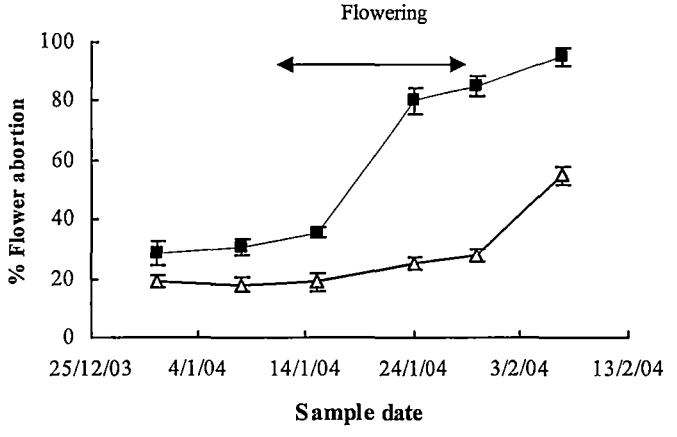


Figure 3.3 – Flower abortion rates during inflorescence development in ON019A (■), ON013A (◊) and ON138A (Δ) grown at a) Swan Hill (site 1), b) Swan Hill (site 2) and c) University Farm (Cambridge) in 2004. Flowering periods are annotated on the graphs. Error bars are standard errors (n = 9).

Under field conditions severity of flower abortion in ON019A varied widely between different sites; the effect of these differences in flower abortion was evident as dramatic differences in umbel appearance after flowering. For example, good seed set was evident at Old Beach (2005) where a flower abortion rate of 6.1% was recorded, while few seeds set at Cambridge (University Farm, 2005) where 84% abortion was recorded (Plate 3.2). Within individual sites large plant-to-plant variation in abortion rate was observed in ON019A, for example abortion rates ranging from 24% to 86% between plants at Cambridge in 2005 (Plate 3.3) though no obvious explanation for this variation observed in the field. Within individual plants variation of abortion rate between primary inflorescences was typically small (less than 10%).

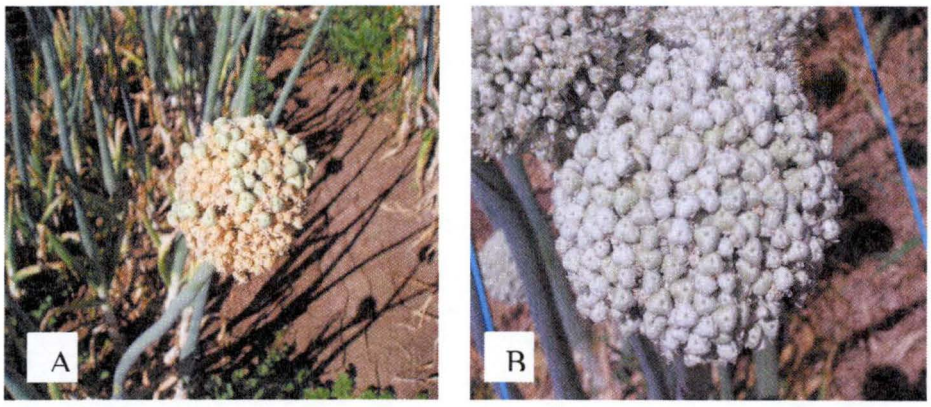


Plate 3.2 – Umbel developments in ON019A after flowering at A) University Farm (Cambridge) and B) Old Beach Tasmania 2005. Flower abortion percentages of 84% and 6.1% respectively were recorded in these crops.

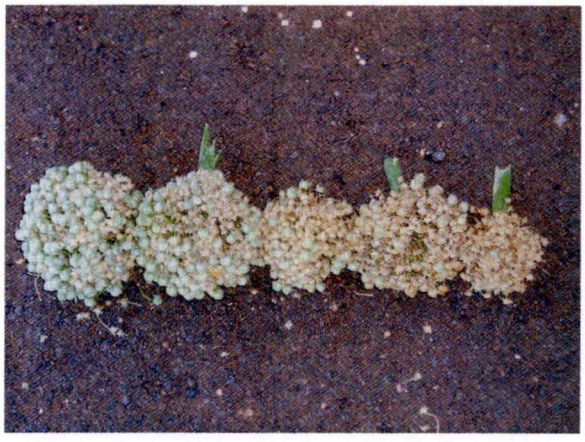


Plate 3.3 – Variation in severity of flower abortion between umbels of different plants of ON019A grown in trial plots in University Farm (Cambridge) in 2005.

Flower abortion was characterised by desiccation of cells on the stigmatic and ovary surfaces (Plates 3.4-3.6). Affected areas of tissue appeared deformed, with loss of turgidity of cells compared to similar structures in flowers that were developing normally. Examination of flowers using scanning electron microscopy revealed that non–aborted flowers of ON019A developed identically to viable flowers of cv kingswood (Plates 3.7,-3.9). Aborting and viable flowers of ON019A were present in varying proportions within individual umbels but there was no apparent pattern to their distribution within the inflorescence.

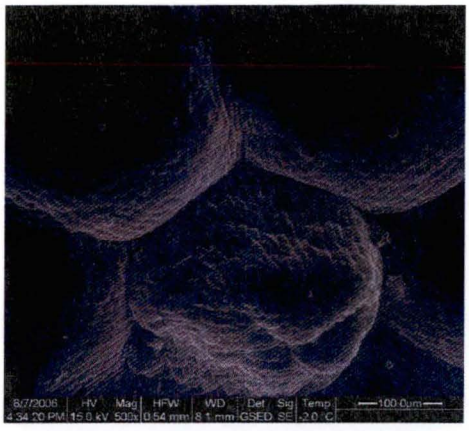


Plate 3.4 – Stigmatic surface of aborting ON019A. Note the zone of desiccated tissue.

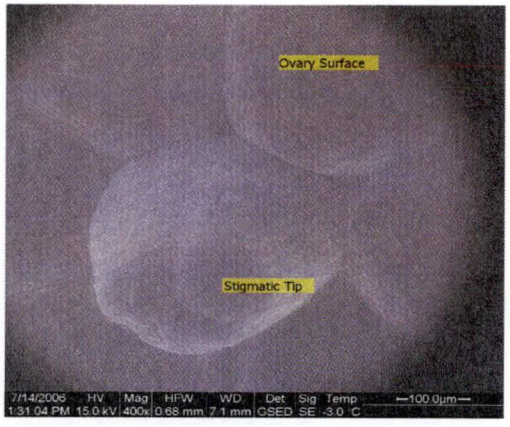


Plate 3.7 – Stigmatic surface of normal flower of Kingswood.

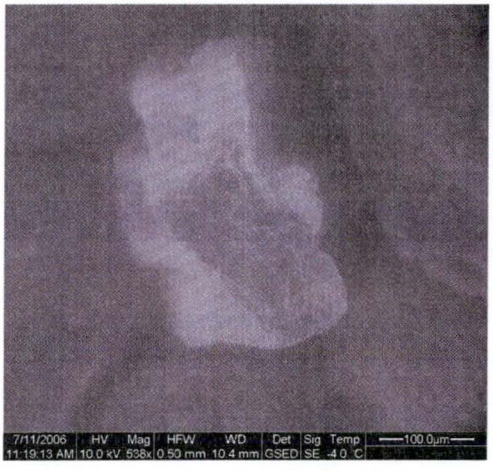


Plate 3.5 – Stigmatic and ovary surface of aborting flower of ON019A.

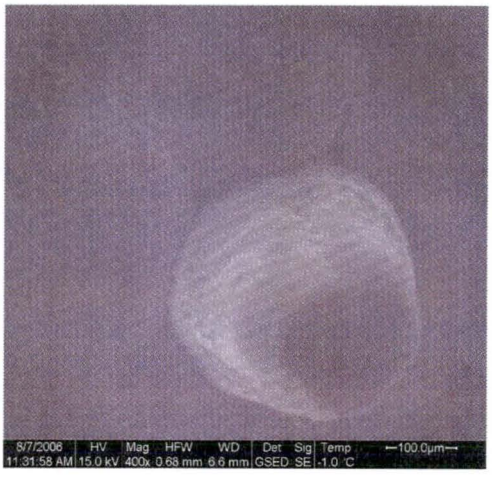


Plate 3.8 – Stigmatic and ovary surface of normal flower of Kingswood.

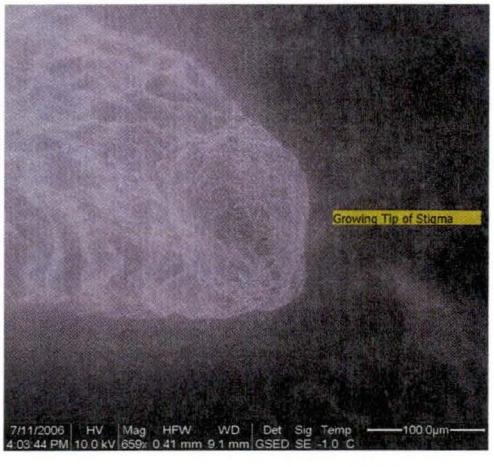


Plate 3.6 – Stigmatic surface of ON019A. Note the desiccated tissue.

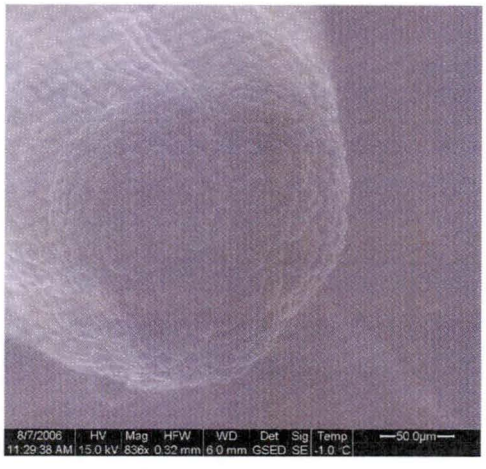


Plate 3.9 – Stigmatic surface of normal flower of Kingswood.

Plate 3.4, 3.5, 3.6, Scanning electron microscopic images of developing aborted flowers.

Plate 3.7, 3.8, 3.9, Scanning electron microscopic images of normally developing flowers.

3.3.3 Flower Abortion and Plant Growth Patterns

Possible relationships between plant growth characteristics assessed at anthesis and incidence of flower abortion in plants from trial plots grown at Cambridge in 2004 and 2005 are summarised in Table 3.1. Of the parameters measured in both seasons flower abortion was most closely correlated with the ratio of partitioning to the floral and bulb components and to total plant size. In these relationships increased partitioning to the bulb correlated to increased rates of flower abortion whilst increased partitioning to the umbels and greater plant size were correlated with reduced rates of flower abortion.

Variables	**Significance and R values**			
	2004 (n = 120)		**2005 (n = 50)**	
	P Value	R	P Value	R
Percentage of total biomass in:				
Roots	<0.05	0.04	NS	
Bulbs	<0.001	0.26	<0.05	0.17
Leaves	<0.01	0.06	<0.05	0.09
Scapes	<0.01	0.06	N/A	N/A
Umbels	<0.001	0.30	N/A	N/A
Scape + Umbels			<0.05	0.16
Total dry weight	<0.001	0.07	<0.05	0.12
Scape length	NS		NS	

Table 3.1 – Summary of correlations between plant growth characteristics and flower abortion rate in plots of ON019A grown at University Farm (Cambridge) in 2004 and 2005.

Consistent with the above correlations high rates of flower abortion in ON019A at Swan Hill and Cambridge in 2005 (75% to 84%) occurred concurrently with large increases in bulb biomass (84 to 88% increases) during the 4 week period prior to anthesis (Figure 3.4). In contrast, a plot of ON019A grown at Old Beach in 2005 in which very low rates of flower abortion was observed (1.2%) featured only a 12% increase in biomass in the bulbs compared with 90% increase in scape and umbel biomass over the 4 weeks preceding anthesis (Figure 3.4). Similarly plants of cv Kingswood

grown with ON019A at Cambridge in 2005 featured a large ratio of biomass gain in the inflorescence compared with biomass gain in the bulbs during the 4 weeks preceding flowering (7:1) and very low rates of flower abortion (<1%) (data not shown).

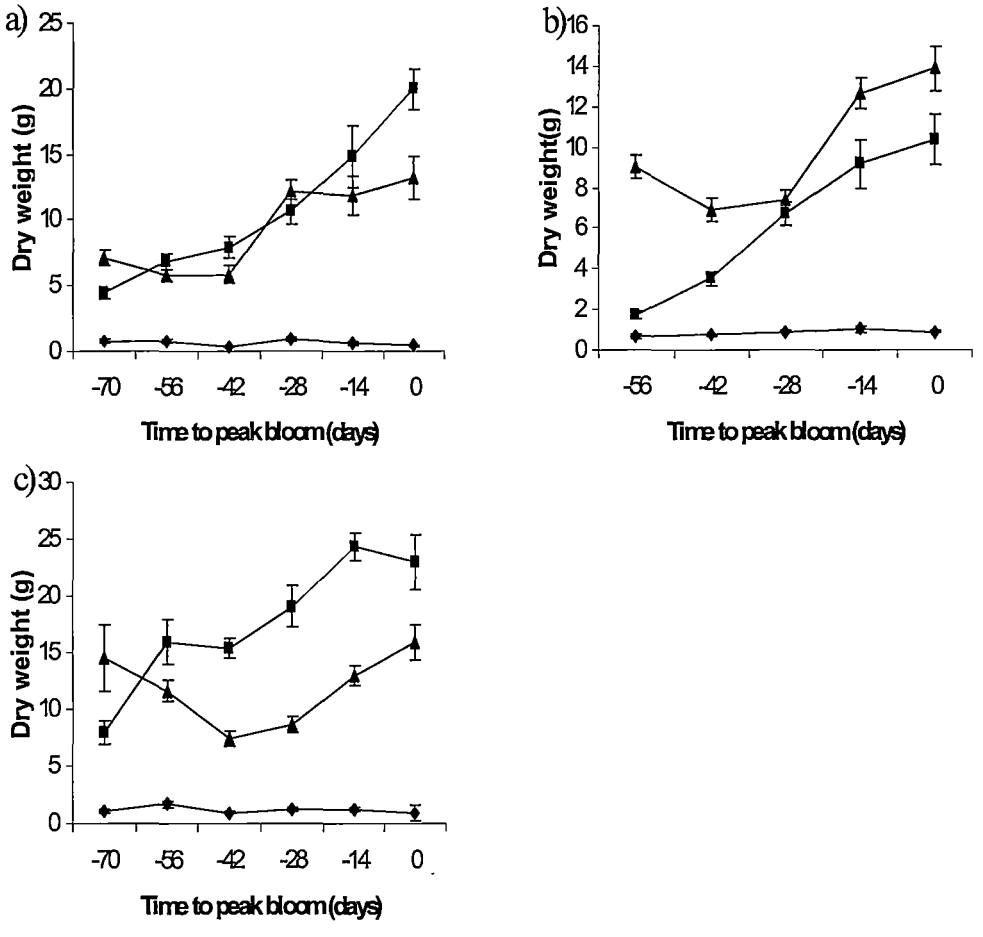


Figure 3.4 – Growth of the reproductive (scape and inflorescence) (■), bulb (▲) and root (♦) components of ON019A in the lead up to flowering (peak bloom) at Old Beach (a), University Farm (Cambridge) (b) and Swan Hill (c) in 2005. Flower abortion rates at peak bloom were: Old Beach = 1.2%, Cambridge = 84%, Swan Hill = 80%. Error bars are standard errors (n = 12).

3.4 Discussion

Low seed yield may result from failure in any of the key stages in flower development, pollen transfer, fertilization and seed set. In the inbred male-sterile onion line ON019A the primary cause of low seed yield was identified as failure of a high percentage of flowers to complete normal flower development. Abortion of flowers during late flower development up to the point of rapid petal expansion and flower opening prevents seed formation. The results from the pollination studies undertaken in several locations revealed that hand pollination did not increase seed yield despite significantly increasing pollen load on the stigmatic surface. Observations made during the trials and in a subsequent survey of trial and commercial plots of ON019A indicated that there was a relationship between the number of abnormal flowers in umbels and poor yield in ON019A. An increased number of defective pistils and decreased number of flowers per umbel have been suggested previously to be the two major factors limiting seed yield in inbred onion lines (Lesley Currah & Ockendon, 1978). The yield differences associated with inbreeding in onion were studied by Erickson & Gabelman, (1954) who found that four inbred lines averaged 420 seeds per plant compared with hybrids that produced 801 seeds per plant. These inbred lines failed to set seeds completely after three generations of inbreeding (Jones & Davis, 1944). Increased incidence of flower abortion may account for the increased risk of low seed yield associated with inbreeding in onion hybrid parent lines.

Floral studies in onion have previously described differences in reproductive structure between male-sterile and male fertile lines; differences in style length between male-sterile and male fertile lines have been documented and suggested as a possible reason for poor seed set (Robert, 1954). The receptive area of the stigmatic knob has also been shown to be comparatively less in case of male-sterile lines (Ali *et al.*, 1984). The receptivity period of the

onion flower has also been considered as a factor affecting seed set and the style length might also be related to the receptivity period (Robert, 1954). While these studies have not linked variations in patterns of stigma development with flower abortion they are consistent with abnormal flower development being a significant factor in low seed yield in onion seed crops.

Pollen viability, and compatibility between pollen and stigma is required for successful fertilization. Pollen viability could not explain the low seed set in the crops used in the hand pollination study as the pollen used had greater than 25% fertility in *in-vitro* tests with Brewbacker and Kwack pollen germination medium (Brewbacker & Kwack, 1963) and was applied in large quantities to greater than 95% of flowers.

Incompatibility between the pollen source and the male-sterile line ON019A was also unlikely as high seed yields are possible using the pollen parent as well as a range of other pollen parents. Examination of *in-vivo* pollen germination and pollen tube growth on hand pollinated flowers of ON019A. Pollen germination percentages from the *in-vitro* method for the same pollen samples were greater than 25% indicating that the stigmatic surface environment was not conducive to pollen germination.

According to (Brewster, 1994) twelve or more initial pollen tubes are needed to archive maximum seed set since the flowers contain six ovules and about half of the pollen tubes at the top of a stigma grow as far as the ovary, with three or four seeds per ovary necessary to achieve good seed yields in a crop. The observed desiccation of the stigmatic surface in aborting flowers may explain the poor *in-vivo* pollen germination results as this variation from normal floral structure development may affect the pollen germination and therefore prevent fertilisation. Desiccation of umbels through high temperatures and / or water stress have previously been shown to prevent seed set and are responsible for the disorder known as "dollar spot" or "hot spot" (Tanner and Goltz, 1972).

The morphological characteristics of the developing flowers differed markedly between aborted and normally developing flowers, the first visible sign occurred prior to petal opening when elongation of the style through the petal gap at the tip of the bud was evident in normally developing flowers but negligible in aborting flowers. Normal and aborting flowers observed at this stage displayed differences in the surface morphology of the stigma, style and ovary surface. Aborting flowers displayed areas of desiccated tissue in all floral parts while normally developing flowers consisted of fully turgid structures. This characteristic growth pattern associated with flower abortion in a mal-sterile inbred line has not been previously reported. The development of normal flowers was consistent with previously published descriptions (Currah *et al.*, 1990; Preiss, 1982; Rabinowitch & Brewster, 1990).

Abortion of flowers characterised by desiccation of the floral organs during the latter stages of flower development when rapid elongation of petals and styles associated with flower opening occurs may explain the poor seed yield often reported for inbred male-sterile onion lines as it would prevent pollen germination and therefore fertilization and seed set. The present study confirmed that pollination was not the major problem causing unreliable seed set in the male-sterile onion line ON019A. Abnormal flower development prior to flower opening was characterised as flower abortion and was concluded to be the primary cause of poor seed set in this line. Although generally less severe, flower abortion was also observed in other inbred male-sterile onion lines used in this study and may therefore be a more significant factor in poor yields from hybrid onion seed crops than previously recognised. The variation of flower abortion was shown to vary between locations and seasons indicating that growing environment and/or crop management practices may influence the processes leading to flower abortion. While inbred lines may have a greater predisposition to abort flowers compared to non-inbred lines the processes may be modified during production of the seed crop. Identification of environmental and crop management factors influencing the flower abortion process would permit development of crop

management strategies to minimise the percentage of flower abortion and therefore increase seed yields. Identification of these factors was therefore the focus of the next set of experiments in this project.

Chapter 4

Agronomic Approaches to Better Understand Flower Abortion and Improve Seed Yield in ON019A

4.1 Introduction

In Chapter 3, flower abortion was identified as the major factor contributing to poor seed yield in the male sterile onion parent line ON019A. Flower abortion was shown to be quite variable between ON019A crops raising the possibility of identifying crop management practices to minimise flower abortion and improve seed yield. The approaches taken in this study included examining crop management practices known to vary within the industry as well as application of growth hormones known to influence flower development.

The aims of the agronomic trials were firstly to identify crop management practices with potential to reduce the flower abortion rate and increase seed yield and secondly to gain insight into processes that contribute to flower abortion through comparison of site and treatment effects. The trials were divided into four main categories; i) pre - planting bulb characteristics, ii) time of planting, iii) crop management practices and iv) gibberellin application trials.

4.1.1 Bulb Characteristics

The size and physiological status of the parent bulb are important characteristics to be considered for successful onion seed production; selection of bulb source, size, time of lifting and storage prior to planting are all important aspects of onion seed production when using the bulb to seed production strategy.

The size of bulb planted has been shown to affect seed production in onions with several studies examining the correlation between bulb size and seed yield (Jones & Emsweller, 1936; Orlowski, 1974). Larger bulbs have been shown to produce more flower stalks (scapes) and a higher seed yield with bulbs weighing 80-85 g producing almost twice as much seed as bulbs weighing 20-25 g (Currah, 1981). The seed yield obtained from bulbs of 6 cm diameter was higher than those of bulbs of 4.5 cm diameter (Balraj Singh *et al.*, 2005). In contrast no significant difference in seed yield was found between bulbs of 50-00 g, and 100-150 g with yields of 3.11 and 3.27 g per plant respectively (Green, 1972). In commercial practice very large bulbs are not preferred for seed production because they are more difficult to store (Currah, 1981).

Bulb size has been shown to be an important factor in determining flower number, size and flowering percentage in most but not all ornamental bulb species. In *Narcissus* the size of the bulb plays a vital role in growth and flowering (Hanks *et al.*, 2001; Rees, 1986). The size of the bulb may not be the limiting factor for flowering once flowering initiation has occurred in Iris, because Iris flower development mostly depends on current assimilates rather than the reserves in the bulb (Rees, 1985).

The effect of bulb storage and forcing temperature on growth, inflorescence development and flower abortion (blast) was investigated in inbred onion by Hesse *et al.*, (1979). Three inbred lines were given four different storage treatments (2 °C for 24 weeks, 2 °C for 12 weeks followed by 10 °C for 12 weeks, 10 °C followed by 2 °C for 12 weeks each, and 10 °C for 24 weeks). The lines differed in their response to the storage treatments. The storage treatment of 10 °C for 12 weeks followed by 2 °C for 12 weeks resulted in a significantly higher seed yield compared with bulbs maintained at 2 °C

throughout the storage period. The highest seed yield was from the inbred line that flowered earliest, had highest number of leaves per bulb, tallest seed

stalk and the highest number of florets per bulb. In contrast, Stirling, (1997) reported that there was little difference in flowering time, flowering percentage and stem length from Dutch Iris bulbs exposed to a wide range of conditioning treatments. In *Gladiolus*, the effect of storage conditions was studied by Gonzalez *et al.*, (1998) and it was shown that bulbs stored at 5°C and 90% relative humidity for 3 to 6 weeks flowered 20 and 11 days respectively before untreated control. Tulip bulbs require stratification for shoot elongation following completion of floral organ formation with duration also greatly affecting the quality of the cut flowers (Moe & Wickstrom, 1973; Rees & Charles-Edwards, 1975). As these studies focussed on flower rather than seed yield further study is clearly necessary to establish the effect of bulb storage and forcing treatments on flower abortion in hybrid onion parent lines.

While bulb production and storage treatments may influence seed crop performance many growers of onion seed crops are only able to influence seed yield by manipulating the crop after planting. Choice of planting date is the first major decision that may impact on crop productivity. Few studies of effect of planting date have been published with Ruggeri *et al.* (1994) suggesting that early July sowing gave the highest seed yield at latitude 37°N, while Singh *et al.* (2005) suggested that October planting produced maximum seed yield in Rajasthan, India (26.9° N). Yield differences were recorded with differing planting dates, but extrapolation of results to predict optimum planting dates at other production locations may prove problematic.

In Australia time of planting for ON019A crops varies significantly between different production locations, for example from April in Swan Hill (35.4°S) to as late as September in Tasmania (42.5°S). Anecdotal evidence suggests that in onion the later planting dates tend to promote bulb rather than inflorescence development. No scientific studies on effect of bulb planting date on onion flower abortion or seed yield in Australia have been published.

4.1.2 Management Practices

The management practices considered by onion seed producers as most likely to affect seed yield are irrigation and nutritional management. Supply of adequate water and nutrients to the plant is necessary to maintain crop health and to sustain the seed yield. Anecdotal evidence suggests that management of irrigation and nutrition may also affect the rate of flower abortion in hybrid onion seed crops.

To maximise and to stabilise the seed yield, irrigation is one of the most crucial factors and timely application of irrigation is essential in onion seed production (Hawthorn & Woodbury, 1969; MacGillivary, 1948). Exposure of onion plants to water deficits at the 'milky' seed stage either through insufficient irrigation or excessive transpirational water loss under adverse temperature and humidity conditions resulted in the seed shrivelling and dying (Harington, 1974). Exposure to periods of soil moisture deficit or periods of waterlogging through poor irrigation management can reduce crop yield so the irrigation management strategy can also affect crop disease status. If the humidity is increased in the field the risk of the crop being affected by foliar disease is increased; in order to reduce this risk in onion seed crops furrow or drip irrigation are used in preference to overhead irrigation in onion seed production (Brewster, 1994).

To maintain crop health and to achieve high yields, proper nutrition management is necessary. Studies of nitrogen application rates ranging from 40-120 kg/hectare in onion seed crops have shown that the highest nitrogen application rate increased seed yield by up to 80 kg per hectare (Tiwari *et al.*, 2002). Split application of nitrogen over three application dates increased the seed yield linearly from 830 to 1100 kg per hectare (Cuocolo &

Barbieri, 1988). Nitrogen nutrition is affected by soil and plant water status resulting in higher nitrogen application recommendations where rainfall and / or irrigation are adequate.

4.1.3 Gibberellin Applications

The gibberellins are a group of chemicals referred to as plant hormones that play an important role in regulating many plant developmental processes. Plant hormones or phytohormones are defined as "organic substances which are synthesized in minute quantities in one part of the plant tissues and transported to another part where they influence specific physiological processes" (Srivastava, 1996). There are five major groups of plant hormones and they have been associated with many physiological changes in plant tissues (Kinet, 1993). Gibberellins are classified on the basis of structure as well as function. All gibberellins are derived from the ent-gibberellane skeleton and are named GA1.....GAn in order of discovery. Gibberellic acid GA_3 was the first gibberellin to be structurally characterised (Figure 4.1). There are currently 136 GAs identified from plants, fungi and bacteria, although only a few of these are physiologically active in most plant species.

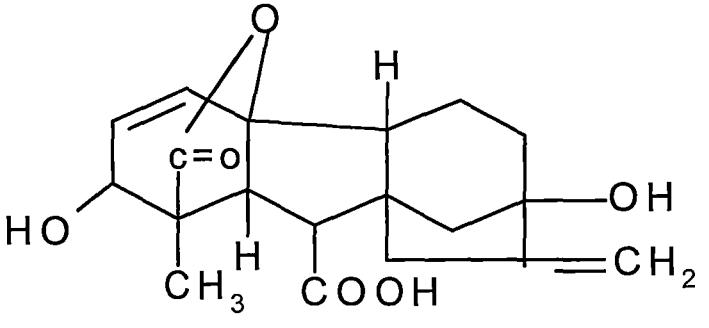


Figure 4.1 – Structure of gibberellic acid (GA_3) from Srivastava, (1996).

Active forms of gibberellin produce many physiological effects each depending on the type of gibberellin present as well as the species of plant. Some of the physiological processes stimulated by gibberellins are outlined below (Raven *et al.*, 1992; Salisbury & Ross, 1992):

- Stimulation of stem elongation by stimulating cell division and elongation.
- Stimulation of bolting / flowering in response to long days.
- Break seed dormancy in some plants which require stratification or light to induce germination.
- Stimulate enzyme production (α-amylase) in germinating cereals for mobilization of seed reserves.
- Induction of maleness in dioecious flowers (sex expression).
- Parthenocarpic (seed-less) fruit development.
- Senescence in leaves and citrus fruits.

Exogenous application of gibberellins to horticultural species is used commercially to manipulate crop development and particularly to manage flower and fruit development. Exogenous gibberellin application elicits a number of responses in onion including increased stem elongation, synchronization of flowering, reduction in the time to flowering and increases in the number and size of flowers and seed yield (Bhople *et al.*, 1999; Thomas & Isenberg, 1972). The responses are, however, concentration dependant; use of certain concentrations may lead to poor quality plants (Hopkins, 1995; Naamni *et al.*, 1980; Neumaier *et al.*, 1987) and potentially reduced seed yields. GA_3 sprays at concentrations in the range 0-450 ppm (Naamni *et al.*, 1980) (50, 150, 450 ppm) applied during early flower

development increased the length of the flower stalk, number of flowers per umbels, number of umbels per plant, number of seeds per umbel, seed weight produced per plant and 100 seed weight (Bhople *et al.*, 1999; Thomas & Isenberg, 1972). Soaking of the onion mother bulb in gibberellic acid prior to planting has also been demonstrated to increase the flower stalk length, seed weight per plant and 100 seed weight (Agwah *et al.*, 1994).

The time of application of gibberellic acid is also important with early spraying increasing the rate of flower induction and therefore number of flowers per umbel, while later applications influence developmental events such as rate of scape elongation (Corgan & Montano, 1972; Naamni *et al.*, 1980). Gibberellins do not appear to be involved in the stimulation of sprouting but application to the bulb promotes further sprout extension. A peak in endogenous GA concentration was observed some time before sprouting and this was concluded to be involved in promotion of flower initiation (Thomas & Isenberg, 1972). Gibberellins also have the capacity to overcome unfavourable photoperiod and temperature conditions in promoting flowering in onions (Naamni *et al.*, 1980; Thomas & Isenberg, 1972). Despite the evidence from several research studies of the involvement of gibberellins in flower initiation and development in onion commercial application of gibberellic acid in onion crops are rarely used. Variability in responses due to concentration and timing of application and the consistent yields achieved in most hybrid seed lines without costly chemical treatment may restrict the practical use of the hormone. The scope for use of gibberellic acid application in ON019A crops may be greater given the low levels of seed set in most crops and the evidence supporting the efficacy of applications in promoting flower development.

4.1.4 Research Objectives

Anecdotal and literature evidence suggests that improvements in seed yield in ON019A crops through reduced rates of flower abortion may be possible using a range of bulb and crop management strategies. The objective of the research covered in this chapter was to assess the effect of parent bulb size and source, bulb storage treatments, crop planting date, selected irrigation and nutrition management practices, and gibberellic acid applications on flower abortion rate and seed yield. A second objective was to undertake a comparison of results between the agronomic experiments to better understand the process of flower abortion in the inbred male sterile onion parent ON019A.

4.2 Materials and Methods

A range of agronomic treatments were applied under field conditions in order to assess their effects on the flower abortion rate and seed yield. The treatments were applied to gain an improved understanding of flower abortion, including the underlying physiology of the problem and to identify treatments that could be adopted in commercial onion seed crop production. The treatments used are detailed in Table 4.1.

Variables	Treatment
Bulb Characteristics	Bulb source Bulb maturity at lifting Bulb size Bulb storage
Transplant Time	Transplant time
Nutritional Survey	Commercial nutrition programs, examined by nutrient testing of commercial and experimental plots from transplanting to full bloom stage
Growth Regulator	1) GA_3 (commercial formulation pro-Gibb) 2) GA_4, GA_7 and benzyladenine application

Table 4.1 – Variables examined in agronomic studies of flower abortion.

4.2.1 Bulb Characteristics

Bulb Source

ON019A bulbs were sourced from commercial bulb producers located at Swan Hill in Victoria, Mt Gambier in South Australia, Narromine in New South Wales and two sites in northern Tasmania in 2005. After lifting all bulbs were air-dried, bulbs sourced from mainland Australia shipped to Tasmania, bulbs were stored in net bags under glasshouse conditions until transplanting in August, 2005. All bulbs were sized and quality graded with firm dormant bulbs between 40 and 60 mm retained for planting. 30 bulbs from each source were transplanted into 200 mm plastic pots filled with standard potting mix. Pots were arranged in a completely randomised block design with five blocks, on benches in a white house at the Australian Quarantine and Inspection Service (AQIS) facility in Kingston, Tasmania, pots were arranged in five blocks and hand watered by AQIS staff. Temperature conditions were ambient air temperature. A single primary umbel was collected from each plant at peak bloom and the percentage of aborted and normally developed flowers determined. Field trials were conducted at Swan Hill in April 2005; the mother bulbs used in the field was sourced from Mt Gambier, Narromine and Swan Hill. The treatment was replicated five times with 15 bulbs in each plot. During the peak bloom stage flower abortion percentage was assessed with five randomly selected primary umbels from each plot. Seed yield assessment was not possible because of the over- zealous farmer who harvested trial plots with the bulk seed lot before the SPS field officer arrived.

Bulb Maturity at Lifting

The effect of bulb maturity at lifting on flower abortion rate was tested using ON019A bulbs sourced from a commercial bulb producer at Wesley Vale, Tasmania, in 2005. The treatments applied were four bulb lifting dates at

approximately 10 day intervals from March, 2005, treatments were timed to extend approximately 2 weeks before and after commercial lifting time. Bulbs were dried and stored under ambient glasshouse conditions until transplanting at Old Beach and Cambridge (University of Tasmania farm) in August 2005. The bulbs from the four bulb lifting time treatments were planted in a randomised block design with five replicates, each replicate contained 15 bulbs in single rows and all plots were located within 1.6 m of pollinator plants (cv Kingswood). At peak bloom umbels from five plants per replicate were collected and assessed for number of aborted and normal flowers in order to calculate the percentage of aborted and normal flowers. The remaining 10 plants were left until seed maturity for the determination of seed yield.

Bulb Size

The effect of bulb size on the rate of flower abortion and on seed yield was tested under field conditions at Old Beach and University Farm (Cambridge) in 2005. Commercially grown bulbs sourced from a producer at Wesley Vale, Tasmania were graded into three size classes; Large (>60 mm diameter), Medium (40-60 mm), and Small (<40 mm). Five replicates of 15 bulbs of each size grade were planted in a completely randomised block design in August 2005; each row was adjacent to a row of pollinator plants (cv. Kingswood). At peak bloom stage individual umbels from five plants of each treatment were collected and assessed for the presence of aborted and normal flowers. The remaining 10 plants were allowed to develop until maturity and were then harvested to determine seed yield.

Bulb Storage Conditions

The effects of bulb storage on inflorescence development, flower abortion percentage and seed yield were assessed in field trials conducted at Old Beach and Cambridge in 2005. The bulbs used in the experiments were

medium size class (40-60 mm diameter), bulbs are sourced from a commercial bulb producer located at Wesley Vale, Tasmania. Bulbs were lifted in April air-dried and stored in a warehouse under ambient conditions (room temperature) until commencement of the storage treatments. The storage treatments were of 12 weeks duration, commencing in the 1st week of May. The treatments were; 4 °C, 10 °C, ambient air temperature (commercial storage conditions), 10 °C for 8 weeks followed by 30 °C for 4 weeks, and 30 °C for 4 weeks followed by 10 °C for 8 weeks. In each treatment the bulbs were stored in darkness in onion nets (open weave bags). Transplanting took place in August of each year, 4 replicate plots of the 5 treatments, each consisting of 20 bulbs were planted in a completely randomised block design. Each plot was located adjacent to a row of pollinator plants (cv. Kingswood) to enable pollination and assessment of seed yield.

4.2.2 Transplant Time

The effects of time of transplanting on inflorescence development, flower abortion percentage and seed yield were assessed in trials conducted in field conditions at both the Old Beach and University Farm (Cambridge) sites in the 2005-06 growing season. Medium grade sized bulbs ranging between 40-60mm diameter were used, bulbs were sourced from a commercial bulb producer at Wesley Vale, Tasmania. They were lifted in the first week of April, air-dried and stored under ambient temperature warehouse condition until transplanting. Firm disease-free bulbs were transplanted at 4 week intervals from May to October. The field experiments were arranged in a completely randomised block design with five replications, each replicate plot contained 30 bulbs arranged in two rows either side of a central row of 15 male pollinator bulbs of cultivar Kingswood. At peak bloom a single primary umbel was collected from each of five plants for flower abortion assessment. The remaining plants from each plot were harvested at maturity for the determination of seed yield.

4.2.3 Plant Nutrition Survey

In order to identify any relationships between plant tissue nutrient levels and rates of flower abortion in commercial crops a survey was conducted in the 2005-2006 season. Leaf and umbel tissue analysis was undertaken on samples collected from ON019A plants grown at 4 commercial sites; two sites from Swan Hill in Victoria and two sites from southern Tasmania, Old Beach and University Farm (Cambridge). Samples were collected every two weeks commencing at the onset of piping (appearance of the developing inflorescence above the leaves) and terminating at seed set. At the Old Beach site corresponding samples from the pollinator line (cv Kingswood) were collected, these samples were used for comparison purposes in the survey as cv Kingswood has a very low rate of flower abortion. Leaf tissue samples were the youngest fully expanded leaves of 10 randomly selected plants, umbel samples were single primary umbels taken from the same 10 plants. Tissue samples were oven dried, ground and sent for analysis for major (N, P, K, S, Ca, Mg,) and minor nutrients (Na, Cu, Zn, Mn, Fe, B, and Al) by a certified plant testing laboratory operated by the Department of Primary Industries, Victoria.

4.2.4 Plant Growth Hormone Trials

The effects of application of growth regulators on flower abortion and seed yield were assessed in field trials in Southern Tasmania and Swan Hill in the 2004–05 and 2005-06 seasons. In an initial experiment conducted at the University of Tasmania farm at Cambridge and Old Beach sites in 2004-2005 treatments were applied as a single application at the stage of development where 30% of plants had a visibly developing inflorescence (30% piping). This was based on previous reports of improved seed yields in onion from GA_3 applied at or near this stage of development (Naami *et al*, 1980). Laboratory grade GA_3 (Sigma, Aldrich, Victoria, Australia) was dissolved in 20 ml of ethanol and diluted in 5 L of deionised water to give

stock solutions of 50, 150 and 450 ppm (W:V). A solution consisting of 20 mL of ethanol diluted in 5 L of deionised water was used for the control treatment. At time of application, 10 ml/litre of Synetrol® crop oil was added to each treatment as a wetting agent, plants were sprayed to the point of run off using a knapsack sprayer. Treatments were applied from lowest to highest concentration and the sprayer was carefully rinsed between treatments. The trials were arranged in a completely randomised block design with 4 replicates, each plot consisted of 20 plants of ON019A in a single row adjacent to a row of pollinator (cv Kingswood). At peak bloom individual primary umbels were collected from five plants in each plot to determine the percentage of flower abortion, the remaining plants were harvested at maturity for seed yield determination.

Effect of GA Formulation

The treatments include Progibb® (a commercial formulation of GA_3), Cytolin (GA_4, GA_7 and Benzyl adenine) and laboratory grade GA_3 prepared as above with(10 mL/L) Synetrol oil in all treatments. Treatments were applied at 30% piping with four replicates at four times following a completely randomised block design.

Effect of Time and Concentration of GA_3 Application on Flower Abortion Rate

The effects of time of application of GA_3 (using the commercial formulation ProGibb®) at concentrations of 450 and 1000 ppm were examined in trials at both Old Beach and University Farm (Cambridge) in 2005-2006. The trials were designed as split plots with time of application as the main plots and application rates as sub plots. Times of application were pre-transplanting (soaking of the bulbs in aerated solution for 8 hrs), 30% piping, and 100% piping and after spathe break (approximately 14 days prior to anthesis).

Plot sizes and sampling procedures were the same as in previous GA experiments (above). The same application rate by time of spraying trial was duplicated in a commercial trial at Swan Hill in 2005 with the exception that a pre-transplanting treatment was not included; application prior to transplanting was not included in this trial.

Effect of Multiple GA_3 Applications on Flower Abortion Rate

The effect of multiple applications of GA_3 at concentrations of 150 and 450 ppm was assessed during the 2005-06 season at Old Beach, University Farm (Cambridge) and Swan Hill. Treatments were applied once (at 30% piping), twice (at 30% piping and 14 days thereafter) or three times (at 30% piping and 14 and 28 days thereafter). Each treatment was replicated four times in a split plot design, with the number of applications as main plots and application rate as split plots. Plot sizes and sampling procedures were the same as in previous GA experiments (above).

4.3 Results

4.3.1 Bulb Characteristics

Bulb characteristics significantly affected the rate of flower abortion and the seed yield in ON019A. The source of mother bulb and the time of lifting of the mother bulb influenced the rate of flower abortion, while the size of the mother bulb has a significant effect on seed yield. Storage treatments applied prior to planting had no significant effect on flower abortion rate. The responses of bulbs of different characteristics, or receiving different treatments, were not consistent between sites, suggesting that crop growing conditions and/or management practices may modify the plant development responses triggered by the pre-planting bulb treatments and characteristics.

Bulb Source

In shade house conditions flower abortion rates varied significantly between plants grown from different parent bulb lots (Figure 4.2); flower abortion rates were lowest for bulbs sourced from Mt Gambier (29%) and ranged up to 61% for bulbs sourced from Tasmania. Under field conditions in Swan Hill the effect of bulb source was apparent but the magnitude of differences was reduced in comparison to the shade house trial. At Swan Hill, plants of parent bulbs from Mt Gambier had an average abortion percent of 55% whilst plants of bulbs sourced from Narromine and Swan Hill had (57% and 62%) flower abortion respectively. Tasmanian bulbs were not grown at the Swan Hill field site; the bulbs grown at Old Beach and University Farm were sourced from Tasmania.

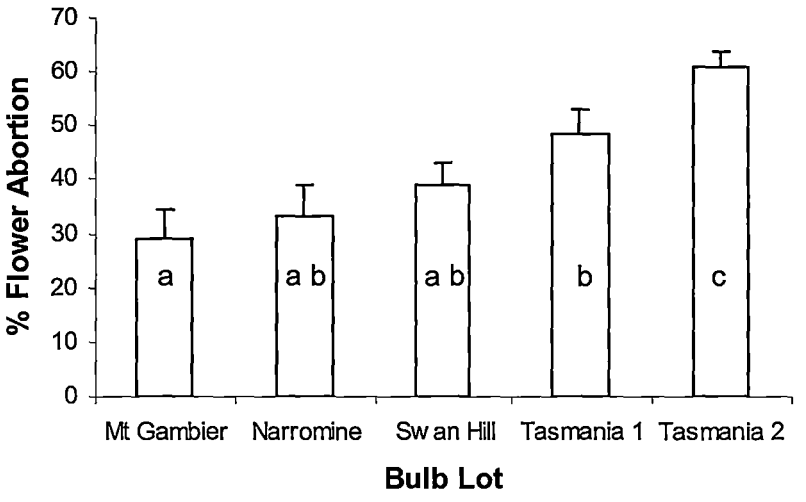


Figure 4.2 – Flower abortion percentages under shade house conditions of primary umbels of plants grown from parent bulbs of different origins. Error bars indicate standard errors (n=24). The lower case letter indicates the significant difference at ($P<0.05$)

Bulb Maturity at Lifting

Time of lifting of mother bulbs did not significantly affect flower abortion, although there was a small reduction in flower abortion rate with delayed bulb lifting. Bulbs lifted 2 weeks prior to commercial harvest aborted 11% of flowers on the primary umbels compared with bulbs lifted 4 weeks later which had a flower abortion rate on primary umbels of 4.9%. These differences did not translate into a significant difference in seed yield which ranged from 6.5 g to 8.7 g/plant (Table 4.2).

Bulb Lifting Date	1°Umbel diameter (mm)	% Flower Abortion	Seed yield /Plant (g)
March 1st	64.2 ± *3.2*	10.8 ± *3.2*	7.9 ± *1.1*
March 11th	71.0 ± *4.3*	12.5 ± *4.3*	8.7 ± *0.5*
March 21st	64.3 ± *4.1*	7.3 ± *4.1*	6.5 ± *0.9*
March 31st	69.5 ± *1.3*	4.9 ± *1.3*	7.7 ± *1.3*
LSD(P<0.05)	NS	NS	NS

Table 4.2 – The effect of mother bulb lifting date on primary umbel size, flower abortion rate and seed yield of ON019A at Old Beach in the 2004 – 05 season. Figures in italics denote standard errors (n = 5).

Bulb Size

At Old Beach mother bulb size significantly affected seed yield (P< 0.001) with yields ranging from 10.5 g/plant for large grade bulbs (>60 mm) to 3.2 g/plant for small grade bulbs (<40mm). Higher yields from larger parent bulbs were mainly due to an increase in the number of primary umbels per plant (Table 4.3). Whilst flower abortion rates declined with increasing bulb size from 9.8% for small bulbs (<40 mm) to 5.9% for large bulbs (>60 mm) this trend was not statistically significant (Table 4.3). The same effect of parent bulb size on seed yield was also observed at the University of Tasmania farm site, although differences in seed yield were less with plants grown from small parent bulbs (<40 mm) yielding 0.5 g of seed compared with 2.8 g from plants grown from large parent bulbs (>60 mm). At this site plants grown from large parent bulbs (>60 mm) also produced greater numbers of primary umbels (Table 4.3) but, in contrast to Old Beach also had markedly reduced rates of flower abortion at anthesis compared with small bulbs (Table 4.3). The extent of this reduction was from 81.0% flower abortion for small (<40 mm) bulbs to 40.1% for large (>60 mm) bulbs.

During 2005–06 the effect of bulb size was studied in the University Farm (Cambridge); the percentage of flower abortion was recorded as 95.6 from the small sized bulb and 39 percent from the large sized bulb which significantly differ at P<0.001 and seed yield of 0.12 gram and 0.64 grams per plant was recorded respectively from small and large sized bulb.

Year	Locations	Bulb size Grade	No. of Umbel/ Plant	Umbel Diameter (mm)	Yield/ plant (g)	Yield/ umbel (g)	% of flower abortion
2004	Old Beach	<40 mm	1.3 ± *0.10*	70.9 ± *2.0*	3.2 ± *0.4*	2.5 ± *0.3*	9.8 ± *0.2*
		40 - 60 mm	1.8 ± *0.11*	69.0 ± *3.9*	5.9 ± *0.3*	3.3 ± *0.2*	6.1 ± *0.4*
		>60 mm	3.2 ± *0.18*	66.4 ± *1.9*	10.5± *1.3*	3.3 ± *0.4*	5.9 ± *0.4*
		LSD (P<0.05)	0.3	NS	1.8	0.5	NS
2004	Cambridge	<40 mm	1.5 ± *0.03*	57.1 ± *1.8*	0.5 ± *0.1*	0.33 ±*0.04*	81.0 ± *3.9*
		40 - 60 mm	2.3 ± *0.03*	54.3 ± *3.5*	1.3 ± *0.1*	0.56 ±*0.06*	54.3 ± *3.1*
		>60 mm	3.1 ± *0.12*	58.2 ± *3.2*	2.8 ± *0.2*	0.90 ±*0.06*	40.1 ± *0.7*
		LSD (P<0.05)	0.5	NS	0.3	0.12	12.3
2005	Cambridge	<40 mm	NR	NR	0.12 ± *0.1*	NR	95.6 ± *4.4*
		40 - 60 mm	NR	NR	0.64 ± *0.2*	NR	39 ± *1.2*
		LSD (P<0.05)			0.3		14.3

Table 4.3 – Effect of ON019A mother bulb size on number of umbels per plant, umbel diameter, percentage of flower abortion and seed yield at Old Beach and University Farm (Cambridge) 2004 and University Farm (Cambridge) 2005,. Figures in italics denote standard error (n = 4). NR=Not recorded.

Bulb Storage Conditions

Bulb storage temperature during the 12 weeks period between curing and transplanting had no significant effect on flower abortion rate or seed yield either or at University Farm (Cambridge)(Table 4.5) or at Old Beach (Table 4.5). At Cambridge flower abortion rates were high (>70%) across all treatments. Whilst a 4 week period of high temperature (30 °C) following 8 weeks storage at 10 °C was effective in reducing the number of vegetative divisions within the parent bulb (Table 4.4)

Storage Temp °C	No. of Umbels /plant	No. of Vegetative divisions/ plant	Yield/plant (g)	Yield/Umbel (g)	% Flower Abortion
Ambient	2.1 ± *0.3*	1.6 ± *0.4*	6.9 ± *1.6*	3.3 ± *0.9*	1.8 ± *0.6*
4	2.3 ± *0.4*	1.9 ± *0.3*	8.7 ± *1.1*	3.8 ± *0.8*	1.2 ± *0.6*
10	2.8 ± *0.3*	1.2 ± *0.3*	7.3 ± *1.3*	2.6 ± *1.8*	1.1 ± *0.5*
30 to 10	2.0 ± *0.3*	1.4 ± *0.3*	6.4 ± *1.1*	3.4 ± *1.3*	1.5 ± *0.9*
10 to 30	1.9 ± *0.2*	0.9 ± *0.3*	6.0 ± *0.9*	3.2 ± *1.0*	1.7 ± *0.8*
LSD (P<0.05)	NS	NS	NS	NS	NS

Table 4.4 – Effect of mother bulb at different storage temperatures on number of umbels per plant; yield per plant and per umbel and percentage of flower abortion at Old Beach 2004. Figures in italics denote standard errors (n = 4).

Locations	Storage Temp °C	Yield/plant (g)
Old Beach	Ambient	3.9 ± *1.0*
	4	3.3 ± *0.4*
	10	2.6 ± *0.4*
	30 to 10	3.0 ± *0.8*
	10 to 30	2.9 ± *0.8*
LSD (P<0.05)		NS
Cambridge	Ambient	0.13 ± *0.1*
	4	0.15 ± *0.1*
	10	0.28 ± *0.2*
	30 to 10	0.45 ± *0.2*
	10 to 30	0.35 ± *0.2*
LSD (P<0.05)		NS

Table 4.5 – Effect of mother bulb storage temperature on seed yield at Old Beach and University Farm (Cambridge) from 2005. Figures in italics denote standard errors (n = 4).

4.3.2 Transplant Time

There was a progressive reduction in seed yield from 7.1 g/plant to 0.4g/ (Table 4.6).Flower abortion data was recorded from the both sites Old Beach and Cambridge. The seed yield from the Cambridge sites is poor when compare to the Old Beach. The maximum seed yield obtained from the Cambridge 0.22 grams per plant during the September.

Months 2005	Old Beach Seed Yield / Plant (g)	University Farm Seed Yield / Plant (g)
May	5.5 ± *2.2*	0.08 ± *0.08*
June	4.9 ± *1.3*	0.06 ± *0.04*
July	7.1 ± *1.7*	0.13 ± *0.10*
August	4.4 ± *3.2*	0.12 ± *0.07*
September	1.4 ± *0.5*	0.22 ± *0.17*
October	0.4 ± *0.1*	0.13 ± *0.11*
LSD(P<0.05)	1.97	NS

Table 4.6 – Effect of different dates of trans-planting at Old Beach and University Farm (Cambridge) on seed yield per plant in 2005 growing season between six different dates. Figures in italics denotes standard errors (n = 5).

4.3.3 Plant Nutrition Surveys

Surveys of the nutritional status of young and fully expand leaves and developing umbels of ON019A between piping and seed set were undertaken at a range of sites encompassing crops that displayed a wide range of flower abortion rates (Table 4.7). The relation-ship between and site condition and abortion percentage was not investigated, but it was evident that were no obvious differences in nutritional status between plants of ON019A and Kingswood (an open-pollinated cultivar) with very low rate of flower abortion.

Sites	Old Beach ON019A	Cambridge ON019A	Hardy ON019A	Grivell ON019A	Cambridge Kingswood
Flower Abortion %	**1.0%**	**84.3%**	**75%**	**77%**	**<1.0%**
		Macronutrient			
N (%w/w)	3.8 – 1.5	4.1 – 1.6	4.0 – 2.0	2.9 – 1.9	3.0 – 1.5
P (%w/w)	0.5 – 0.2	0.5 – 0.2	0.4 – 0.2	0.4 – 0.3	0.5 – 0.2
K (%w/w)	3.9 – 1.3	4.3 – 1.8	4.0 – 2.2	3.6 – 2.2	3.3 – 1.5
S (%w/w)	0.6 – 0.3	0.8 – 0.3	1.0 – 0.5	0.8 – 0.5	0.6 – 0.2
Ca (%w/w)	1.8 – 1.0	1.8 – 0.9	1.3 – 1.1	1.8 – 0.9	0.9 – 0.5
Mg (%w/w)	0.6 – 0.3	1.0 – 0.4	0.7 – 0.4	0.6 – 0.4	0.6 – 0.4
Na (%w/w)	0.7 – 0.3	0.3 – 0.1	0.6 – 0.2	0.3 – 0.1	0.4 – 0.1
		Trace element			
Cu (mg)	5 – 3	7 - 12	12 - 6	9 - 4	6 - 2
Zn (mg)	52 – 26	26 - 11	46 - 25	41 - 27	23 - 6
Mn (mg)	130 – 58	99 - 16	170 - 100	210 - 100	22 - 13
Fe (mg)	920 – 450	340 - 160	750 - 380	590 - 330	310 - 200
B (mg)	49 – 39	47 - 30	57 - 40	45 - 35	35 - 51

Table 4.7 – The macro and trace element concentration of ON019A and Kingswood from 2005 crops. Samples from Old Beach, University Farm (Cambridge) and Swan Hill.

4.3.4 Plant Growth Hormone Trials

Application of GA_3 at 450 ppm at 30% piping significantly ($P<0.05$) reduced flower abortion at anthesis at the Old Beach site in the 2004 – 05 season. In contrast 50 and 150 ppm of GA_3 applied at the same stage had no effect on flower abortion rates. From the preliminary trials conducted at the Tasmanian locations, Generally low levels of flower abortion and high seed yields were recorded in all treatments at Old Beach so reductions in flower abortion did not translate into increased seed yield (Table 4.8). At University Farm (Cambridge) seed yield increased significantly from 0.78 g / plant (control) to 1.3 g / plant in the 450 ppm GA_3 treated plants. Umbels treated with 450 ppm GA_3 had larger more elongated flowers in which the petals were opened fully and the anthers and stigmas extended fully (Plate 4.1).

Site	GA_3 Concentration (ppm)	% Flower Abortion at peak Bloom	Seed Yield /Plant (g)
Old Beach	0	15.6 ± *0.1*	5.9 ± *0.8*
	50	14.6 ± *1.8*	6.1 ± *1.0*
	150	14.3 ± *0.1*	5.9 ± *0.7*
	450	4.1 ± *1.7*	6.0 ± *0.8*
LSD(P<0.05)		3.8	NS
Cambridge	0	NR	0.8 ± *0.1*
	50	NR	0.8 ± *0.2*
	150	NR	1.0 ± *0.2*
	450	NR	1.3 ± *0.2*
LSD(P<0.05)			0.3

Table 4.8 – Effect of GA_3 at single application of 50, 150, and 450 ppm from Old Beach and University Farm (Cambridge) during the 2004 growing season. Figures in italics denote standard errors (n = 4). NR= preliminary trial, data not recorded.

Plate 4.1 – An umbel from the plant treated with 450 ppm compared to non treated umbels at University Farm (Cambridge) site in 2005. Note the failure of anthers and petals to open fully on most flowers in the non-treated umbel.

Progibb® a commercial formulation of GA_3 proved as effective as laboratory grade GA_3 in reducing flower abortion and improving seed yield,

The stage of plant development at which GA_3 was applied significantly ($P<0.05$) affected its efficacy in reducing flower abortion (Table 4.9); later application (up to 100% piping) was more effective in reducing flower abortion and increasing seed yield than earlier application at both Swan Hill and Old Beach. Yield responses to GA_3 application were significant ($P<0.05$) and substantial at Old Beach (from 2.6 g for non treated plants to 4.2 g / plant for plants treated with 1000 ppm GA_3 at 100% piping) but, although significant ($P<0.05$) relatively small at Swan Hill (from 0.28 g for non treated plants to 0.77 g / plant for plants treated with 1000ppm GA_3 at 100% piping) and Cambridge ($P<0.05$) despite marked reductions in flower abortion at anthesis at Swan Hill and Old Beach sites (Table 4.9) and University Farm (Table 4.10). Soaking of bulbs in GA_3 prior to transplanting had a significant negative effect on seed yield at both Old Beach and University Farm (1.6 g/plant at Old Beach and 0.16 g/plant at Cambridge).

Location	GA_3 Rate (ppm)	Stage of Development	% Flower Abortion at Peak Bloom	Seed Yield / Plant (g)
Swan Hill	Control (0ppm)		77 ± *5.1*	0.28 ± *0.1*
	450	15% piping	70 ± *5.3*	0.36 ± *0.2*
		60% piping	76 ± *9.9*	0.26 ± *0.1*
		100% piping	60 ± *2.6*	0.80 ± *0.1*
		Spathe break	62 ± *4.6*	0.42 ± *0.2*
	1000	15% piping	75 ± *1.7*	0.18 ± *0.04*
		60% piping	71 ± *3.2*	0.27 ± *0.1*
		100% piping	48 ± *0.8*	0.77 ± *0.2*
		Spathe break	54 ± *4.2*	0.55 ± *0.3*
	LSD (P<0.05)		12.41	0.39
Old Beach	Control (0ppm)			2.6 ± *0.2*
	450	Transplanting	NR	1.6 ± *0.1*
		30% piping	NR	3.0 ± *0.3*
		60% piping	NR	3.8 ± *0.2*
		100% piping	NR	4.0 ± *0.1*
	1000	Transplanting	NR	1.8 ± *0.1*
		30% piping	NR	3.2 ± *0.2*
		60% piping	NR	3.6 ± *0.2*
		100% piping	NR	4.2 ± *0.4*
	LSD (P<0.05)			0.5

Table 4.9 – Effect of time of application and concentration of GA_3 on flower abortion rate and seed yield at Swan Hill and Old Beach in 2005. Figures in italics denote standard errors (n = 4). The treatment differed at the two sites to check the effects at different times during flowering. NR= Not Recorded.

GA_3 concentration	Time of Application	Seed Yield/plant (g)
Control		0.24
150 ppm	Transplanting	0.16
	15% piping	0.20
	60% piping	0.40
	100% piping	0.24
	Spathe break	0.43
450 ppm	Transplanting	0.33
	15% piping	0.23
	60% piping	0.18
	100% piping	0.22
	Spathe break	0.16
LSD($P<0.05$)		NS

Table 4.10 – Effect of GA_3 (commercial formulation ProGibb®) at time of application at different stage of plant growth at 2005 season in University Farm (Cambridge) on seed yield errors (n = 4).

Despite the apparent benefit of two or three successive multiple applications at 14 day intervals from 30% piping, they did not reduce flower abortion or improve seed yields beyond that achieved with a single application applied at 60% piping at either Old Beach or University Farm (Cambridge) in 2005 (Table 4.11).

Locations	Multiple application	% of Flower Abortion	Seed yield /plant (g)
Swan Hill	Control	75 ± *9.5*	0.11 ± *0.5*
	1 X 150 ppm	36 ± *11*	0.17 ± *0.4*
	2 X 150 ppm	56 ± *14*	0.11 ± *0.3*
	3 X 150 ppm	62 ± *10*	0.13 ± *0.2*
	4 X 150 ppm	71 ± *2.1*	0.12 ± *1.0*
	1 X 450 ppm	42 ± *10*	0.36 ± *0.7*
	2 X 450 ppm	56 ± *14*	0.47 ± *3.2*
	3 X 450 ppm	69 ± *9.1*	0.28 ± *2.0*
	4 X 450 ppm	62 ± *17*	0.11 ± *0.5*
LSD(P<0.05)		31.8	NS
Old Beach	Control	NR	1.90
	1 X 150 ppm	NR	2.64
	2 X 150 ppm	NR	1.70
	3 X 150 ppm	NR	2.80
	4 X 150 ppm	NR	1.18
	1 X 450 ppm	NR	3.01
	2 X 450 ppm	NR	1.87
	3 X 450 ppm	NR	0.84
	4 X 450 ppm	NR	1.05
LSD(P<0.05)			0.97

Table 4.11 – Effect of multiple applications of GA_3 at various concentrations Swan Hill and Old Beach during the 2005 season on flower abortion and seed yield. Figures in italics denote standard errors (n = 4). NR=Not Recorded.

4.4 Discussion

Bulb size and bulb source had a significant effect on flower abortion percentage and therefore seed yield, while bulb size also influenced seed yield through an effect on umbel and flower number. The difference in seed yield between small and large sized mother bulbs was consistent between seasons and sites with bulb size less than 40 mm diameter producing lower yield than bulb size greater than 60 mm diameter; this effect of bulb size on seed yield was consistent with that found in other studies (Green, 1972; Jones & Emsweller, 1937; Singh & Ahmed, 2005; Orlowski, 1974; Reghin *et al.*, 2005; Van der Meer & Van Bennekorn, 1969). While yield increases were obtained from using larger sized mother bulbs at both field sites the variation in yield between the two sites from Tasmania was very high. There was no consistency in the percentage of flower abortion with bulb size grades between the two sites indicating that the bulb size effect on flower abortion may be modified by some other site specific factor. The effect of site factors on crop responses to bulb characteristic treatments was also evident in the bulb source trial with significant treatment effects recorded at all sites but, also large differences in flower abortion percentage and seed yield between sites irrespective of treatment.

Storage of mother bulbs at different temperatures did not result in a significant change in flower abortion percentage, this result was consistent with previously published studies with onions (Currah *et al.*, 1990; Reghin *et al.*, 2005; Van der Meer & Van Bennekorn, 1969; Van Kampen, 1970). As found in the bulb size and source trials large differences in seed yield were found between sites supporting the conclusion that environmental factors play a major role during the flowering time in determining seed yield. Time of lifting of the mother bulb had a minor effect on flower abortion with percentages ranging from 11% to 4.9% from the bulbs lifted from 4 weeks and 6 weeks before commercial harvest; this difference is unlikely to account

for the variation noted between sites and seasons in ON019A crop performance.

Time of planting had a significant effect on seed yield and flower abortion with mother bulbs planted in June producing a higher seed yield per plant of 7.9 grams than bulbs planted in October (0.42 gram per plant). Delayed sowing has been shown previously to reduce the seed yield and both number of flowers and umbels (Corgan & Kedar, 1990; Farghail, 1995; Wurr *et al.*, 2001). The results from the present study also demonstrate that time of planting can affect rate of flower abortion, the effect on flower abortion was evident under field conditions in the October planting with 42% flower abortion compared to 0.8 % flower abortion June planted bulbs. In contrast, under constant environmental conditions abortion percentage remained between 16% and 24% for all transplanting dates. Therefore it was concluded that the response observed in the field reflected a negative impact of environmental factors such as increasing day length, incident radiation and temperature on inflorescence development following transplanting.

This is consistent with a previous report of long day and warm temperature favouring bulb growth over inflorescence growth during the competition phase of onion plant development (Brewester, 1982). Samples collected for biomass partitioning shows that reduction of dry weight above the ground level and increased partitioning to the bulb for the later planting dates. The data from the Old Beach (7.1 g from July planting and 0.4 grams in October planting) trial support a role for competition for reserves in induction of flower abortion and demonstrate that the delayed time of planting can induce flower abortion. The nutritional survey of plant tissue and umbel samples showed there was no obvious differences in tissue nutrient levels between the male sterile line (ON019A) and the pollinator line but, very large differences in rates of flower abortion. Furthermore at all sites examined nutrient concentrations in ON019A were within or slightly above the range recommended as optimal for onion (Boyhan *et al.*, 2001); it was therefore

concluded that a nutritional factor was unlikely to be the critical factor for ON019A in induction of flower abortion.

Application of GA_3 was found to be a promising tool for management of flower abortion in ON019A; a single application of GA_3 at 450 ppm at 60% piping reduced the flower abortion percentage, but later application of GA_3 either once or twice at 14 day intervals commencing at 30% piping did not reduce the rate of flower abortion or improve the seed yield. Soaking of the mother bulb in GA_3 prior to planting significantly reduced seed yield. Application of GA_3 at later stages of scape elongation was effective, but less so in reducing the rate of flower abortion and increasing seed yield. Previous studies have shown that GA_3 helps to increase the number of umbels and number of flower per umbels and therefore seed yield (Looper & Waller, 1982; Naamni *et al.*, 1980; Ruggeri *et al.*, 1994) but this is the first study to document the beneficial effect of GA_3 application on reducing flower abortion. The concentration and time of application of GA_3 affect efficacy of the treatment with a single application of GA_3 at 450 ppm at later stages of flowering improving seed yield. The effect also depends on the prevalent environmental conditions with variation in plant response recorded between field sites. Gibberellin acid application increases availability of carbohydrate to the growing parts from the source (Theron & Jacobs, 1996) and therefore may affect tissue osmotic potential (Millar *et al.*, 1971) suggesting that changes in plant water relations may be involved in flower abortion of the weak inbred male sterile line ON019A.

Overall it was concluded that the seed yield from ON019A could be improved by selection of large mother bulb size, early planting and the application of gibberellic acid at late flowering stage to reduce the incidence of flower abortion. These recommendations are however not a complete solution for flower abortion as considerable inconsistency in response to treatments between sites demonstrates that some other factors could also be associated with the seed yield problem.

Chapter 5

Role of Water Relations in Flower Abortion in Inbred Male Sterile Onion Parental Line ON019A

5.1 Introduction

Low yield and failure of seed set in open pollinated and hybrid cultivars have been attributed to poor husbandry, including pest and disease management, whereas in inbred parent lines with poor reproductive capacity the mechanisms behind unreliable seed yield appear to be more complex (Brown *et al.*, 1977). One management factor thought to be important in production of both open pollinated and hybrid crops is irrigation, with the timing of irrigation and type of irrigation system affecting crop growth rate and therefore potentially influencing seed yield. The onion root system is considered to be very shallow, absorbing water from the top 25 cm of sandy soil (Goltz *et al.*, 1971) and consequently onion crops require plentiful soil water in that zone to maximize growth rate (Levy *et al.*, 1981).

Irrigation is an important crop management tool in onion seed production (Hawthorn & Woodbury, 1969; MacGillivary, 1948; Millar *et al.*, 1971). The recommended method of irrigation varies with locality and farmers (Brewster, 1994). Common irrigation methods followed are furrow irrigation, drip irrigation and sprinkler irrigation. Furrow and drip irrigation have been reported to be the most common strategies used on short day onion crops in dry regions (Corgan & Kedar, 1990). The required amount and frequency of irrigation depends on the method of irrigation, soil type and condition and weather (rainfall amount and timing, temperature, evapotranspiration).

In trials utilizing furrow irrigation, the highest onion seed yield was obtained with 0.5 bars of soil moisture tension whereas furrow irrigation with 0.4 bars of soil moisture tension reduced seed yield by 26% (Brown *et al.*, 1977). The possibility of obtaining reasonable onion seed production under sprinkler irrigation is heavily influenced by the timing of irrigation as there is a risk of promoting diseases such as downy mildew and adverse effects on pollination if the sprinkler irrigation results in dilution of nectar and subsequent failure to attract the bees (Brown *et al.*,1977). Alternatively, irrigation may have a positive effect as onion nectar is generally more attractive to bees when it is diluted due to high potassium content. Drip irrigation has also been recommended for onion seed production as it makes more efficient use of water than the open furrow system and has been widely adopted in Israel (Corgan & Kedar, 1990) and Australia.

Buried drip irrigation is also possible but is not suitable for wide onion beds (1.5-2.0 m) unless a sprinkler irrigation system is used to supply additional water at critical stages of crop growth (May *et al.*, 1994). In less developed regions where the technology for the large scale irrigation channels is not available irrigation systems using small ditches and sluices are used to apply water to small beds on which crops are grown (Currah *et al.*, 1990). In many arid and semi-arid countries water availability is the most important factor for onion seed production and different soil mulches such as polyethylene film and organic mulches (paddy straw, ground nut shell, millet straw) are used to maintain the soil moisture and increase the availability of moisture to plants (Adetunji, 1994); covering of the soil surface with polyethylene reduced the amount of irrigation water needed for an onion seed crop by 70% (Abu-Awward, 1999).

Millar *et al,*(1971) explained that the lowest water potentials in the onion plant existed in onion flowers and pedicles, indicating there is a considerable barrier to flow of water from the plant system to the florets. Stomatal conductance of onion leaves displays a three times greater response to a one bar change in turgor than snap bean, indicating that onion stomata are very sensitive to water deficits. Water stress during the peak period of flower

development leads to the poor fertilisation and seed development in onion reported by (Brown *et al.*,1977) and may also affect the pollen viability, Stigma receptivity can be adversely affected reducing seed yield due to abortion of developing seeds (Brown *et al.*,1977).

Temperature is another important climatic factor to be considered when examining the processes associated with flower abortion and low seed yield in onion. High daytime temperature and low night time temperature as well as low day time and high night time temperatures have been shown to have a major effect on flower abortion in iris (Fortainer & Zevenbergen, 1973); Stirling, (1997). Evidence from an earlier study in *Iris* suggested that plants were more susceptible to water stress during the reproductive stage rather than in the vegetative phase and that exposure to high temperature may induce abortion during the susceptible phase (Hartsema & Luyten,1955). In *Lilium,* high night temperatures have been found to result in a high rate of flower abortion (Wilkins, 1986) .

The onion plant has been demonstrated to have a very low water potential in the flowers and pedicel with considerable resistance to the flow of water from the soil to the florets (Brown *et a.*, 1977). Soil moisture deficits through insufficient irrigation increase the likelihood of low flower and pedicel water potential, while conditions such as high temperature and low humidity would also favour low water potential. Even under condition of high soil moisture levels, hydraulic resistance within the plant could lead to transpiration losses exceeding water uptake. High temperatures, promoting rapid tissue development and subsequent requirement for regulation of turgor during expansion growth may also predispose the flowers to damage associated with declining water potential.

If flower abortion in onion involves loss of turgor in the expanding floral tissue in the latter stages of flower development then it would be expected that improved irrigation management would increase yield under such environmental conditions. Strategies to reduce the risk of water stress in

crops include effective irrigation management, selection of sites with conditions that favour low rates of evapotranspiration and application of chemicals to slow transpiration. The range of chemical substances used to spray on the plants to reduce the loss of water through transpiration are known as anti-transpirants (Srivastava, 1996).

There are two different types of ant- transpirant; metabolic inhibitors which reduce the stomatal opening and film-forming anti-transpirants that form a thin film on the surface of the plant tissue to reduce moisture loss through transpiration (http://en.wikipedia.org/wiki/Antitranspirant). While anti-transpirants may reduce water loss for short periods after application the duration of the effect may not be sufficient for crop yield or water saving benefits (Srivastava 1996). Anti-transpirant applied under an arid climate situation were not effective in improving sunflower seed production (Thakuria *et al.*, 2004).

Application of the anti-transpirant 'vapour guard' before flowering in tomato had no effect on the fruit yield (Irmak *et al.*, 1999). The efficacy of anti-transpirants in reducing flower abortion rates in bulb crops such as onion has not been investigated but given the short window in which the developmental events occur, the likelihood of reduced transpirational water loss at the critical stage of flower development may be high.

5.1.1 Research Objectives

Literature evidence suggests that there is may be a relationship between plant water status and flower abortion and therefore to the seed yield in onion. The objective of the research covered in this chapter was to assess the effect of irrigation management, shading, GA_3 application and anti-transpirant application on flower abortion percentage and seed yield. A second objective was to use the knowledge gathered from the effect of the applied treatments to gain some understanding the physiological basis of flower abortion in the inbred male sterile line ON019A parental line.

5.2 Materials and Method

5.2.1 Plant Materials

The plant materials were supplied by the commercial seed production company South Pacific Seeds from a commercial parent bulb crop grown in Mt Gambier (cultivar ON019A) and purchased from a locally grown North West Tasmania bulb crop (cultivar Creamgold). Field trials were conducted in at the University Farm and South Pacific Seeds Farm (42.5° S, 147.3° E) Cambridge, Tasmania and Swan Hill (35.34° S, 143.5°E) Victoria, Australia in the 2006-2007 season. Current management practices were followed to maintain crop health. Glasshouse trials were conducted in the University of Tasmania (Hobart campus) (Table 5.1).

Variable	Treatment
Glasshouse Experiment	
Irrigation	High Irrigation Low Irrigation
Shading	Shading the whole plant
Blowing Air	Maintaining air movement around umbel to maximise evapo-transpiration.
Shade House Temperature between (28°C)	Low temperature regime
Glasshouse Temperature between (38°C)	High temperature regime
Field Experiments	
Irrigation	High irrigation rate Low irrigation rate
Wind sheltering	Protect the plant with vertical shade cloth (50% grade)
GA_3 and anti-transpirant application	GA_3 alone GA_3 applied as single application @ 450 ppm Anti-transpirant (Envy) and GA_3 in combination Anti – stress Vapour Guard (Anti-transpirant) @SPSsite. Anti-transpirant (Envy) alone Envy applied @ 50 ml per litre of water applied at 400 L/Ha

Table 5.1 – Variables examined in the experiments described in Chapter 5.

5.2.2 Glasshouse Experiments

Glasshouse experiments were conducted at the School of Agricultural Science at the Hobart campus of the University of Tasmania in the 2007 growing season. Experiments were conducted with two different environments with mean maximum temperatures of 28°C (shade house) and 38°C (glasshouse) respectively. Single bulbs were planted in 4.5 L plastic pots filled with standard potting mixture (Appendix 1). Plant water status was manipulated within the two environments by imposing shading and irrigation treatments. High and low rates of irrigation were adjusted using two different sized drippers and varying the number of drippers in each pot. Shading treatment was imposed using 50% shade cloth in order to keep the umbel surface shaded; shade cloth was hung from the top irrigation pipe and was arranged to completely cover pots. The percentage of flower abortion was examined in the treatments which were imposed to vary the rate of transpiration.

The effect of removal of free surface moisture from inflorescences was examined by blowing the air from the two edges of the bench with a fan to keep the umbel surfaces dry. The five treatments (high and low irrigation, shading, removal of surface moisture and control) were arranged in a completely randomised block design with five pots per treatment. During the peak bloom stage the flower abortion percentage was calculated for plants in all treatments under both environmental conditions. The prevailing environmental conditions were given in the Figures 5.1 and 5.2

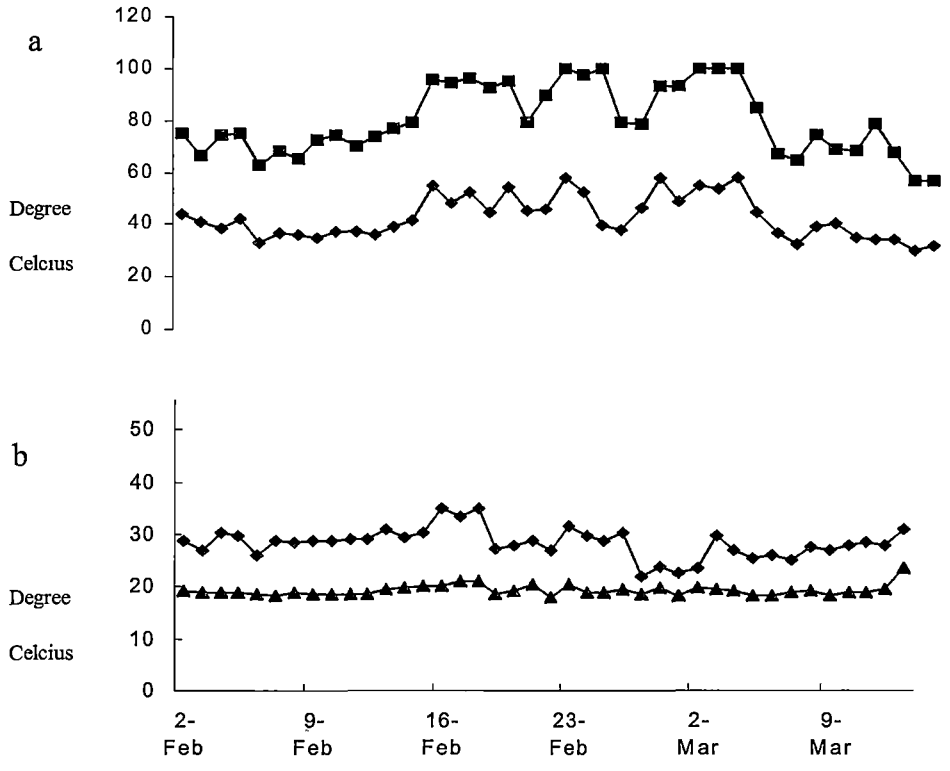


Figure 5.1 –Daily maximum (■) and minimum (♦) relative humidity (a) and the maximum (♦) and minimum (▲) temperature (b) during the flowering period inside the shade house number (8) (Feb-Mar 2007).

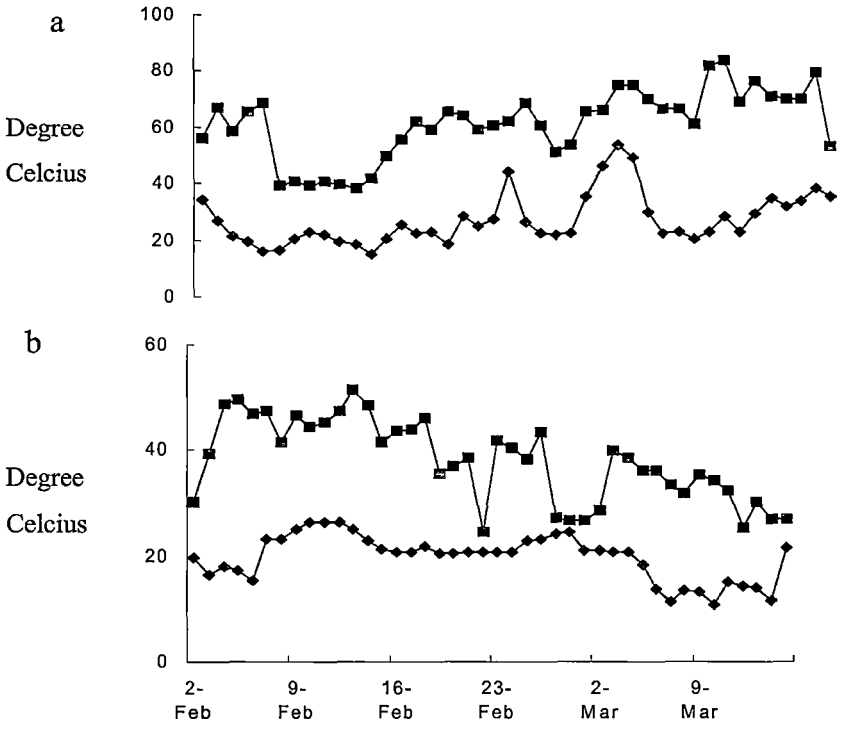


Figure 5.2 – Daily maximum (■) and minimum (♦) relative humidity (a) and the maximum (■) and minimum (♦) temperature (b) during the flowering period inside the glasshouse number 12 (Feb – Mar 2007).

Field Trials

5.2.3 Wind Shelter

Under field conditions at the SPS Site (Cambridge) and University Farm (Cambridge) during the 2006-2007 growing season a shelter treatment was imposed using 50 percent shade cloth with four sides pegged with star pickets such that plants were surrounded on all sides. The plot size was 6 m long x 3 m wide consisting of two rows of ON019A and a central row of the pollinator Creamgold. At the University Farm the sheltered plants were sprayed with a single application of GA_3 at 450 ppm, with four replications in a completely randomised block design. Flower abortion and seed yield data were collected from the SPS Farm (Cambridge) and seed yield data were collected from the University Farm (Cambridge) as outlined in chapter 2.

5.2.4 Irrigation Treatment

Irrigation trials were conducted in 2005-06 seasons at the University Farm (Cambridge) site. The treatments used in both seasons were cycles of different duration and intensity of water stress generated by varying the interval between irrigation and therefore events (2, 4, 8, and 12 days). Irrigation was applied by surface drip tape at the rate of 70% replacement of cumulative pan evaporation. Evaporation data were sourced from the Australian Bureau of Meteorology, Hobart Airport weather station, located less then 5 km from the trial site. Individual plots consisted of 30 plants in two rows, adjacent to a single row of pollinator line (cvs Kingswood and Creamgold). Each treatment was replicated five times in a completely randomised block design. Following transplanting all plots was maintained under a commercial irrigation regime until sprouting, and at this point the four treatments were applied.

Soil water potential data were collected at 2, 4, 8, and 12 days from three replicates of each treatment by gypsum block (MEA GB Reader) positioned in the root zone (150 mm below the soil surface).

During the 2006-07 season, an irrigation trial was carried out at the SPS farm; two irrigation treatments, low and high irrigation rates, were imposed using automatic timer Hunter SVC automatic solenoid (Hunter Industries, The irrigation Innovators, South Australia) controlled irrigation systems. The treatments were conducted over a 7-day irrigation schedule. The low irrigation schedule was irrigated on one day during the 7-day period (9 am, 12 noon and 3 pm) (70% of cumulative pan evaporation determined at the Hobart airport). The high irrigation treatment received the same total volume of water per day (9 am, 12 noon and 3 pm) three times in a week. Treatments were applied from spathe break to seed maturity. Treatments were replicated four times in a completely randomised block design. At the peak bloom stage, five umbels samples were collected and the percentage flower abortion scored, the remaining samples were left until full maturity for the determination of seed yield.

5.2.5 Ant-Transpirant Application and GA_3

The effect of application of the anti-transpirant 'Envy' with and without GA_3 application was studied in three different locations: two locations in Tasmania [SPS Farm (Cambridge) and University Farm (Cambridge)] and one in [Victoria (Swan Hill)]. The chemical treatments were 450 ppm GA_3 as a single application and 50 ml of Envy per litre of water, applied at 400L/Ha.

Specific treatments

1) Control – no treatment
2) +GA_3
3) +Envy
4) + GA_3 and Envy

Treatments were replicated four times at each location and the experimental design was a completely randomised block. A standard plot size of two rows consisting of twenty bulbs of ON019A on each side of a single row of pollinator Creamgold bulbs was used. During the peak flowering stage umbel samples were collected from the five primary umbels from each replication for calculation of flower abortion percentage. The remaining plants were harvested at maturity for the calculation of seed yield per plant.

5.2.6 *In vitro* Pollen Germination and Seed Set

During the peak flowering season in the field trials at Cambridge SPS Farm, non-aborted ON019A flowers were hand-pollinated in all four replicates for shelter, GA_3 and control treatments. To check the effect of GA_3 and shelter on seed set and to make sure the pollen germination and fertilisation on the hand-pollinated plants, twenty five flowers from each replication were hand-pollinated and 10 of the hand-pollinated flowers were collected after 48 h and checked for pollen grain germination using fluorescence microscopy. Hand-pollinated flowers were dissected under light microscopy and the stigma and style surface was placed on a glass slide with a drop of aniline blue dye (0.01 g of aniline blue in 20 ml of 0.1 M K_3PO_4) and covered with a glass cover slip. Samples were examined under ultraviolet light using a fluorescent microscope (Leica, Heerbrugg, Switzerland) and scored for the number of germinated pollen grains. The remaining hand-pollinated flowers were collected at seed maturity to confirm seed setting rates under the different treatments.

5.3 Experiment Results

5.3.1 Glasshouse Experiments

The glasshouse experiments included two different irrigation treatments, shading and removal of moisture from the umbel by blowing air on the surface of umbel. The results showed that there were significant differences between the treatments at $P<0.05$.

The maximum flower abortion percentage was obtained in the shading treatment was 22 percentage of flowers aborted compared to the control 18 percent (Table 5.2)

Location	Treatment	% Flower Abortion in ON019A
Glass House	Air Blowing	39.5 ± *6.6*
	Shading	21.7 ± *4.7*
	Low irrigation	32.0 ± *8.8*
	High Irrigation	29.7 ± *13.0*
	Control	18.0 ± *4.4*
LSD(P<0.05)		3.78

Table 5.2 – Flower abortion percentages for ON019A from the glasshouse experiment conducted during the 2006-07. Figures in italics denotes standard error (n=5).

The mean level of flower abortion in ON019A plants for all treatments in the shadehouse and glasshouse environments were 20% and 47% respectively. In contrast, the pollinator cultivar Creamgold, had less than 5% flower abortion on average in both environments because it is more adaptable to different locations. Differences in temperature, light levels and humidity existed between shade house and glasshouse environments (Figure 5.3)

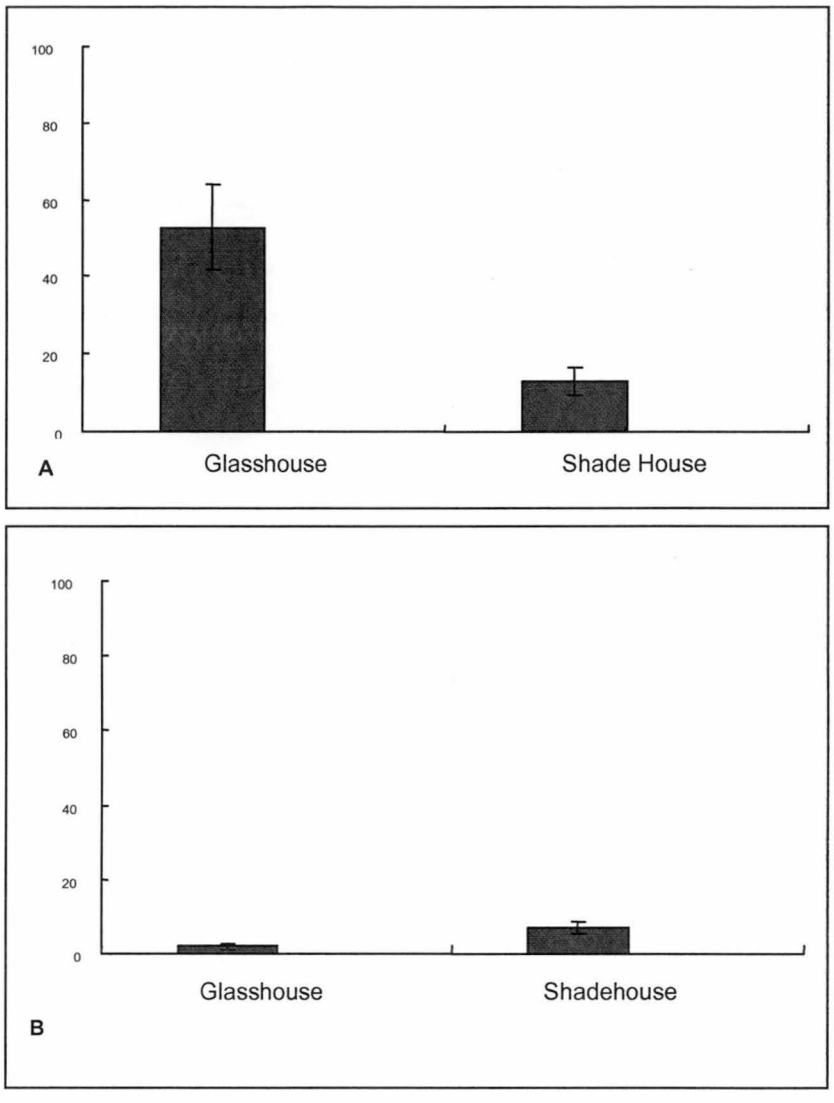


Figure 5.3 – Flower abortion percentage carried out in the Glasshouse and Shadehouse, A) ON019A, B) Creamgold error bars show standard error (n = 5).

5.3.2 Wind Shelter

A significant difference in percentage flower abortion was found between sheltered and non-sheltered plants under field conditions from the Cambridge site (SPS Farm) at P value $P<0.001$. Whilst the shade cloth was set in the vertical plane around the plot edges for shelter and only extended 200 – 300

mm above umbel height, some shading effects were unavoidable. The design of the treatments meant that these would have been relatively small.

A flower abortion percentage of 30 %, compared to 18 % in control plants was recorded at the SPS Farm site (Table 5.3)

At both the University Farm and SPS sites, plants within the wind shelter treatments had significant increases in the seed yield per plant at P<0.01. At the University farm shelter increased the yield from 1.61 g/per plant to 4.23 g/ per plant. At the South Pacific Seeds site, seed yield from sheltered plants was 1.98 g/per plant compared with 0.79 g/plant for control plants.

Location	Treatment	% Flower abortion ± se	Seed Yield Per Plant (g) ± se
SPS Farm	Wind Sheltered	18.40 ± *8.2*	1.98 ± *0.65*
	Non Sheltered	30.05 ± *8.8*	0.79 ± *0.14*
		LSD (P<0.001) 5.0	LSD (P<0.05) 0.9
University Farm	Wind Sheltered	NA	4.23 ± *1.5*
	Non Sheltered	NA	1.61 ± *0.4*
LSD (P<0.05)			1.9

Table 5.3 – Flower abortion percentage and seed yield from the wind sheltered and non sheltered (control) treatments during the 2006-2007 season at SPS Farm (n=4) and University Farm (Cambridge) (n=3).

5.3.3 Irrigation Treatment

Irrigation treatments applied at Cambridge in 2005–06 consisting of different durations and therefore intensities of water stress generated by different intervals between irrigation events (2, 4, 8 and 12 days) (Figure 5.4) had no effect on flower abortion rate or seed yield (Table 5.4). Across all treatments flower abortion rates were between 54 and 60% and low seed yields (1.1 and 1.6 grams per plant) were observed (Table 5.4).

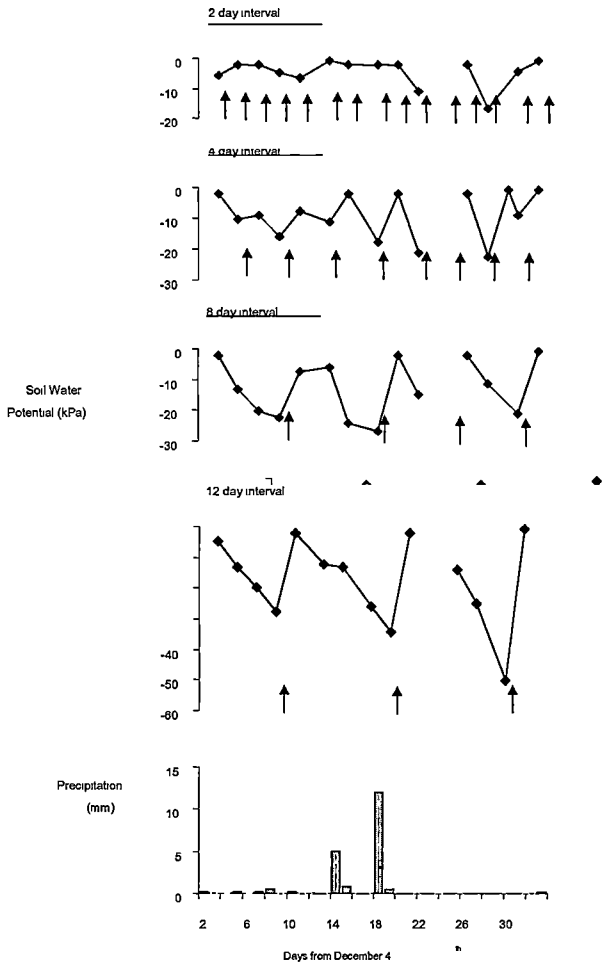


Figure 5.4 – Soil moisture deficits generated in the 2, 4, 8 and 12 day intervals between irrigation in the 2005–06 (University Farm) Cambridge irrigation trial during a one month period commencing 14 days prior to anthesis. Each point is the mean of the readings of 3 tensiometers positioned in the onion root zone 150 mm below the soil surface. Irrigation rates in each treatment were 70% of cumulative pan evaporation.

Intervals between irrigation events	% flower abortion at anthesis	Seed yield / plant (g)
2 days	54.0 ± *4.6*	1.23 ± 0.*23*
4 days	57.2 ± *3.2*	1.12 ± 0.*28*
8 days	60.0 ± *3.5*	1.10 ± 0.*13*
12 days	55.4 ± *5.1*	1.58 ± 0.*40*
LSD (P<0.05)	NS	NS

Table 5.4 – Effect of irrigation interval on flower abortion at anthesis and seed yield in ON019A grown at University Farm (Cambridge) in the 2005–06 season. Figures in italics are standard errors (n=5).

The results from the SPS Farm site in the 2006-07 season trial with two rates of irrigation showed significant differences in flower abortion rate between the treatments of low and high frequency irrigation (50% and 20% respectively), whereas the seed yield per plant did not show any significant difference (Table 5.5).

Location	Treatment	% Flower Abortion	Seed yield /plant (g)
SPS site	Low Irrigation	50.9 ± *4.6*	0.79 ± *0.2*
	High Irrigation	20.2 ± *4.0*	0.68 ± *0.1*
LSD (P<0.05)		18.5	NS

Table 5.5 – Effect of irrigation on flower abortion and seed yield during the 2006-07 growing seasons at SPS Farm.

5.3 4 Anti-transpirant Application in Combination with GA_3

Both the anti-transpirant and GA_3 treatments were effective at reducing flower abortion in ON019A at SPS Farm ($P<0.001$) and Swan Hill ($P<0.001$) sites (Table 5.6). At Swan Hill the percentage of flower abortion was 80.27 in untreated plants compared to the 29.26 and 29.96 in the GA_3 and GA_3+Envy. At the SPS site, the control flower abortion percentage was 63%, which was significantly different from the plants treated with GA_3 and GA_3+Envy, which had flower abortion rate of 24.4 and 23.2 respectively.

There was no significant difference in the seed yield obtained from the Swan Hill site (Table 5.6). Seed yields ranged from 1.6 to 1.7 g/plant in all the treatments and 1.9 g/plant for the control. A significant difference ($P<0.05$) was obtained at the SPS Farm (Cambridge) between the treatments. Seed yield of 0.7 g/plant was obtained in the control which was significantly lower than those from the GA_3+ Envy and Envy treatments at 1.7 and 1.6 g/plant, respectively (Table 5.6). At the University Farm (Cambridge), application of GA_3 and Anti-transpirant did not significantly increase seed yield.

Locations	Treatment	% Flower Abortion	Seed Yield Per Plant (g)
SPS Farm	Envy	35.8 ± *15.6*	1.6 ± *0.1*
	GA_3	24.4 ± *15.6*	1.58 ± *0.1*
	GA_3 +Envy	23.2 ± *8.0*	1.77 ± *0.1*
	Vapour Guard	NA	1.6 ± *0.2*
	Control	63.30 ± *8.8*	0.7 ± *0.2*
LSD(P<0.05)		13.2	0.36
University	Envy	NA	2.4 ± *0.1*
Farm	GA_3	NA	3.4 ± *0.4*
	GA_3 + Envy	NA	2.8 ± *0.5*
	Control	NA	3.14 ± *0.4*
LSD (P<0.05)		NA	NS
Swan Hill	Control	80.3 ± *3.5*	1.9 ± *0.1*
	GA_3 +Anti stress 1	23.8 ± *3.7*	1.64 ± *0.1*
	GA_3 + Anti stress 2	NS	1.5 ± *0.1*
	GA_3 + Envy 1 application	29.9 ± *3.1*	1.66 ± *0.1*
	GA_3 + Envy 2 application	NS	1.63 ± *0.1*
	GA_3	29.2 ± 2.2	1.5 ± *0.12*
LSD(P<0.05)		12.8	NS

Table 5.6 – Effect of GA_3, and anti-transpirant, and their combination on the flower abortion percentage and seed yield from the Swan Hill, SPS and University Farm (Cambridge) sites during the 2006-07 growing season. Figures in italics denote standard errors (n=4).
Vapour guard is an anti-transpirant used in one of the trial at SPS farm

5.3.5 *In vitro* Pollen Germination and Seed Set Assessment

Assessment of hand-pollinated flowers 48 h after pollination revealed that flowers collected from the sheltered treatment had a higher level of pollen germination (31.8%) than flowers from the GA_3 treated and control plants at 3% and 11.1% respectively under the fluorescence microscopy. This was confirmed by data for seed set, with 72% of hand-pollinated flowers in the shelter treatment at SPS Farm (Cambridge) setting seeds compared to 11 and 13% respectively for the GA_3 and control treatments (Figure 5.5).

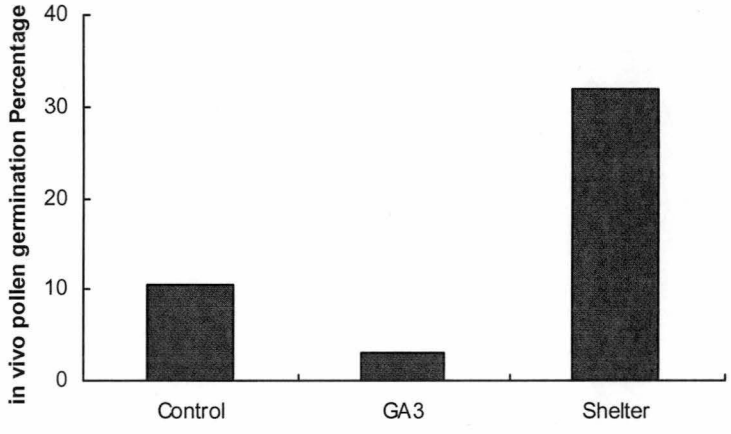


Figure 5.5 – The percentage of pollen germinated from the different treatments; shelter, GA_3 and control.

5.4 Discussion

Large variation in flower abortion percentage was recorded under field conditions compared with more consistent low flower abortion percentages recorded in the controlled environment trials. Treatments aimed at limiting water stress in umbels late in flower development had little effect on flower abortion percentage under conditions where flower abortion percentages in control plants were low but significantly reduced flower abortion percentage when conditions favoured flower abortion. Commercially acceptable seed yields were achieved under field conditions when plants were sheltered by shade cloth wind breaks and flower abortion percentage was reduced following application of anti-transpirant or gibberellic acid. The results were consistent with the hypothesis that flower abortion was a response to water stress in the flowers during the stage of rapid elongation of floral parts in late flower development.

Under shade house conditions flower abortion percentages of less than 20% were recorded both for control and treated plants, there was no significant difference in flower abortion percentage between the irrigation, shading and umbel drying treatments. A trend towards increased flower abortion under the treatment imposed to increase water stress was noted with a flower abortion percentage of 39% for the umbel drying treatment compared with 18% for the untreated plants.

Under glasshouse conditions a flower abortion percentage of 52% was recorded and while this percentage was lower than that observed for ON019A under most field conditions, it was much higher than that of the cultivar Creamgold under controlled environment conditions again demonstrating that ON019A was very susceptible to flower abortion. The response of the inbred line to more frequent irrigation, and reduced air movement with use of wind shelters under field conditions was reduced flower abortion percentage and improved seed yield. The treatments were

applied to plants to reduce risk of exposure to high rates of transpiration and thereby increase the water flow inside the plant system. The results were consistent with the hypothesis that plant stress during the late flowering period due to exposure of hot dry conditions and / or scarcity of water is one of the causal factors of flower abortion in ON019A. The methods of irrigation and its importance in onion seed production have been discussed by many authors (eg Goltz, 1971; Millar, 1971; Corgan and Kedar, 1990) but its significance in flower abortion has not been previously documented in onion.

Trial conducted by South Pacific Seeds in a commercial crop in Swan Hill during 2003–04 found no effect of a 25% increase in irrigation rate relative to current commercial practice. The frequent irrigation treatment at the SPS Farm (Cambridge) site produced a significant reduction in flower abortion and use of shade cloth windbreaks also significantly reduced flower abortion percentage and increased seed yield. While the windbreak treatment may have had its effect by reducing transpirational water loss from the plants, the 50 percent shade cloth was erected to a height just above umbel height and would therefore have also resulted in some shading of the plants and possible reduction in temperature. The possibility that direct shading effects or responses to reduced temperature (Goltz *et al.*, 1971, Peterson & Trammell, 1976) contributed to the improvement in flowering percentage cannot be discounted. The effectiveness of the growth hormone and anti-transpirant application treatments in improving the flowering percentage indicated that they may have a useful role in commercial onion seed production.

A single late application of gibberellic acid at 450 ppm significantly reduced the incidence of flower abortion. The combination of gibberellic acid and anti-transpirant produced a similar improvement in seed yield to gibberellic acid or anti-transpirant alone. Two issues associated with the use of gibberellic acid and anti-transpirant were noted in the trials and warrant further investigation if the treatments are to be developed into commercial applications. Firstly the anti-transpirant 'Envy' had a sticky nature and application on the umbel surface could have an effect on pollination with

pollinating insects avoiding the sticky surface. Secondly application of gibberellic acid appeared to reduce pollen germination rate on the stigmatic surface and this may reduce seed set under conditions where pollen transfer rates are low. These effects may account for the failure to achieve improvements in seed yield despite effectiveness in reducing flower abortion percentage. Further research on timing and rates of application of gibberellic acid and anti-transpirant are required to ensure these issues do not preclude the use of the treatments commercially.

From the research presented in this chapter, it was concluded that water relations plays a major role in flower abortion in ON019A. While further research is required on the physiological basis of this response, better selection and management of field conditions to reduce the risk of high transpiration and application of gibberellic acid and/or anti-transpirant sprays at the late flowering stage were shown to be effective treatments to reduce the flower abortion percentage in the inbred male sterile onion parent line ON019A.

Chapter 6

General Discussion

Large variability in seed yield between crops and frequent very low seed yields is a significant issue for companies producing hybrid seeds of certain vegetables such as onions. The problem has been attributed to inbreeding depression (Ali *et al.* 1984; Campbell *et al.*, 1968; Erickson & Gabelman, 1954; Franklin, 1970), with the inbred parent lines used in hybrid seed production displaying low vigour, high susceptibility to diseases and a narrow range of environmental and cultural conditions for optimum performance. The inbred, male sterile onion parent line ON019A used in the present studies was characteristic of inbred parent lines in that seed yields obtained in commercial production ranged widely and were sometimes very low. The line was therefore a useful model system for investigating seed set processes in inbred lines. The two major objectives of the investigation were

i) to identify the cause of the variability in seed yield between crops and

ii) to improve the seed yield by developing commercially applicable management practices.

In order to achieve commercially acceptable seed yields the crop must progress through a series of flower development, pollination, fertilization and seed development processes. Low seed yield will occur when there is a problem in one or more of the component steps in this reproductive pathway. Field observations in ON019A crops indicated that initiation and early development of flowers was unlikely to be the barrier to seed set as adequate numbers of umbels were routinely produced by the plants and timing of flowering between ON019A and pollinator lines was synchronous. Normal seed development was also reported following seed set indicating that the

barrier to high seed yield occurred around the time of flowering, pollination and fertilization. Initial experiments performed in the 2004 and 2005 seasons focussed on pollination as the possible developmental stage that determined seed yield in ON019A. Low yields in onion seed crops due to low rates of pollen transfer (Shaw and Bourne, 1936) or low pollen viability (Ali *et al.*, 1984; Ockendon & Gates, 1976) following exposure of onion flowers to high temperatures, have been reported previously. Hand pollination of ON019A umbels significantly increased the percentage of flowers within the umbels that were pollinated but, did not increase seed set. Pollen viability was high indicating that either the stigmas were not competent to support pollen germination and development, or that processes occurring after pollen transfer were responsible for low seed set.

High seed yields were obtained in other trials in the project using the same pollen parent crossed with ON019A, indicating that genetic incompatibility was unlikely to be the basis for any incompetence of the stigma to support pollen germination. Observations during the initial trials suggested that abortion of flowers around the time of flower opening was occurring and it was hypothesized that failure to set seed was linked to incompetence of aborting flowers to support pollen germination and / or pollen tube growth. Abortion of flowers in ornamental *Allium* species has been reported and has been recognised as the principal physiological disorder in *Allium* crops grown for cut flowers (De Hertogh & Zimmer, 1993.)

Two different classes of flowers were observed in ON019A, one with an elongated style and fully opened petals, and another with short styles often with hook tips. It was possible to separate the flowers for quantitative assessment of flower development on the basis of style length, with the former class characterised by styles protruding from the flowers >4 mm and the latter by styles shorter than 4 mm. Flowers with short styles at flower opening were observed to senesce rapidly leading to the conclusion that retarded style elongation was an early symptom of likely flower abortion.

Abortion of flowers during the late flower developmental stage was identified as the primary process determining the seed set in ON019A; the flowers that abort at that stage fail to develop viable seeds. Hand-pollinated flowers displaying normal development (styles >4 mm long) set a high percentage of seeds, whereas hand-pollinated flowers displaying the early signs of abortion failed to set seed. The symptoms of flower abortion were only observed in later stages of flower development at the time when rapid elongation of the style and rapid expansion of the petals during flower opening was occurring.

Flower abortion was not limited to ON019A with a percentage of flowers on each umbel of all lines investigated in the project observed to abort. Flower abortion was more frequent in ON019A than in other inbred onions lines and the propensity of lines to abort was consistent with the yield variability noted by commercial producers for the lines. Large differences in the flower abortion percentage in ON019A were recorded between crops and between treatments imposed in the project, again supporting the conclusion that yield variability was primarily due to variability in percentage flower abortion. Examination of aborting flowers using scanning electron microscopy revealed areas of desiccated and in some cases apparently necrotic tissue on the stigmatic surface, the style and the ovary surface. Affected areas of tissue appeared deformed, with loss of turgidity of cells when compared to similar structures in flowers that were developing normally. Non–aborted flowers of ON019A developed identically to viable flowers of onion cultivars that had low propensity to abort.

A possible relationship between the flower abortion and pattern of dry matter allocation was identified. Allocation of reserves to the bulbs rather than flowers at the peak flowering time occurred under conditions causing a high flower abortion percentage; competition between the bulb and the florets has been considered to be one of the major factors causing flower abortion in bulb species (Fortanizer and Zevenbergen, 1973). This mechanism would be

consistent with the effect of flower bulb size on flower abortion percentage, with larger bulbs having greater resources to allocate and therefore reduced risk of insufficient supply to support flower development. The seed yield was higher in large-sized bulbs than small-sized bulbs as noted previously for onion (Currah, 1981; Jones & Emsweller, 1937; Orlowski, 1974). Application of gibberellic acid was demonstrated to reduce abortion and increase seed set and this finding was consistent with the resource allocation theory as gibberellins have been shown to increase carbohydrate allocation to the area in which they are applied (Theron & Jacobs, 1996).

Evidence was also obtained in the project linking water stress with flower abortion. Onion plants are highly susceptible to water stress and considerable resistance to flow of water from soil to florets has been documented (Brown *et al.*, 1977). High temperature and water stress during the peak flower development stage may lead to the abortion of onion flowers; this relationship has been demonstrated for a range of bulbous species (Brown *et al.*, 1977; Goltz *et al.*, 1971; Hartsema & Luyten, 1955; Stirling, 1997; Wilkins, 1986). Conditions that limit water loss such as shading, avoiding high temperatures and reducing air movement around developing umbels reduced flower abortion percentage more so than irrigation treatments aimed at supplying adequate root zone moisture. It was concluded that the poor capacity of the plant to replace transpirational water loss from umbels was a greater issue than soil moisture deficits.

From the research it can be concluded that flower abortion in the ON019A male sterile parent is manageable considering certain factors like the temperature effect and cultural practices including bulb size which plays a major role in the success of the seed production, sheltering the plant during the peak blooming stage and protecting the umbel from the effects of sunlight. There is a possible link between water stress during the peak blooming stage in onions and flower abortion and failure of seed production.

Some of the major findings and key conclusions from the research are:

- **Pollination was not the issue behind the poor seed set in the male sterile line ON019A.**

- **Flower abortion is the major reason for poor seed set and is likely to happen from a week before the anthesis until after anthesis in the ON019A.**

- **Increasing biomass in the bulb at the peak flowering stage is the main reason for the plant having insufficient resources at the flowering stage leading to the abortion of growing flowers.**

- **Larger mother bulb size of ON019A reduces the flower abortion problem and increase the seed yield per plant.**

- **There is a possible relationship between water and temperature in flower abortion of ON019A.**

- **The GA_3 application at the rate of 450 ppm as a single application at 100 % piping will reduce flower abortion and increase the seed yield per plant.**

- **Application of GA_3 was not consistent at all locations and is not a silver bullet for the problem of flower abortion in ON019A.**

- **Sheltering the plant with shade cloth wind barriers reduced the flower abortion percentage.**

Conclusion and Recommendations

The results presented in this thesis provide an explanation for the unreliable seed yield obtained with the inbred male sterile onion line ON019A. Flower abortion possibly induced by water stress and/or other high temperature effects of the flowers in the late stages of development prevents fertilization and seed set in affected umbels. ON019A appears to be very susceptible to flower abortion and this susceptibility was considered likely to be associated with poor ability to control water loss or maintain cell functions during periods of water deficit. The mechanism of flower abortion requires further study and it is recommended that further studies concentrate on water relations in flowers in the late stages of flower development. Continued studies of the physiological basis of flower abortion in onion may lead to the development of improved management practises for the crop. On the basis of trials undertaken in this study it is recommended that growers of inbred onion lines that suffer from large yield variability choose sites where transpirational water losses are likely to be low. Use of large sized parent bulbs planted early in the season is also recommended along with shade cloth wind shelters and management of irrigation to avoid soil moisture deficits during flowering. Application of anti-transpirants may be effective in some cases as is the use of gibberellic acid application. Further refinement of these treatments is required as it is likely the gains will be additive provided high rates of water loss from plants during late flower development are avoided.

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Appendix 1

Potting Medium- Standard Mix (P^H approx 6.0) Ingredients

Ingredients		Per 50 L (one mixer full)
Composted Pine Bark	80 %	40 L (4.0 level buckets)
Coarse Sand	20 %	10 L (1.0 level bucket)
	Per m^3	
Limil	1.8 kg	90 g
Dolomite	1.8 kg	90 g
Osmocote Plus, 5-6 Month	6.0 kg	300 g
Ferrous sulphate	0.5 kg	25 g
Nitram	1.5 kg	75 g
Hydraflow	0.6 kg	30 g
Zeolite	0.8 kg	40 g

Appendix 2

Hoaglands No.1 Solution (P^H=5.8; EC=1.70 sd/m)

Chemical	Stock solution concentration	Volume of stock solution added to 100 litres
Magnesium Sulphate	9.860 kg / 40 L	200 ml
Calcium Nitrate	9.448 kg /40 L	500 ml
Potassium Nitrate	4.040 kg / 40 L	500 ml
Potassium Di-hydrogen Phosphate	2.722 kg / 20 L	100 ml
Iron Chelate	6.56 g / 20 L	100 ml
Micro-Nutrients;		
Boric Acid	57.2 g / 20 L	100 ml
Manganese Chloride	36.2 g / 20 L	
Zinc Sulphate	4.4 g / 20 L	
Copper Sulphate	1.6 g / 20 L	
Sodium Molybdate	0.5 g / 20 L	

Appendix 3

Locations	**Mean temperature**	**Mean Day length**	**Mean Relative Humidity**	**Soil**
Tasmania	10.34	8hrs	80	Sandy Loam
Victoria	14.10	9 hrs	80	Loamy
South Australia	19.45	12.5 hrs	70	Sandy Loam