



Know-how for Horticulture™

**Determination of seed
quality for the
vegetable industry**

R Clark
Tasmanian Institute of
Agricultural Research

Project Number: VG98071

VG98071

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry, Botanical Resources Australia, South Pacific Seeds, Fletcher Challenge, Derwent Seeds, Tasmanian Onion Seed Growers and Magnus Kahl Seeds Pty Ltd.

All expressions of opinion are not to be regarded as expressing the opinion of Horticulture Australia Ltd or any authority of the Australian Government.

The Company and the Australian Government accept no responsibility for any of the opinions or the accuracy of the information contained in this report and readers should rely upon their own enquiries in making decisions concerning their own interests.

ISBN 0 7341 0487 1

Published and distributed by:
Horticulture Australia Ltd
Level 1

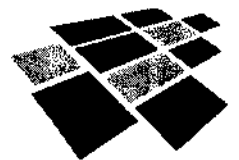
50 Carrington Street
Sydney NSW 2000

Telephone: (02) 8295 2300

Fax: (02) 8295 2399

E-Mail: horticulture@horticulture.com.au

© Copyright 2002



Horticulture Australia

Final Report.

**Determination of seed quality
for the vegetable industry**

Project number: VG98071 (May 2002)

Chief Investigator:

Professor R.J. Clark

Tasmanian Institute of Agricultural Research

Project Number: VG98071

Date: May 2002

Project Leader: Professor R.J. Clark
School of Agricultural Science/Tasmanian Institute of Agricultural Research
GPO Box 252-54 Hobart
Tasmania 7001 Australia
Telephone (03) 6226 2620 Facsimile (03) 6226 2642

The purpose of this report is provide detailed information to the public about the seed quality trials conducted during this study.

Any recommendations contained in this publication do not necessarily represent current Horticulture Australia policy. No person should act on the basis of the contents of this publication, whether as to matters of fact or opinion or other content, without first obtaining specific, independent professional advice in respect of the matters set out in this publication.

Acknowledgments

The chief investigator wishes to acknowledge the major contribution to this project from;

Researchers:

Dr David Fulton, Postdoctoral Research Fellow
Dr Nadine Donovan, Dr Alistair Gracie, Dr Philip Brown
Honours students - Cameron Spurr, Dion McKenzie and Chris Chapman

Industry Partners:

Norske Skog
Botanical Resources Australia
Bejo Seeds
Magnus Karl Seeds
Tasmanian Onion Seed Growers
South Pacific Seeds



Table of Contents

<i>Chapter</i>	<i>Page</i>
Part A	
Media Summary	2
Technical Summary	3
Chapter 1 Introduction	5
Chapter 2 Onion seed quality	6
2.1 Introduction	6
2.2 The changes in seed yield and quality with time of harvest	7
2.3 Umbel maturity and post-harvest drying effects on seedlot quality	8
2.4 Mitigation of stunted root abnormality in onions using seed priming treatments	12
2.5 Hydropriming to overcome stunted root abnormality in onion seedlings	14
2.6 Conclusions	16
Chapter 3 Carrot seed quality	17
Chapter 4 Brassica seed quality	27
Chapter 5 Radiata pine seed quality	36
5.1 Introduction	36
5.2 Improvement of germination by stratification	38
5.3 Cold hydropriming to improve germination	42
5.4 Hydropriming at 5 °C and 20 °C to improve laboratory germination and field emergence	45
5.5 Conclusions	51
Chapter 6 Recommendations	52
Chapter 7 Technology transfer	54
References	57
Appendix 1. Germination parameters	65
Appendix 2. Literature Review	66
Part B. Pyrethrum seed quality	

Media Summary

Much of the Australian vegetable industry depends on seed for crop establishment. Seed emergence percentage, time and uniformity, and seedling growth rate are critical aspects of crop establishment that are influenced by seed quality. This project focused on increasing the understanding of quality parameters in seed; identifying critical seed production and establishment practices; and incorporation of these research findings into commercial operations.

Aspects of seed quality including seedling abnormality, seed dormancy, variability of seed vigour effects on crop yield and quality, harvest technique, harvest time, and seed pretreatment were investigated with onion, carrot, brassica, pyrethrum and radiata pine seeds.

Major outcomes of the project include:

- Identification of factors affecting the level of abnormal seedlings produced by onion seedlots. Pretreatment application reduced the incidence of abnormality.
- Variability in carrot embryo size is related to variability in plant size early in establishment, but by 120 days after sowing the relationship diminishes which suggests that other factors may be influencing the final variability of taproot size.
- The production of quality brassica seed depends on minimisation of damage during threshing. This study indicated that choice of harvester is a critical factor determining the level of damage sustained.
- Identification of poor seed quality and narrow germination temperature range as causes of poor field emergence of radiata pine seeds. Application of appropriate pretreatment prior to sowing increased field nursery emergence rate, and percentage germination of some seedlots.
- Establishment of a Seed Research Group at the University of Tasmania/Tasmanian Institute of Agricultural Research and ongoing industry commitment to seed research at the university.
- Contribution Research Higher Degree and honours training within the School of Agricultural Science.

Technical Summary

Much of the Australian vegetable industry depends on seed for crop establishment. Seed is either sown directly into the field or used to produce seedlings in nurseries. Seed germination, seedling emergence percentage, time, and uniformity, and seedling growth rate are all critical aspects of crop establishment that depend to some extent on seed quality. This project recognised the increasing importance of seed performance to vegetable and plant industries, and focused on increasing the understanding of quality parameters in seed and seed/environment interactions; identifying critical seed production and establishment practice; and incorporation of these research findings into commercial operations.

Research focused on onion, carrot, brassica, pyrethrum and radiata pine seed quality. Pyrethrum seed quality studies are reported separately (Part 2). Some Tasmanian onion seedlots when grown, produce a high percentage of abnormal seedlings. Much of this abnormality relates to stunted root growth. The percentage of abnormal seedlings is considered by industry to be an indicator of seedlot quality. A high incidence of abnormal seedlings therefore reduces the value of onion seedlots and the value of the seed to industry. Trials were conducted in commercial onion seed crops, and under glasshouse and laboratory conditions using a range of onion cultivars. Time of harvest, variation in umbel maturity at harvest and umbel drying regime were found to be factors affecting seed quality, including the incidence of abnormal seedlings. Priming treatments, particularly hydropriming, were found to reduce the incidence of abnormality in seedlings produced from a range of seedlots. The trials also found that seedlings with stunted roots were able to survive when grown in glasshouse pot trials, although percentage emergence was lower and growth rates slower than normal seedlings. Hydropriming techniques may have further advantages in the onion sprout industry where uniformity of germination is a critical issue.

The fresh carrot market pays a premium for high quality taproots falling within stringent size ranges. Taproot size variability can be accounted for by differences in time of emergence of individual seedlings, which can be attributed to some extent to seed quality. Commercial seedlots (Kuroda and Nantes cultivars) were graded to produce seedlots varying in embryo size. Relatively large differences in plant size variability were observed between seed grades 40 days after sowing (DAS), but by 120 DAS the differences between seed treatments had diminished. This reduction suggests that factors between the establishment phase and taproot maturity are having a major impact on the final variability of taproot size.

Local seed companies indicated that seed coat damage during threshing is an area of particular concern with brassica seed. The effects of two combine harvesters on seed damage were compared. The rotary combine harvester (International 1420) was superior to the conventional combine harvester (Allcrop) in terms of harvested seed quality (germination percentage) and damage levels. The higher leachate conductivity, lower mean time to complete germination and higher level of fragmented seed pieces found in seedlots harvested with the Allcrop indicate greater physical damage and lower seed quality. Small improvements in seed quality were obtained through changes in harvester settings,

Radiata pine seedlots sown in commercial nurseries occasionally show low and variable germination and there are industry concerns that genetic gains have been achieved at the expense of seed quality. Trials tested germination of pretreated seeds in the laboratory, glasshouse and field nursery. The germination test at 10 °C demonstrated that radiata pine seeds have a relatively narrow range of optimal germination temperatures. The time to germination

was greatly increased and percentage germination decreased by dropping the germination test temperature from 20 °C to 10 °C. Pre-soaking reduced the germination of some seedlots. Treatments combining chilling and hydropriming for 12 weeks were most effective in improving germination and overcoming soak injury. When germinated at 20 °C the effect of this treatment was not lost following 4 weeks storage, and there was relatively little effect of redrying before storage. Hydropriming at 5 °C and 100% relative humidity for 12 weeks overcame the effect of reducing the temperature to some extent.

An important project outcome was the establishment of communication between seed companies and researchers, allowing research to address problems identified by industry. Another important outcome was the development of a Seed Research Group within the School of Agricultural Science and Tasmanian Institute of Agricultural Research (TIAR), which represents an ongoing commitment of researchers, research providers and industry to continue the investment in seed research. Neither this focus nor commitment existed prior to this project.

Chapter 1. Introduction

Many Australian plant industries depend on seed for crop establishment. Seed is either sown directly into the field or used to produce seedlings in nurseries, for later field transplanting. Seed germination, seedling emergence, emergence time, vigour and uniformity of emergence are all critical aspects of crop establishment that depend on seed quality. The establishment phase of crop growth has a profound effect on crop yield and quality. Failure during establishment may necessitate resowing, may limit crop yield and quality and will adversely affect the economics of crop production and erode the competitive position of cropping enterprises. Furthermore, if future productivity gains arising from biotechnology, plant breeding and precision farming are to be captured by the cropping industry, significant improvements in crop establishment and hence seed quality, is essential.

This project recognised the increasing importance of seed performance to plant industries and focused on ;

- increasing the understanding of seed quality parameters in seed and seed/environment interactions
- identification of critical seed production and establishment practices, and
- incorporation of these research findings into commercial protocols

By working with industry to identify and focus on real establishment problems, the project generated information aimed at informing crop decision makers about risks, better management strategies and the importance of seed quality. The project identified critical seed quality parameters and optimal management practices in a range of seeded crops. These objectives were achieved through the conduct of field trials, glasshouse studies and detailed laboratory germination studies. Issues investigated included germination capacity, uniformity and vigour. These quality parameters were investigated across several crops.

An important output from the project was value adding to seed by seed treatment and through provision of a system of information packages on seed quality which inform management decisions. The milestone reports previously submitted to Horticulture Australia Limited contain information that is not included in the final report, including an extensive review of the literature relating to seed quality in the crops included in the project and in seeds in general, but may be of interest to seed producers and seed users.

Chapter 2. Onion seed quality

2.1 Introduction

The Tasmanian onion seed industry, primarily located in the Derwent Valley, produces up to ten tonnes of onion seed per annum. In this region, a large proportion of the production is based on two varieties (early cream gold and cream gold), produced for premium local and export markets. The seedlots produced are generally perceived to be of high quality, however, premium markets demand a minimum of 85% normal germinants. That is, 85% of the seeds must produce seedlings with the necessary structural components to grow as a normal seedling within 12 days. One of the difficulties in attaining this standard is the high proportion of abnormal seedlings that develop. Seedlings with stunted root growth account for the majority of the abnormal seedlings (Spurr 1998). The percentage of abnormal seedlings is considered by industry to be an indicator of seedlot quality. Abnormal onion seedlings are identified as part of the routine germination test required for the sale of seedlots. A high incidence of abnormal seedlings therefore reduces the value of onion seedlots. Identification of the cause of high levels of abnormal onion seedlings is critical for the industry. The physiological basis and the field practices responsible for the production of abnormal onion seedlings remain unclear. The implications of seedling abnormality on performance are also unclear.

Earlier work (Spurr 1998) examined the pattern of change in seed yield, seedlot germination and seedling quality characteristics with maturity in onion seed. Seed yield and quality were improved by delaying harvest until the seed moisture content was close to 30 %. Time of seed harvest has been found to determine to some extent the level of abnormal seedlings produced. Time of harvest was also found to affect germination rate, which is an indicator of seedlot quality in onions (Finch-Savage 1986).

Variation in onion umbel maturity at harvest has been observed, but the effect of this variation on seedlot quality has not been determined. Differences in seed maturity within crops may be the result of variation in flowering time within umbels, between umbels on a plant, or between plants (Currah 1982; Currah and Ockendon 1978). Variation in deterioration has also been proposed as a source of variation in germination within a population of onion seeds (Ellis and Butcher 1988). The last seeds to germinate within a population are believed to be the most deteriorated, as these seeds tend to produce weaker seedlings and fewer normal healthy seedlings (Finch-Savage 1986; Ellis and Butcher 1988). Mohamed-Yasseen and Splittstoesser (1990) reported that deterioration led to the death of root tip cells and stunted root systems.

Few management options are available to reduce the incidence of stunted root abnormality in onion seedlots. Selection of appropriate production environments (Brewster 1982) and harvesting dates (Spurr et al. 2002) have been examined but no post harvest treatments have been described. Increased rate and uniformity of germination of onion seeds following priming has previously been reported (Ellis and Butcher 1988; Gray et al. 1990). However, no reports of the effect of priming on seedling abnormality levels have been found in the literature.

The present study investigated umbel maturity at harvest and the effect of maturity on the level of abnormal seedlings. Further work examined the effect of a range of priming pre-treatments on seedling abnormality, hydro-priming to reduce abnormality in a range of seedlots, and the growth of abnormal seedlings in soil.

2.2 The changes in seed yield and quality with time of harvest

In a preliminary study, the changes in seed yield and quality with maturity were investigated in a commercial onion seed crop in Southern Tasmania to provide an indication of the optimum stage of maturity for harvest. Results of the study were reported in full by Spurr (1998). Samples of seed were collected on six occasions from the 20th of January 1998 to the 18th of March 1998, extending from early in the development of the crop until well after commercial harvest at 54% seed moisture content. The samples were assessed for maturity, yield and quality. The seed quality parameters evaluated included the percentage final germination, the rate and uniformity of germination, seedling weight and the incidence of seedling abnormality.

Both the yield and the quality parameters described were found to be affected by seed maturity. Table 2.1 shows FG and MGT at 10 °C and 20 °C (germination parameters are defined in Appendix 1). There was a definite sequence of quality acquisition starting with the capacity to germinate early in seed development, then the capacity to produce normal seedlings and finally increased rate and uniformity of germination. Rate and uniformity of germination continued to increase until the end of the trial. The quality changes that were observed were associated with increased seed weights throughout the trial, which was also reflected in greater seedling weights. There was no effect of maturity on seedling development after germination and the relatively high levels of abnormal seedlings observed from seed from all harvest dates indicates an effect of additional factors on this aspect of seed quality.

Table 2.1. Percentage germination and mean germination time of seedlots from each harvest date germinated at 10 °C and 20 °C. Germination temperature did not significantly affect germination percentage. Means followed by the same letter are significantly different.

Harvest date	% FG	MGT at 10 °C (days)	MGT at 20 °C (days)
20/1/98	57.9 w	10.74 a b	6.6 d
31/1/98	77.4 x	11.11 a	6.23 d e
11/2/98	86.0 b y	11.02 a b	6.55 d e
22/2/98	96.0 y z	10.04 b	6.17 d e
5/3/98	96.4 y z	8.63 c	5.83 d e
18/3/98	92.2 z	8.42 c	5.55 e

The results obtained indicate an opportunity to improve both seed yield and quality by delaying harvest until the seed moisture content is close to 30%.

2.3 Umbel maturity and post-harvest drying effects on seedlot quality

2.3.1 Introduction

Substantial variation in flowering time and capsule maturity was observed in an onion seed crop in early 1999. The variation was considered to be a possible cause of high levels of abnormal seedlings observed in onion seedlots. In this study, umbel maturity at commercial harvest time was investigated. Umbels were divided into maturity classes, and samples representing these classes were harvested and the seeds germinated. The effect of umbel drying conditions on seedling abnormality levels was also examined.

2.3.2 Materials and Methods

Materials and methods are outlined below, and reported in more detail by McKenzie (1999).

Samples were collected from a commercial onion seed crop (cv. Creamgold) from a field at Bushy Park in southern Tasmania at the time of commercial harvest. The trial was located on four non-edge rows within the field. The trial was designed as a split-plot with a 20 m section from the middle of each row serving as an independent block. Rows were chosen as representative of each quarter of the field. The sub-plots were post-harvest drying treatment. Umbels were harvested by cutting the scape level with the base of the umbel.

Umbels were divided into visual maturity classes according to the proportion of open capsules revealing onion seeds (Table 2.2). Umbels were harvested by two different methods: one sample was harvested by removing all umbels from a randomly selected 1m section of the block and the number of umbels belonging to each maturity class (Table 2.2) was recorded; for the second method, six or seven umbels of each maturity class were selected from a 20m block.

Table 2.2 Umbel maturity classes

Visual Definition	Maturity Class	
All capsules closed and green	Class A	Most immature
Some capsules open (< 50 %)	Class B	
Most capsules open (> 50 %)	Class C	
All capsules open revealing seeds	Class D	Most mature

Drying treatments

Samples were dried using two methods, either: in open paper bags with passive air movement at 28 ° C (slow drying); or, in open paper bags with forced air movement at 38 ° C (rapid drying). Drying treatments were discontinued when moisture loss between assessments was found to be minimal. The forced air drying at 38 ° C lasted for a period of ten days. Passive drying at 28 ° C took 19 days. Samples were re-weighed, transferred to airtight plastic bags and stored at 4 ° C until threshing and cleaning.

Seed moisture content and dry weight

After cleaning, the seedlots were weighed and number of seeds counted (total and number per umbel). Moisture content was determined using the low-constant temperature method (ISTA 1999). Moisture content was calculated as a proportion of the fresh mass.

Germination characteristics

Germination of samples of 110 seeds per petri dish were tested at 10 ° C and 20 ° C, in accordance with ISTA guidelines. Treatments were compared using FG, MGT and CUG (Appendix 1).

Assessment of seedling characteristics

Seedlings were grown in paper rolls saturated with distilled water. The paper rolls were placed in sealed trays with a high humidity environment, and germinated at 20 ° C with a 12:12 hour light/dark regime. Seedlings were assessed at day 7 for normality. At day 14 the remaining seedlings were classified as: normal; small but normal; abnormal; or other, including fresh ungerminated (FUG), diseased, hollow or damaged (ISTA 1999). Small but normal seedlings were defined as those with all the representative features of a normal seedling, except that the length from root tip to knee was less than 30 mm.

Assessment of yield and seedling character distribution

The unsorted sample was divided into maturity classes to estimate the variation of umbel maturity within the commercial crop. Yield per maturity class and total yield were estimated.

Statistical analysis

All data was analysed by analysis of variance (ANOVA) for split-plot design using SAS system® for Windows Release 6.12 software. Fishers' least significant differences (LSD) were calculated at a probability value of 0.05.

2.3.3 Results and Discussion

Drying treatments

Initial decreases in weight were rapid for all class A umbels. The most mature umbels, class D, lost little moisture. The rate of water loss was considerably higher when dried in forced-air at 38 ° C than when passively dried at 28 ° C. The two post-harvest drying treatments led to differences in umbel moisture content. However, the treatments removed a similar amount of water from umbels within the same maturity class. The difference between the two rates approached four times in magnitude. Maturity classes had significantly different moisture contents at harvest. The most immature umbels (class A) had the highest moisture content at the time of harvest. Progressively lower moisture percentages were observed in the umbels that were more mature.

Umbel moisture content has previously been promoted as a criterion for estimating the optimum time of harvest of onion seed crops (Steiner and Akintobi 1986). The optimum range of umbel moisture percentage for harvest was based on maximising seed quality and minimising seed shatter. Globerson et al. (1981) reported that the optimum time of harvest was in the range of 30-40% moisture or when 1-3% of the umbels in the field had split capsules revealing black seeds. The crop in the present experiment was therefore harvested later in development and at moisture contents outside the range previously reported as optimum for maximising yield and seed quality in onion.

Seed moisture content and dry weight

At harvest the more mature umbel classes contained more seeds per umbel and the most immature umbels had the least seeds.

Seed moisture content was significantly affected by umbel maturity and drying treatment. Forced air drying at 38 ° C resulted in a significantly lower moisture content than passive drying. Drying treatment also significantly affected seed dry mass. Forced-air drying at 38 ° C produced seeds that were lighter in comparison with passive drying at 28 ° C. Loss of dry mass suggests that post-harvest development occurred within the umbels during drying. Forced air drying reduced the process of development, resulting in reduced uptake of reserves from the umbels into the seeds, as reported with leeks (Gray et al. 1989).

Germination characteristics

Germination percentage was the least effective measure in separating treatment differences. Germination percentage provided information on seed viability but provided little information on seed quality. Percentage germination of all seedlots at 20 ° C was greater than 90 %. Finch-Savage (1986) reported that viability was not closely related to seed vigour in onion or leek. All seedlots in the present study had a germination percentage greater than 90%, however significant levels of low vigour and poor quality seedlings were present. At both 10 ° C and 20 ° C, immature umbels produced seedlots that took longer to germinate. Germination time is an important trait in the determination of seedlot quality as the speed of germination is an important determinant of field emergence. Wheeler and Ellis (1994) reported that germination rate was the main factor affecting onion seedling size in the field. Finch-Savage (1986) reported that slow germinating seeds produced fewer normal healthy seedlings than fast germinating seeds. Thus seeds from immature umbels were likely to cause slow germination and small and abnormal seedlings in the commercial seedlot.

Forced air drying of umbels lengthened the time to germination, an affect that has been reported in leek seeds (Gray et al. 1989). Germination percentage at 10 °C and 20 °C was not significantly affected by drying treatment, except when seeds were germinated in paper rolls. Forced air drying at 38 ° C resulted in lower germination than passive air drying at 28 ° C.

Seedling characteristics

The percentage of low vigour seedlings (small but normal and abnormal seedlings) formed a considerable proportion of the population of some seedlots. Umbel maturity interacted significantly with drying treatment, such that forced drying at 38 ° C of immature umbels resulted in more abnormal seedlings than passive drying the umbels at 28 ° C. The effect of drying at 38 ° C was reduced in more mature classes. It is unclear whether the higher temperature or more rapid rate of drying were responsible for the increase in low vigour seedlings. Gray et al (1989) observed deteriorative effects in leek seedlots dried at high temperatures. Low vigour seedlings were produced by all umbels within the population with the highest percentage from the most immature umbels.

The number of small but otherwise normal seedlings was a good indicator of relative seedlot quality. Forced air drying reduced seedling vigour resulting in higher percentages of small but normal seedlings. The small but normal seedling classification was based on practices of the current commercial seed test used by the local industry (E. Pascoe pers. comm.). Under ISTA (1999) recommendations these seedlings would be classified as normal.

Yield and seedling character distribution

Table 2.3 indicates that the most immature umbels contributed the least to the total yield of the commercial crop. The majority of the yield was from seed of the umbels from classes B and C. The most mature umbels contributed little to the total yield. The most immature umbels had a

considerably higher percentage of low vigour seedlings in comparison with more mature umbels. However, the more mature umbels still had a high percentage of low vigour seedlings.

Seedlot quality could be improved by removing the most immature umbels from harvest. The results suggest that a 13% decrease in crop yield would reduce the percentage of low vigour seedlings by 1.4%. This small reduction suggests that maturity is only one of a number of factors influencing the production of low vigour seedlings.

Table 2.3 Mean yield and abnormal seedlings for each umbel maturity class.

Umbel maturity class	Mean yield (dry g of seed m ⁻¹ of row)	Mean low vigour seedlings (% m ⁻¹ of row ⁸)
A	24.30	28.27
B	67.31	15.27
C	76.56	17.74
D	30.64	15.09
Total	199.10	17.70

⁸Number of low vigour seedlings per maturity class as a percentage of the number of seeds per maturity class.

The seeds from the most immature umbel class contributed about 10% of the total yield. The seeds from that class had a lower germination percentage, were slower to germinate, produced more abnormal seedlings and more small but normal seedlings than seeds from more mature umbels. High quality traits include high germination percentage, rapid germination and production of normal seedlings. Thus, seeds from the immature umbels were of low quality compared with seeds from mature umbels, perhaps as seed growth and development was not complete in immature umbels at harvest.

Post-harvest drying treatment had a significant impact on seed quality. Rapidly drying the umbels reduced seedlot quality by decreasing the mean dry mass of seeds, increasing the mean germination time, and increasing the proportion of abnormal and small but normal seedlings in the seedlots. Adamson (1960) and Gray et al (1989) reported similar post-harvest effects on seedlot quality in leek. These studies reported reduced viability, decreased seed dry mass, increased mean germination time and reduced seed vigour. The results suggest that high temperatures reduce resource uptake and possibly development during dehydration of the seed.

2.4 Mitigation of stunted root abnormality in onion using seed priming treatments

2.4.1 Introduction

The occurrence of abnormal seedlings in onion seed germination tests is an important quality issue for onion seed producers. A high incidence of abnormal seedlings reduces the value of onion seedlots. Seedlings with short primary roots are considered abnormal, and constitute up to 35% of imbibed seeds and 88% of all seedling abnormalities in recently harvested seed of the cultivar "Creamgold" produced in Southern Tasmania (Spurr et al. 2002). There is little information pertaining to the longer term growth and survival characteristics of onion seedlings with short primary roots.

Seed pretreatments are widely used by the seed industry to increase germination rate and uniformity of seedling emergence (Bujalski and Nienow 1991) and benefits of various treatments have been demonstrated in onion (Drew et al, 1997; Ali et al. 1990; Gray et al. 1990; Dearman et al. 1986). Effects of the pretreatments used in these studies on abnormality were not noted. The potential for using seed priming or seed pretreatments to overcome short roots in onion seedlings has not been reported.

This study investigated the effects of hydro and osmotic priming, hardening and gibberellic acid application on the incidence of abnormal seedlings compared with untreated seed.

2.4.2 Materials and Methods

Materials and methods are given in brief below. Tajbakhsh et al. (2002a) give a detailed account of the materials and methods used in this study.

Two seedlots (seedlots A & B) with greater than 75% germination combined with a significant proportion of abnormal seedlings were selected for the study. Seedlots were characterised using standard germination tests (ISTA 1993). A second experiment was conducted to record the timing of abnormal seedling development within a seedlot population. One thousand seeds of each seedlot were imbibed on filter paper. Seeds were examined daily and the number of germinants recorded. Germinated seeds were transferred to new petri dishes. Assessment and transfer were continued until 12 days after imbibition. This procedure resulted in segregation of the seedlot populations into sub lots based on germination time. The percentage and classification of abnormal seedlings was recorded for each sub lot 8 days after germination (actual date of counting varied according to date of germination) and 12 days after imbibition (all sub lots counted on the same day).

Survival and early development of normal seedlings and seedlings with stunted root abnormality were compared following transplanting to potting mixture. Four replicates of 24 seedlings were selected 12 days after imbibition from the seedlot A population in the above experiment. Seedling survival percentage and dry weights were recorded 22 days after transplanting.

Five pre-germination treatments were given to samples from seedlots A and B. The five treatments were: 1) osmotic priming using -1.2 MPa NaCl solution; 2) osmotic priming using 1% KNO₃ solution; 3) hydropriming; 4) hardening; and 5) soaking in gibberellic acid solution.

2.4.3 Results and Discussion

The percentage of abnormal seedlings recorded in standard germination tests for onions was significantly reduced by priming of seedlots. Hydropriming caused the greatest improvement in seedlot quality, and resulted in a 50% reduction in percentage abnormal seedlings. Reductions of 20% to 47% were obtained for the other treatments. Hydropriming and osmotic priming with NaCl produced significantly lower percentage abnormal seedlings than hardening or gibberellic acid pretreatment for both seedlots (see Tajbaksh et al. 2002 for further detail). Hydro priming also resulted in significant improvement in other quality characteristics including germination percentage and rate and uniformity of germination.

There was a trend towards increased levels of abnormality in slower germinating sub lots, but levels of stunted root abnormality did not vary significantly with later germination date. The percentage of normal seedlings decreased with later germination dates due to an increase in the frequency of abnormalities other than stunted root with later germination times. The rate of germination has been demonstrated as the key facet of seed quality affecting onion seedlings size (Wheeler and Ellis 1994). The weak relationship between rate of germination and stunted root abnormality indicated that expression of the abnormality was not associated with overall seedling growth rate but was specific to radicle expansion. In addition, hydropriming increased mean root length of normal seedlings while osmotic priming decreased root length compared with control seedlings.

Seedlings with severely stunted root systems were shown to be able to develop normal root systems within 22 days of transplanting to potting media. Ninety percent of normal seedlings survived compared with 68% of abnormal seedlings, and the mean dry weights of surviving seedlings were 10.3 ± 0.7 mg and 5.4 ± 1.3 mg respectively. It is apparent, that under the conditions of this trial, that seedlings were able to grow despite having stunted root abnormality, albeit more slowly during seedling establishment.

This study demonstrated that priming may have commercial application in salvaging onion seedlots with unacceptable percentages of abnormal seedlings.

2.5 Hydropriming to overcome stunted root abnormality in onion seedlings

2.5.1 Introduction

The previous trial (Chapter 2.4) demonstrated that in addition to improving germination percentage and the rate and uniformity of germination, hydropriming reduced the incidence of seedlings with stunted root abnormality in a seedlot. The treatment has the potential to be applied on a commercial scale to onion seedlots with unacceptable percentages of abnormal seedlings. However, it has yet to be demonstrated to be effective on a range of genotypes. Another consideration before adopting the treatment for commercial application is the effect of re-drying after treatment. Re-drying onion seeds after priming treatments has previously led to loss of pre-treatment effects (Peterson 1976; Haigh et al. 1986; Bujalski and Nienow 1991). However, in some cases the primed and re-dried seeds germinated more rapidly than untreated seeds (Bujalski and Nienow 1991). We have found no reports in the literature of the effects of re-drying after hydropriming on onion seed germination and the percentage of abnormal seedlings.

In this study, the effect of hydro priming treatments on germination and incidence of abnormal seedlings was examined with a range of commercially available onion genotypes. The survival of hydroprimed seedlings in saline conditions and in soil was also investigated.

2.5.2 Materials and Methods

Materials and methods are given in brief below. Tajbakhsh et al. (2002b) give a detailed account of the materials and methods used in this study.

Ten commercially available onion seedlots with viability levels below 85% normal seedlings were selected for this study. Seedlots were placed on saturated filter paper for 5 hours, surface-dried, then held at 100% relative humidity and room temperature (22 ± 2 °C) for 3 days. For each seedlot the germination characteristics of hydroprimed seed, with and without post priming drying, were compared with non primed seed. Five treatments were applied to seedlots: non-primed (untreated control); hydroprimed and not re-dried; and hydroprimed and dried to approximately 10% moisture content using three separate conditions for a 7 day period (20 °C with forced airflow; 30 °C with forced airflow; room temperature with no forced air flow). Seedlots were germinated according to International Seed Testing Association (ISTA, 1999) procedures.

Performance of hydroprimed and non-primed planted seed in a soil media were compared for the 10 onion seedlots assessed in this study. Hydropriming was undertaken as described above and the seed was re-dried at room temperature for 7 days. Emergence was recorded daily.

2.5.3 Results and Discussion

A higher proportion of hydroprimed seeds developed as normal seedlings than untreated seed. This result occurred regardless of whether or not the seeds were re-dried to moisture contents comparable or lower than that in the untreated seedlots.

No significant difference in the level of normal seedlings was recorded between the 3 post-priming drying regimes examined in this study. While a significantly higher proportion of

normal seedlings was detected when the seeds were germinated immediately after hydropriming, compared with hydroprimed seeds dried at room temperature, the difference was only small (3%) compared with a difference of approximately 20% between hydroprimed seeds and untreated seeds.

The increase in proportion of normal seedlings exceeded 40% for some hydroprimed seedlots. These were seedlots with normal seedling level ranging from 9.5% to 33% when untreated. The increase in the proportion of normal seedlings associated with priming appears to be a function of an increase in the total number of seeds that germinated, and a lower proportion of abnormal seedlings categorised as under-developed and lacking a definitive 'knee'.

A significantly higher proportion of stunted primary roots was recorded in the following order of magnitude: hydroprimed and not re-dried (10.1%) > hydroprimed and re-dried (5.8%) > untreated seed (4.9%). As a proportion of seeds that germinated, this represents 15.7%, 10.5% and 11.5% for hydroprimed and not re-dried, hydroprimed and re-dried, and untreated seeds respectively. The higher proportion of seedlings with stunted roots indicates that the additional seeds that germinated in the hydroprimed (non-dried) treatment may have had higher propensity for stunted primary roots.

Consistent with an increase in seedlings classified as normal in the laboratory based studies, a significantly greater proportion of seedlings emerged from soil media when seeds were hydroprimed prior to sowing. The increase in level of seedling emergence for hydroprimed seeds ranged from 5% through to 35%.

2.6 Conclusions

Tasmanian onion seedlots produce a high percentage of abnormal seedlings, particularly those with stunted root growth. The percentage of abnormal seedlings is considered by industry to be an indicator of seedlot quality. A high incidence of abnormal seedlings therefore reduces the value of onion seedlots.

Identification of the cause of high levels of abnormal onion seedlings is critical for the industry. Seed yield and quality were improved by delaying harvest until seed moisture content was close to 30 %. Seed quality improved with harvest date, starting with the capacity to germinate early in seed development, then the capacity to produce normal seedlings and finally increased rate and uniformity of germination. Rate and uniformity of germination continued to increase until the end of the trial. The quality changes that were observed were associated with increased seed weight, which was also reflected in greater seedling weights. However, there was no effect of maturity on seedling development after germination, relatively high levels of abnormal seedlings were observed from seed from all harvest dates.

Onion umbel maturity was variable at harvest. The seeds from the most immature umbel class contributed about 10% of the total yield, and had a lower germination percentage, were slower to germinate, produced more abnormal seedlings and more small but normal seedlings than seeds from more mature umbels. High quality traits include high germination percentage, rapid germination and production of normal seedlings. Thus, seeds from the immature umbels were of low quality compared with seeds from mature umbels. Post-harvest drying treatment also had a significant impact on seed quality. Rapidly drying the umbels reduced seedlot quality by decreasing the mean dry mass of seeds, increasing the mean germination time, and increasing the proportion of abnormal and small but normal seedlings in the seedlots.

Few management options, beyond selection of appropriate harvest date and umbel drying treatment, are available to reduce the incidence of stunted root abnormality in onion seedlots at present. This study demonstrated that priming may have commercial application in salvaging onion seedlots with unacceptable percentages of abnormal seedlings. Investigation of the effect of priming on seedling abnormality found that the percentage of abnormal seedlings was significantly reduced by priming. Hydropriming caused the greatest improvement in seedlot quality. Hydropriming also resulted in significant improvement in other quality characteristics including germination percentage and rate and uniformity of germination. Decreased levels of abnormal seedlings occurred regardless of whether or not the seeds were re-dried to moisture contents comparable or lower than that in the untreated seedlots. The increase in proportion of normal seedlings exceeded 40% for some hydroprimed seedlots. These were seedlots with normal seedling level ranging from 9.5% to 33% when untreated. The increase in the proportion of normal seedlings associated with priming appears to be a function of an increase in the total number of seeds that germinated, and a lower proportion of abnormal seedlings categorised as under-developed and lacking a definitive 'knee'.

Seedlings with stunted root systems were able to develop normal root systems within 22 days of transplanting to potting media. Ninety percent of normal seedlings survived compared with 68% of abnormal seedlings. The mean dry weights of surviving seedlings were 10.3 ± 0.7 mg and 5.4 ± 1.3 mg respectively. It is apparent that, under the conditions of this trial, seedlings were able to grow, albeit more slowly, despite having stunted root abnormality.

Chapter 3. Carrot seed quality

3.1 Introduction

The production of carrot taproots for international markets is an expanding industry. The fresh carrot market pays a premium for high quality taproots falling within stringent size ranges. Taproots falling outside the specified size requirements represent a potential loss of income. Carrots like many other vegetable crops are established from seed. The quality of the seed sown has been reported as having an impact on the uniformity of seedling emergence (Gray and Steckel, 1983), stand density (Benjamin, 1982), and in some cases marketable yield of taproots (Currah and Salter, 1973). In trials akin to commercial practice from 3 to 72% of the variability in taproot size at harvest can be accounted for by differences in time of emergence of individual seedlings depending on the time of year trials were undertaken (Benjamin, 1984). Values ranging from *ca.* 20 to 50% tend to be the most common (Salter *et al.*, 1981; Benjamin, 1982; Benjamin, 1984). Therefore the establishment phase can be considered one of the most important factors governing the uniformity of mature taproots (Benjamin, 1984 and Salter *et al.*, 1981).

The spread in time of seedling emergence can be attributed to the quality of the seed (Gray *et al.*, 1991) and environmental conditions. The environmental conditions that the seed germinate under are commonly referred to as 'field factors' and encompass variables such as temperature, soil moisture and physical impedance (Finch-Savage and Pill, 1990; Tamet *et al.*, 1996; Hegarty, 1978). Seed quality refers to the performance of seed under a range of environmental conditions.

The internationally recognised method of evaluating carrot seed quality is to germinate seeds at 20°C and after 14 days record the proportion of seedlings with the necessary structural components for development as a normal plant (ISTA, 1999). This test provides a measure of the level of seed viability, however, given that the viability test is done under ideal germination conditions it does not always equate to the performance of the seed under sub optimal conditions for germination (Hegarty, 1971; Matthews, 1980). Therefore alternative methods to the ISTA viability test have been examined to assess seed performance under field conditions. Some researchers have shown that germinating carrots seeds under a sub-optimal temperature of 10°C, or a controlled deterioration test to provide a better measure of seed vigour than the ISTA test (Hegarty, 1971; Matthews, 1980).

Gray and Steckel (1983a) undertook an extensive comparison of methods for evaluating seed vigour on 18 separate carrot seedlots displaying a similar range of viability to that used commercially in the United Kingdom. They found that the results from the ISTA test were equivalent and sometimes better than other common methods of assessing seed vigour (i.e., cold test (10°C), slope test, controlled deterioration test). Of particular note was the finding that the variability of individual seedling weights at establishment was strongly correlated with the coefficient of variation (CV) of embryo length within the seed. Additional work by Gray and colleagues indicated that a measure of CV of embryo length combined with a level of seed viability could be used to predict seedling size variability and stand density under a wide range of conditions (Gray and Steckel, 1983b; Gray *et al.*, 1986; Gray *et al.*, 1991). From their assessment of commercially available seed they proposed a universal classification of carrot seed with low, medium and high size variability as follows: CV of embryo length less than 20% 'low'; 21 to 30% 'medium'; and above 30% 'high' (Gray *et al.*, 1986). Approximately 5% of

commercially available seed in the UK were categorised as low, 70% as medium and 25% as high (Gray *et al.*, 1986).

Further field trials assessing the effect of variability of embryo size on the variability of mature taproots showed that the CV of seedling sizes 20 days after 50% emergence was positively correlated with CV of embryo size and spread in emergence regardless of stand density (Gray *et al.*, 1991). While there have been some reports of a positive correlation between CV of plant sizes soon after emergence and the CV of taproot sizes at maturity (Salter *et al.*, 1981) this has not always been found to be the case (Gray *et al.*, 1986). Further evidence suggests that this relationship is more likely to exist at high densities ($>200 \text{ plants.m}^{-2}$) (Gray *et al.*, 1991). These findings indicate that competition *per se* is not a primary source of variation in taproot size but magnifies any initial variation within the crop at the time of seedling emergence (Salter *et al.*, 1981; Li *et al.*, 1996; Weiner and Thomas, 1986; Benjamin, 1982). Figure 3.1 illustrates the relationship between variability of taproots at harvest and variability of embryo length within planted seed at a high and low density.

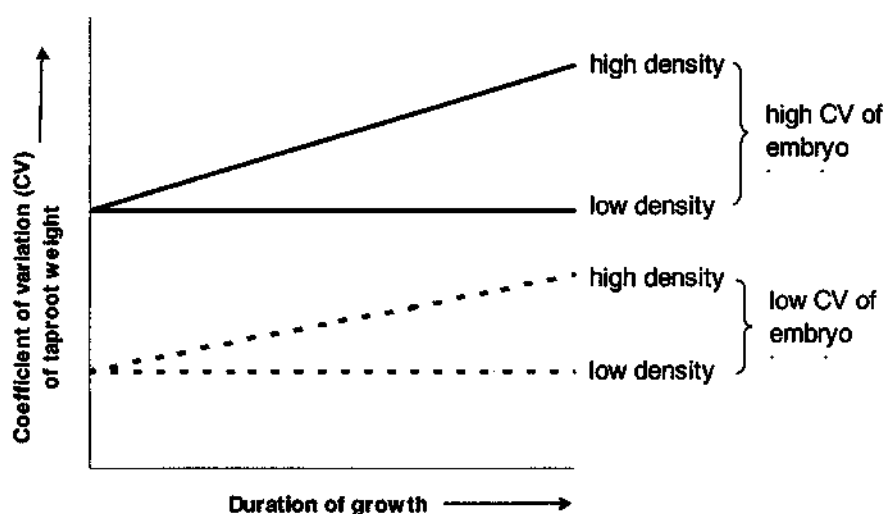


Figure 3.1 Illustration of the variation in taproot size with crop growth, associated with low ($<20\%$) or high ($>30\%$) CV of embryo size at a low (50 plants.m^{-2}) or high ($>250 \text{ plants.m}^{-2}$) stand density. Adapted from Gray and Benjamin (1993).

Physiological treatments to enhance the performance of vegetable seed in the field have been widely trialed. The seed treatments trialed to enhance carrot seed performance have primarily been based on hydration and include; osmo-conditioning, matri-conditioning and hardening. All three seed treatments generally improve the rate of seedling development, synchronicity of emergence and the proportion emerging in the field (Currah and Salter, 1973; Finch-Savage and Pill, 1990; Khan *et al.*, 1992), sometimes resulting in higher yields of carrot (Szafirowska *et al.*, 1981; Currah and Salter, 1973). However, in some trials osmo-conditioning seeds has increased seedling size variability (Finch-Savage, 1990). This was thought to be due to the germination of seed that developed into small seedlings following treatment whereas similar seed in the control (untreated) group failed to germinate. Further, any advantage gained from pre-conditioning seeds often diminishes when either ambient air temperature increases (above approximately 10°C), field conditions become more favourable to germination (Currah *et al.*, 1974; Szafirowska *et al.* 1981; Finch-Savage and McQuistan, 1989; Bodsworth and Bewley, 1979), or

seed is dried back to original water potential to plant through conventional precision air seeders (Murray, 1989).

An alternative planting technique has been developed that enables sowing of pre-germinated seed suspended in a gel medium to protect the emerging radicles - this is referred to as fluid drilling. While a more rapid emergence and predictable stand density can be achieved (Pill, 1995), the lack of precision in spacing and short amount of time to plant seeds have proved to be two obstacles to the wider use of fluid drilling in vegetable production (Rubatzky *et al*, 1999). The latter problem can be partially overcome by the addition of the plant hormone abscisic acid to pre-germinated seeds to inhibit further development prior to planting (Finch-Savage and McQuistan, 1989). However, this technique has not been proven to be economically advantageous in commercial production of carrots and is therefore not widely used.

In summary, factors influencing the variation in carrot taproot sizes have received some attention in the literature, particularly in the past 20 years. The most comprehensive studies have been undertaken in the UK on soils of a sandy loam or light sandy texture. Outcomes of these studies have shown that the variation in taproot size at harvest is largely influenced by the initial variability in plant size at emergence and the magnitude of competition between plants, i.e., stand density. While field factors have a large impact on the rate and spread in seedling emergence, seed quality is also important. The variation in embryo size within a seedlot has been shown to provide a reliable measure of the potential variation in seedling size.

This study was initiated by key stakeholders in the fresh carrot industry in Tasmania, Australia, to identify the major causes of size variability under local conditions and develop strategies to reduce variability. Unlike many other regions of the world this production system is located on a soil of high clay content. While density and yield responses have been examined no consideration has been given to variability in sizes under this production system. This study reports on the effect of seed quality on the variation in seedling size at establishment and the subsequent variation in mature taproots at harvest under commercial conditions. In order to achieve a range of seed quality consistent with those in commerce seed was graded by density and size. The results are discussed in relation to previous research and recommendations.

3.2 Materials and Methods

Seed grading

Fifteen kilograms of commercially supplied seed of Nantes and Kuroda genotypes were graded by density and size in a 3 x 3 factorial design (Figure 3.2). Each seedlot was initially graded into 3 density classes of approximately equal quantity using a gravity table (Oliver 70) and then by size using square sieves, resulting in 9 separate sub groups. For Kuroda seed the sieve sizes were; <1.6mm (small), 1.6 - 2mm (medium) and >2mm (large). For Nantes seed the sieve sizes were <1.3mm (small), 1.3-1.6mm (medium) and >1.6 mm (large). The weights of 1000 seeds from each of the 9 classes were recorded enabling the proportion of seed in each grouping by number and weight basis to be calculated.

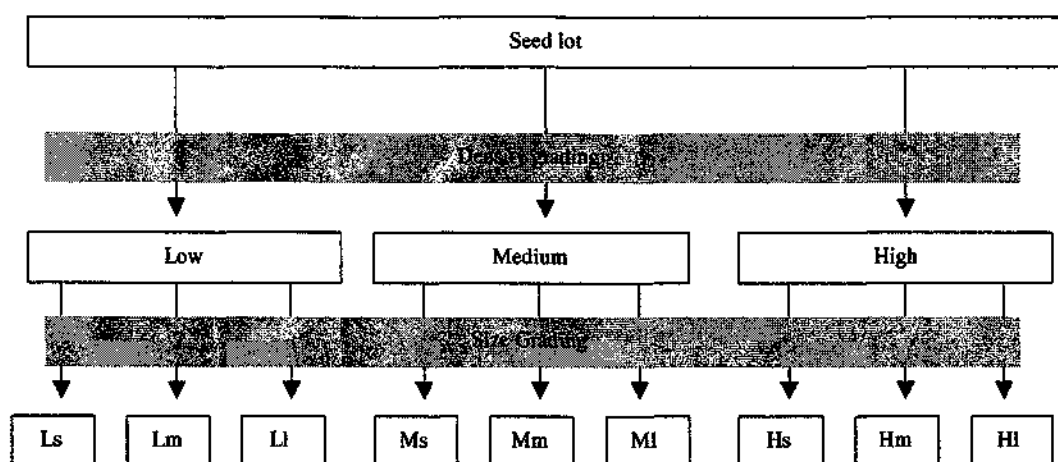


Figure 3.2 Carrot seedlots were graded by density into Low (L), Medium (M) and High (H) density groups and then by 3 sizes; Small (s), Medium (m) and Large (l).

Seed germination

The germination characteristics of carrot seeds from each of the graded seed class were assessed at constant temperatures of 10°C and 20°C. Each replicate consisted of 100 seeds germinated on double Whatman No.1 filter paper in the dark. This was replicated 4 times for each seed weight class under both temperatures. The wetting of the filter paper with distilled water was taken as day 0 and the number of germinated seeds, as evidenced by radicle emergence, was recorded each day. The percentage germination, mean time to complete germination (MGT) and the coefficient of uniformity of germination (CUG) were calculated (Appendix 1).

Embryo measurement

The technique used to extract and measure embryo size was similar to that outlined by Gray and Steckel (1983a). The length of 100 embryos (25 embryos x 4 replicates) from each graded seedlot was recorded after soaking seeds in formalin acetic acid (FAA v/v; 50% ethanol, 40% water, 5% glacial acetic acid, 5% formalin) for a minimum of 48 hours. Embryos were carefully extracted from individual seeds by removing the caruncle then pressing slightly on the back of the seed using the blunt side of a scalpel to extrude the embryo under a stereomicroscope. The length of each individual embryo was recorded by measuring the distance from the base of the hypocotyl to the tip of the cotyledons after projecting the image onto a monitor (13" Sanyo model VMC 7514) using a video camera (Videolabs® Teachcam™). The magnification of the image was calibrated using a slide graticule. CV of embryo length was calculated for the 100 embryos examined.

Field trials

Three graded seed groups (Ls, Mm and Hl) and a combination (Mx) of the 3 in proportion to that in the seedlot prior to grading were selected for field trials. The 4 seedlots were replicated 3 times in a randomised complete block design for each cultivar. Each block consisted of 4r adjacent 10m lengths of beds/mounds. Trials were established within commercial crops and the distance between each block was approximately 100m. The commercial crop provided a buffer around the plots. Seedlots were sown in a manner consistent with commercial practice for both carrot types using Stanhay precision air seeders. Kuroda seedlots were sown on the 12th October. Nantes seedlots were sown on the 26th November .

Seedling emergence

A 1m length of the bed/mound was marked out in each plot. Within this area the number of carrot seedlings was recorded every 2nd or 3rd day during the seedling establishment phase. Seedlings were recorded as having emerged when the cotyledons were upright and had separated.

Size variability

Size variability of plants was assessed 40, 80 and 120 DAS (Days After Sowing). At each of the 3 dates carrot plants were hand harvested from a 0.5m length of bed/mound from each plot. The dry weight of individual seedlings was recorded at 40 DAS while fresh taproot weight was recorded at 80 and 120 DAS. Percentage Coefficient of Variation ($CV = (\text{standard deviation}/\text{mean}) \times 100$) was calculated for each sample and the mean of the 3 replicate plots for each commercial crop recorded.

Seedlings harvested at 40 DAS were carefully removed from the soil using a trowel. Soil was gently washed from the plant roots and the weight of individual seedlings was recorded after drying at 70°C for 72 hours. At 80 and 120 DAS hand harvested taproots were washed in water, surface dried using paper toweling and then weighed. One hundred and twenty DAS coincided with the approximate time of commercial harvest. A sub sample of fresh taproots was dried at 70°C to determine moisture content.

Statistical analysis

The frequency distributions of embryo lengths, seedling weights and taproots weights across treatments were assessed using SAS proc univariate version 6.12 statistical software for individual plots. Only approximately 20% of plots deviated from a normal distribution. However, there did not appear to be any systematic departures from a normal distribution across all treatments. The one exception to this was the embryo length of a graded seedlot. The treatment of this data is discussed in the results section. Given that most plots were normally distributed, transformation of the data for analysis of variance was not considered necessary. Previous reports of carrot embryo length, seedling size and taproot size from graded and ungraded seedlots having a normal distribution have been published (e.g. Gray and Steckel 1983a, Gray and Steckel 1983b, Gray *et al.*, 1986, Gray *et al.*, 1991), justifying the use of CV as an appropriate relative measure of variability.

3.3 Results and Discussion

The effect of size variability of plants soon after emergence on taproot variability at harvest maturity was examined by grading commercial seedlots by density and size and sowing selected graded seedlots in field trials within commercial crops. The germination characteristics and embryo size of graded seedlots are shown in Table 3.1. The percentage germination for all graded seedlots was in excess of 85% by the end of the germination period, 14 days at 20°C and 20 days at 10°C. While a significant difference ($P < 0.05$) in the mean time to complete germination (MGT) was recorded between some of the graded seedlots the maximum difference in MGT was approximately 1.5 days (Table 3.1) at either 10 or 20°C.

A general increase in mean embryo length was recorded with increasing seed density and seed size for both cultivars, indicating that embryo length was closely associated with both grading procedures in the commercial seedlots used in this study (Table 3.1). The range in CV of embryo lengths in graded sub seedlots (18 to 29%) was consistent with the range in variability in lengths found in commercial industry in the United Kingdom (Gray and Benjamin, 1993).

The seedlots Ls, Mm and Hl and their combination (Mx), in the same ratio to that within the pre-graded seedlot, were sown in field trials.

Table 3.1 Characterisation of graded seedlots. Proportion of seeds by weight and number, 1000 seed weight, germination characteristics, embryo length, and coefficient of variation of embryo length (100 embryos). (a) Kuroda and (b) Nantes genotypes. LSD ($P < 0.05$) shown, $n = 4$. MGT = mean time to complete germination, CUG = coefficient of uniformity of germination. Seedlots selected for field trials bolded.

(a) Kuroda	Low density			Medium Density			High Density			LSD
	Size			Size			Size			
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	
1000 seed weight (g)	3.034	3.383	3.535	3.516	3.974	4.045	3.995	4.399	4.602	
<i>Proportion by:</i>										
Number (%)	8.2	19.0	7.5	9.1	17.6	9.9	5.7	14.8	8.2	
Weight (%)	6.5	16.7	7.0	8.3	18.2	10.5	6.0	17.0	9.8	
<i>Germination at 20°C</i>										
% Germination	87.9	88.5	85.0	91.3	90.7	88.9	90.7	90.1	89.1	Ns
MGT	7.31	7.41	7.5	6.86	7.06	6.93	6.65	6.80	6.93	0.41
CUG	0.169	0.161	0.144	0.205	0.150	0.163	0.200	0.177	0.169	ns
<i>Germination at 10°C</i>										
% Germination	86.7	87.8	83.9	90.8	90.5	88.9	91.1	90.3	88.7	Ns
MGT	11.44	11.25	11.84	10.83	10.97	10.67	10.37	10.37	10.97	0.668
CUG	0.136	0.146	0.106	0.161	0.112	0.133	0.139	0.137	0.125	ns
Embryo Length (mm)	1.16	1.33	1.32	1.39	1.42	1.45	1.33	1.46	1.48	0.12
%CV Embryo Length	28.8	29.2	25.8	26.8	21.1	21.6	20.9	20.0	18.4	

(b) Nantes	Low density			Medium Density			High Density			LSD
	Size			Size			Size			
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	
1000 seed weight (g)	1.87	2.11	2.40	2.00	2.26	2.53	2.11	2.42	2.75	
<i>Proportion by:</i>										
Number (%)	11.7	16.2	9.6	9.3	16.8	8.5	5.5	13.7	8.5	
Weight (%)	9.7	15.2	10.2	8.2	16.8	9.6	5.2	14.7	10.4	
<i>Germination at 20°C</i>										
% Germination	87.1	88.3	83.2	90.5	90.3	87.9	90.1	89.6	88.4	ns
MGT	6.4	7.1	7.8	6.7	7.1	7.8	6.8	7.5	7.9	0.8
CUG	0.100	0.094	0.087	0.095	0.118	0.091	0.103	0.118	0.101	ns
<i>Germination at 10°C</i>										
% Germination	87.4	88.2	83.6	90.2	90.1	88.1	90.4	89.6	88.4	4.0
MGT	8.7	8.7	9.5	8.4	8.9	8.9	8.6	9.6	9.2	ns
CUG	0.106	0.144	0.105	0.139	0.130	0.133	0.149	0.125	0.142	ns
Embryo Length (mm)	1.33	1.40	1.53	1.46	1.46	1.54	1.50	1.54	1.58	0.12
CV of Embryo Length	29.2	21.6	24.4	21.7	21.6	21.0	19.8	20.4	22.0	

A similar pattern of seedling emergence occurred for all seedlots in both trials (Figure 3.3). No significant differences ($P>0.05$) in the total number of seedlings emerging between the graded seedlots for both the Kuroda (mean of 109 plants.m⁻¹ of bed) or Nantes (mean of 66 plants.m⁻¹ of mound) field trial was found. This was consistent with the laboratory based germination profiles (Table 3.1). However, within the Kuroda trial, seedling emergence was delayed in one of the 3 replicate blocks (Figure 3.3c) resulting in a significantly ($P<0.05$) lower stand density (97 plants.m⁻¹ of bed) compared with the other 2 blocks (114 and 116 plants.m⁻¹ of bed). The data indicates that 'field factors' had a greater effect on seedling establishment than the differences in seed quality used in this trial.

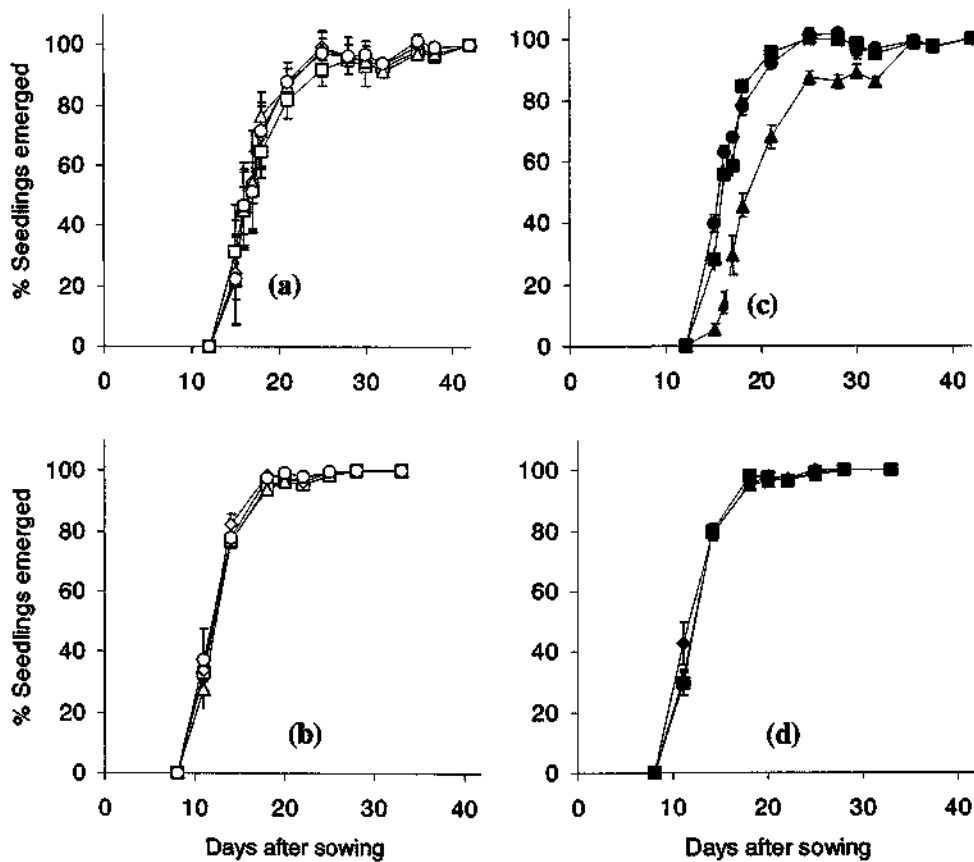


Figure 3.3 Cumulative percentage of seedlings emerged as a proportion of maximum that had emerged for Kuroda (a,b) and Nantes trial (c,d). Seedlots: LS (□); MM (○); HL (Δ); and Mix (◇), and the replicate blocks: 1 (◆), 2 (■) and 3 (▲).

For both genotypes examined, the grading of the seed resulted in the distribution of embryo lengths in seedlot Ls to be positively skewed. Hence the CV of embryo length for the combined seedlot (Mix) had a lower CV than the Ls group (Figure 3.3). An examination of the distribution of seedling sizes for seedlot Ls in the field trial showed a normal distribution within plots. The Kuroda trial was undertaken at a time in the carrot season when conditions are generally most difficult for crop establishment. In this trial the CV of seedling size 40 DAS was positively correlated with the CV of embryo length within the seedlot (Figure 3.4). However, by 120 DAS the mean CV of taproot sizes from all seedlots ranged from only 47 to 53 % and differences in variability between seedlots noted 40 DAS were reduced (Figure 3.5a).

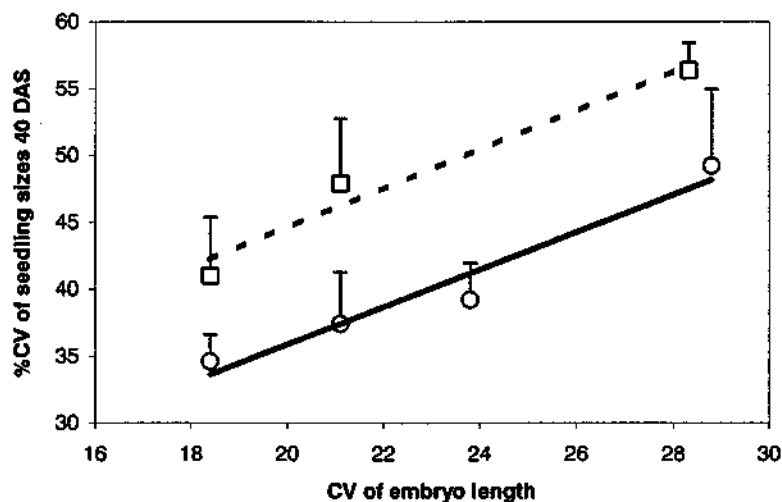


Figure 3.4 Relationship between CV of embryo length and CV of seedling weights 40 DAS for 2 separate field plantings cv. Kuroda. Linear regressions shown for the separate field plantings. Mean values shown. Bars represent SE (n=3).

The Nantes trial was planted under more favorable environmental conditions for field establishment. The CV of seedling size 40 DAS and the CV of taproot size 80 and 120 DAS was approximately the same for this trial (Figure 3.5b). The mean CV of taproots 120 DAS across all seedlots ranged from 41 to 48%. No significant difference ($P>0.1$) in CV of taproot sizes 120 DAS between the graded seedlots resulted in either trial.

The mean seedling dry weight 40 DAS and mean taproot dry weights 80 and 120 DAS from the seed grading trials were recorded (Table 3.2). For the Kuroda trial, a significant difference ($P<0.05$) in seedling dry weight was recorded between seed groups 40 DAS with seedling dry weight corresponding directly to the seed weight (Table 3.2). The same trend existed for the Nantes trial (Table 3.2). While the mean taproot weight 80 and 120 DAS from each of the seedlots remained in the same order of magnitude (Hl > Mm and Mix > Ls) as the seedling dry weight at 40 DAS no significant difference in mean taproot weight were recorded ($P>0.05$).

Table 3.2 Mean dry weight of carrot plants 40 DAS and taproots 80 and 120 DAS of graded seedlots. (a) Kuroda and (b) Nantes. LSD ($P<0.05$).

		Seedlots				
Kuroda		Ls	Mm	Hl	Mix	LSD
40 DAS	Whole plant	0.018	0.024	0.029	0.024	0.0057
80 DAS	Taproots	0.33	0.52	0.59	0.51	Ns
120 DAS	Taproots	7.94	8.13	9.05	7.85	Ns
Nantes		Ls	Mm	Hl	Mix	
40 DAS	Whole plant	0.073	0.089	0.103	0.091	Ns
80 DAS	Taproots	2.88	3.09	3.55	3.26	Ns
120 DAS	Taproots	9.33	9.71	10.81	10.55	Ns

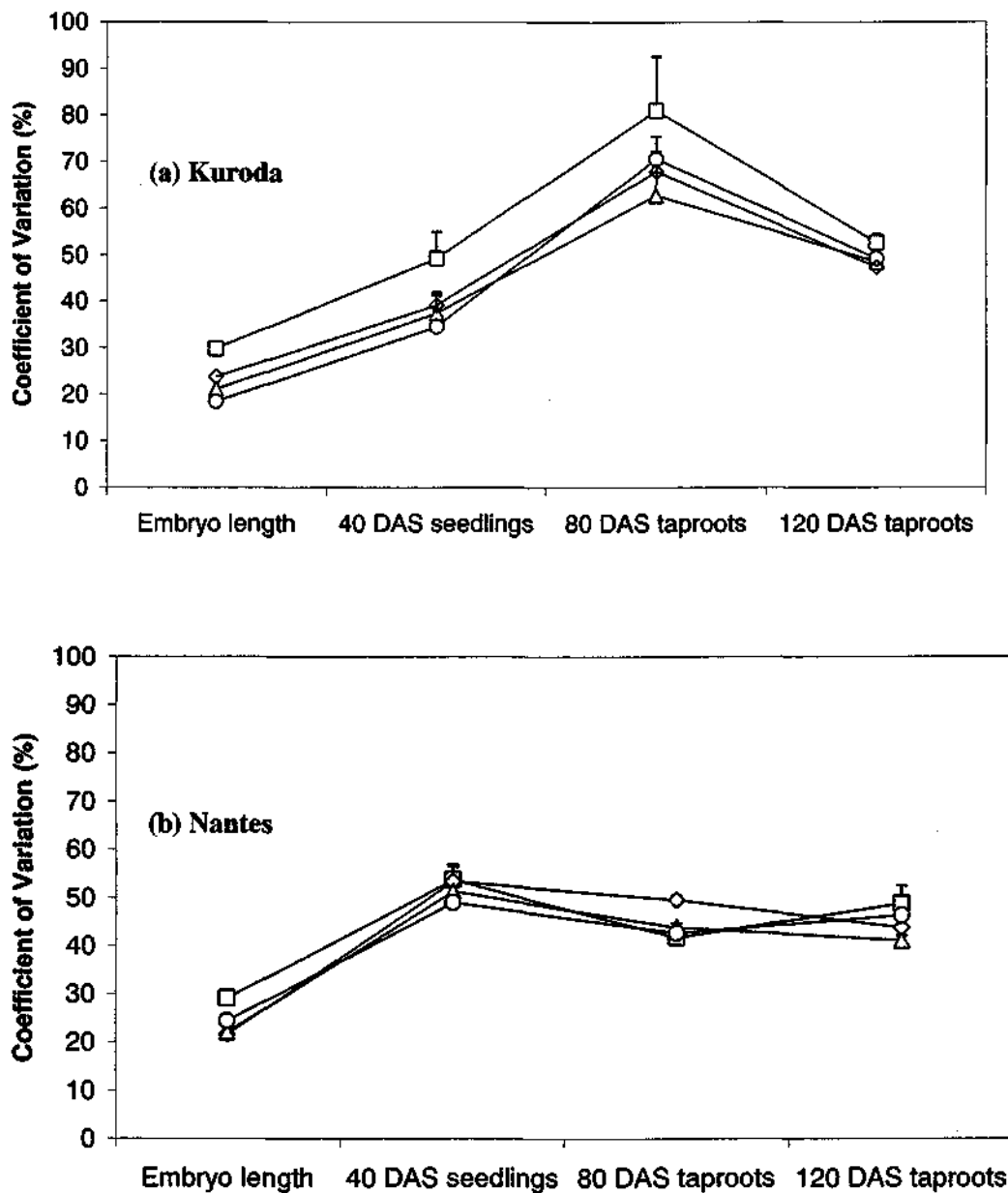


Figure 3.5 CV of embryo length from 100 seeds for each seedlot; Ls (□); MM (◇); HL (△); and Mix (○), and resultant CV of seedling size 40 DAS, and taproots 80 and 120 DAS. (a) Kuroda and (b) Nantes. Mean CV shown, bars represent SE, n=3.

3.4 Conclusions

In order to assess the effect of seedling size variability on mature taproot size variability in this study, commercial seedlots of both the Kuroda and Nantes genotypes were graded to produce seedlots varying in embryo size. The greatest range in CV of embryo length was obtained for the Kuroda seedlot (18 to 29%) and the resultant mean CV of seedling size in field planting was 34.6 and 49.2% for HL (Heavy large seeds) and Ls (Light small seeds) respectively. This range in plant size variability is comparable to the range in seedling size variability from the survey of 12 commercial crops (32.9 to 50.4%) under taken in Tasmania, Australia (Gracie, 2002).

Though relatively large differences in CV of plant sizes between graded seed groups within this trial were recorded 40 DAS, by 120 DAS the differences between seed treatments had diminished with CV of taproot weights ranging from 47 to 53%.

The reduction in differences in CV of taproot size between graded seedlots by 120 DAS suggests that factors between the establishment phase and the taproot maturity may be having an impact on the final CV of taproot size. The degree of competition caused by stand density and evenness of intra row spacing are two such parameters that may be having an impact (Gracie and Brown, 2001).

Further research into carrots size variability should consider; (1) the interaction of seed quality, plant spacing arrangement and density, and (2) the interaction between seed quality and field factors in obtaining the target stand density. Currently the carrot production in Tasmania is strongly seasonal, planting mainly in spring through to early summer and harvesting from mid summer through to autumn. Further expansion of the industry to planting at other times of the year when conditions are less favorable for field establishment may benefit from seed priming in order to obtain target densities and uniform emergence.

Chapter 4. Brassica seed quality

4.1 Introduction

The need for quality seed for vegetable brassica industries has been recognised both for production systems relying on modular transplants and those using direct seeding (McCormac and Keefe, 1990; Jett *et al.*, 1996). The importance of various germination characteristics varies depending on the conditions under which the seed is expected to germinate, but generally, high quality seeds germinate quickly and evenly into normal seedlings while poor quality seeds germinate slowly and less uniformly. Seeds are considered to be of high quality if they have the capacity to germinate rapidly in stressful environments such as high or low temperatures, drought or waterlogging. In addition to capacity to germinate, larger seeds are considered to be of higher quality than smaller seeds. Often germination and seed size are found to be correlated (Heather and Siczka, 1991). Many of the production and harvesting practices and technologies used by brassica seed producers are specifically targeted at achieving the highest possible seed quality in addition to high crop yield.

Until recently, assessment of brassica seed quality has been by determination of germination capacity, that is, germination of seeds under ideal temperature and moisture conditions. However, this measure fails to provide any information on the ability of seeds to perform under less than ideal conditions. Workers have investigated alternative ways of assessing seed quality in brassica species, for example, high quality brassica seedlots germinate rapidly over a range of temperatures (Wilson *et al.* 1992) and water potentials (Still and Bradford, 1998), have slow solute leakage during imbibition (Thornton *et al.*, 1990), and, when coated, the absence of fluorescence due to sinapine leakage (Taylor *et al.*, 1993). Seed companies have developed individual methods of seed quality assessment. One such method is the soil germination test, where seed is sown in soil in a glasshouse and emergence recorded after a period of time. It can be seen, therefore, that although the importance of seed quality has been recognised, there are a range of methods of brassica seed quality assessment that are currently in use, and there is no single measure of seed quality that is widely accepted.

There are many aspects of seed crop management, production environment and harvesting methods that influence the quality of seed produced. Factors identified from the literature as important determinants of seed quality in vegetable brassica crops include: seed size and cultivar (Heather and Siczka, 1991); seed maturity at harvest (Still and Bradford, 1998); seed deterioration or aging (Powell *et al.*, 1991); and seed coat integrity (McCormac and Keefe, 1990). While priming has been found to overcome poor seed quality in brassica species including broccoli (Jett *et al.*, 1996) and a range of canola cultivars (Zheng *et al.*, 1994), the objective of seed producers is to obtain high quality seed. Of the factors affecting brassica seed quality, anecdotal evidence supplied by local seed companies indicated that seed coat damage during threshing is an area of particular concern. Damaged seed coats cause increased rates of water uptake in cauliflower seeds (McCormac and Keefe, 1990). More rapid imbibition led to decreased growth rates and reduced emergence in soil. Therefore seed coat integrity is likely to be an important determinant of seed performance in locally produced brassica crops.

The aim of this study was to compare the effects of two commercially used combine harvesters on seed damage. Preliminary investigations into location of points within the harvester where damage was occurring, and the effects of different harvester settings on levels of seed damage, were also undertaken. The study used scanning electron microscopy (SEM) to identify the degree of physical damage to seed coats after threshing crops using mechanical harvesters. Seed

quality was assessed through assays of leachate conductivity and germination percentage, and yield losses associated with seed fragmentation were determined.

4.2 Materials and Methods

The impact of physical damage on cabbage seed quality during the harvesting process was assessed. Seed quality was recorded as yield loss associated with seed fragmentation, germination capacity and rate, and electrolyte leakage. Seed coat integrity was also examined under ESEM (Environmental Scanning Electron Microscope). All trials were undertaken in commercial cabbage (*Brassica oleracea*) seed crops at the time of commercial harvest. Two of the seed crops were of the same line of Savoy cabbage while the third crop was a line of white cabbage.

Comparison between harvesters

Seed samples were collected after mechanical threshing with a rotary (International 1420) and a conventional combine harvester (Allis Chalmers Allcrop) in three separate cabbage seed crops. The crops were dried on field drying racks prior to harvesting. Four replicate samples were collected from each crop by selecting plant material from four locations on the drying racks and threshing subsamples through each harvester. The experimental design was a randomised complete block. The mechanically threshed samples were collected into paper bags for transport to the laboratory. Additional plant material was collected from the drying racks at each location and gently hand threshed to produce control (non-mechanically threshed) samples. Samples for hand threshing were taken by collecting 4 plants from each replicate and transporting them in feed sacks. All samples were stored in a cool place for 6 days before further processing. Hand threshing of control samples was done carefully in the laboratory, with about 2000 seeds being collected from each replicate. Immediately after harvest or hand threshing seed samples were dried for 6 days at 20°C to a moisture content of approximately 10%. All mechanically harvested seed was cleaned using a Blount Clipper laboratory air screen seed cleaner. Cracked and misshapen seed was subsequently removed using a spiral seed cleaner.

Position within the harvester

A rotary combine harvester (International 1420) was used in this trial. On 4 separate occasions within a single crop the harvester was stopped and seeds were collected from different positions within the harvester. The positions were; before the seed entered the rotor (on the drag chains feeding the rotor), after the seed had been through the rotor (taken as seed exited the concave), after the screens (in the auger feeding the good seed elevator) and from the collection bin. Hand harvested seed was used to determine the quality of the seed prior to harvest.

Harvester Settings

The effects of rotor speed and concave gap on seed quality were examined. This was undertaken for both a rotary (International 1420) and a conventional combine harvester (Allis Chalmers Allcrop). Each setting was replicated 4 times within one crop.

The modified settings assessed on the International 1420 were:

- Rotor speed 500 rpm, concave gap at 3.5 units
- Rotor speed 600 rpm, concave gap at 3.5 units
- Rotor speed 600 rpm, concave gap at 2.5 units

The modified settings assessed on the Allis Chalmers were:

- Rotor speed 680 rpm,
- Rotor speed 800 rpm,
- Rotor speed 600 rpm,
- Rotor speed 680 rpm, concave gap decreased by 1 mm
- Rotor speed 680 rpm, concave gap increased by 1 mm

Yield loss

Samples of seeds collected in each of the trials were cleaned using a spiral seed cleaner. Non-seed material was carefully removed from the sample and the weight of fragmented seed, as a proportion of the total sample weight, was determined.

Seed germination

Seeds were germinated in accordance with International Seed Testing Association (ISTA) guidelines (ISTA, 1999). Each replicate consisted of 100 seeds germinated on saturated filter paper at 20°C with a 12 hour light/dark cycle. The number of seeds that germinated each day, as evidenced by radicle emergence, was recorded. The proportion of seeds germinating (%germination) within 10 days and the mean time to complete germination (MGT) was calculated (Appendix 1).

Leachate conductivity

Conductivity was recorded using a method adapted from the ISTA seed vigour testing procedures (ISTA 1995). Each replicate consisted of 200 seeds soaked in 50 ml of deionised water for 24 hours at 20°C. Solution conductivity readings were recorded using a conductivity meter (Hanna Instruments, model HI 933100).

Seed Coat damage

Observational studies of seed coat damage were undertaken using an Environmental Scanning Electron Microscope (ESEM). Only a small number of seeds were examined in order to determine the types of seed coat damage occurring as a result of the threshing operation.

Statistical analysis

Anova analysis was used to test significance between mean values using Microsoft Excel statistical software. LSD (Least Significant Difference) values were calculated when P<0.05.

4.3 Results and Discussion

Comparison between harvesters

The percentage germination of seeds from the three crops used in this trial exceeded 90%, regardless of the mode of harvesting; by hand or using mechanical harvesters. While germination percentage varied between the three crops sampled, the overall trend in germination percentage was for seed threshed using the conventional harvester to have the lowest germination percentage (Table 4.1).

Table 4.1 Savoy (crops 1 and 2) and white (crop 3) cabbage seeds were either mechanically harvested using an International 1420 or an Allis Chalmers, or hand harvested. Germination percentages were determined using a standard 10 day germination test. Results are expressed as means of four replicates with standard errors.

	Crop 1	Crop 2	Crop 3
Hand threshed	98.5 ±0.6%	98.0 ±1.2%	99.3 ±0.3%
International 1420	92.7 ±1.2%	98.3 ±1.0%	96.8 ±0.8%

Allis Chalmers	93.6 ± 1.6%	94.8 ± 0.5%	94.6 ± 1.1%
----------------	-------------	-------------	-------------

When data from the three crops sampled were combined, the order of magnitude of percentage germination from the different modes of harvesting was hand harvested (98.6%) > International 1420 (95.9%) > Allis Chalmers (94.3%). Statistical analysis of the data showed a significantly higher percentage germination for hand harvested seedlots than seedlots harvested with the Allis Chalmers. No significant difference ($P > 0.05$) in percentage germination between the two mechanical harvesters or between the International 1420 and hand-harvesting was detected. The low level of replication within crops, combined with the level of variability between crops and the small size of any difference in germination percentage between seeds from the two harvesters, lead to a low likelihood of statistically significant differences between harvesters being detected. Further studies with increased replication are recommended if statistical significance in differences between harvesters needs to be proven.

Seedlot quality was also recorded as the mean time to complete germination (MGT) (Figure 4.1b) and leachate conductivity (Figure 4.1c). Lower MGT is associated with higher quality seedlots, while the conductivity test provides an indirect method of assessing seed coat/cell membrane integrity (damage to the seed coat and/or loss of membrane integrity result in higher rate of leakage of soluble material out of the seed and hence higher electrical conductivity in solution in which the seeds are immersed). Analysis of the results revealed that seeds harvested with the Allcrop had a significantly ($P < 0.05$) higher MGT and higher leachate conductivity than seeds harvested with the International 1420 or hand harvested. No significant difference in MGT and leachate conductivity was recorded between hand-harvested seeds and seeds harvested with the International 1420.

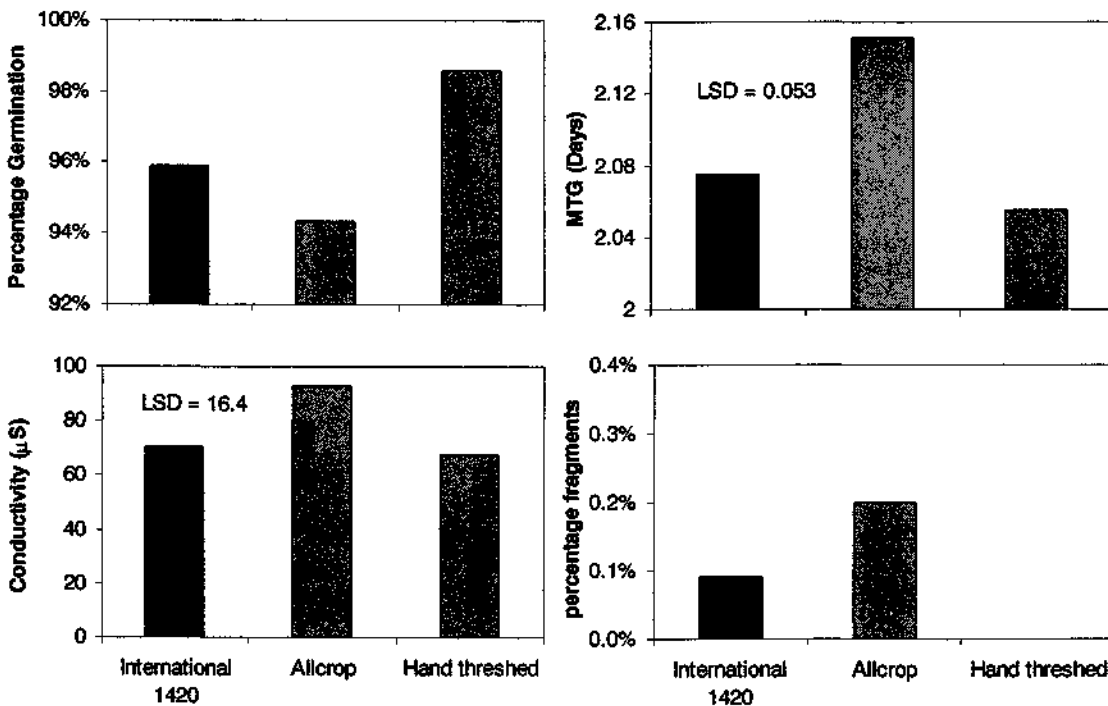


Figure 4.1 Cabbage seeds were either mechanically harvested using an International 1420 or an Allcrop, or hand harvested. Seed quality was recorded as (a) percentage germination, (b) mean time to complete germination, (c) leachate conductivity, and (d) percentage (by weight) of fragmented seeds.

Detailed observational studies using a scanning electron microscope revealed the presence of microscopic fractures on the seed coat and underlying tissue of mechanically harvested seeds (Figure 4.2). A higher degree of physical damage to the seeds provides a likely explanation for the higher leachate conductivity and the lower germination rate recorded for seedlots harvested with the Allcrop. This is further supported by the significantly ($P < 0.05$) higher proportion of fragmented seed pieces within seedlots harvested with the Allcrop than the International 1420 (Figure 4.1d).

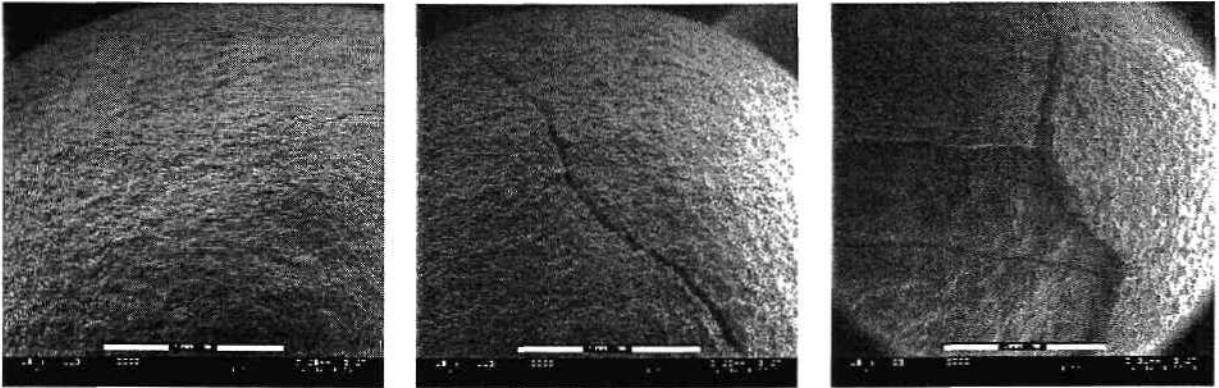


Figure 4.2 Scanning electron micrographs of cabbage seed surfaces. The first image is of an undamaged seed coat (bar = 400 μ m), the second image shows a crack approximately 20 μ m wide in the seed coat (bar = 400 μ m), and the third image shows a seed with a fragment of the seed coat removed and cracking of the internal tissue in the seed (bar = 500 μ m).

Position within the harvester

Seed samples were collected from a number of positions within the International 1420 during harvesting. The positions within the harvester were; (a) just before the rotor, (b) after the rotor, (c) after the screens and (d) from the bin. Hand harvested seeds were also collected to assess the quality of the seeds prior to harvesting. An overall decrease in germination percentage of 5% was recorded from seedlots prior to mechanical harvesting (98.5%) to seeds lots collected in the bin (93.6%) (Figure 4.3a). The major site of the loss of germination within the harvester was between rotor and the screens.

This trial was replicated 4 times in one crop. Further replication in a greater number of crops would be required for a definitive answer as to the location of major seed damage site(s) within the harvester. However, the reduction in germination percentage during the harvesting process was also reflected in a general increase in MGT (Figure 4.3b) and leachate conductivity (Figure 4.3c), suggesting that for the crop tested the impacts received by seed between the rotor and the screens were more damaging than at other positions in the harvester.

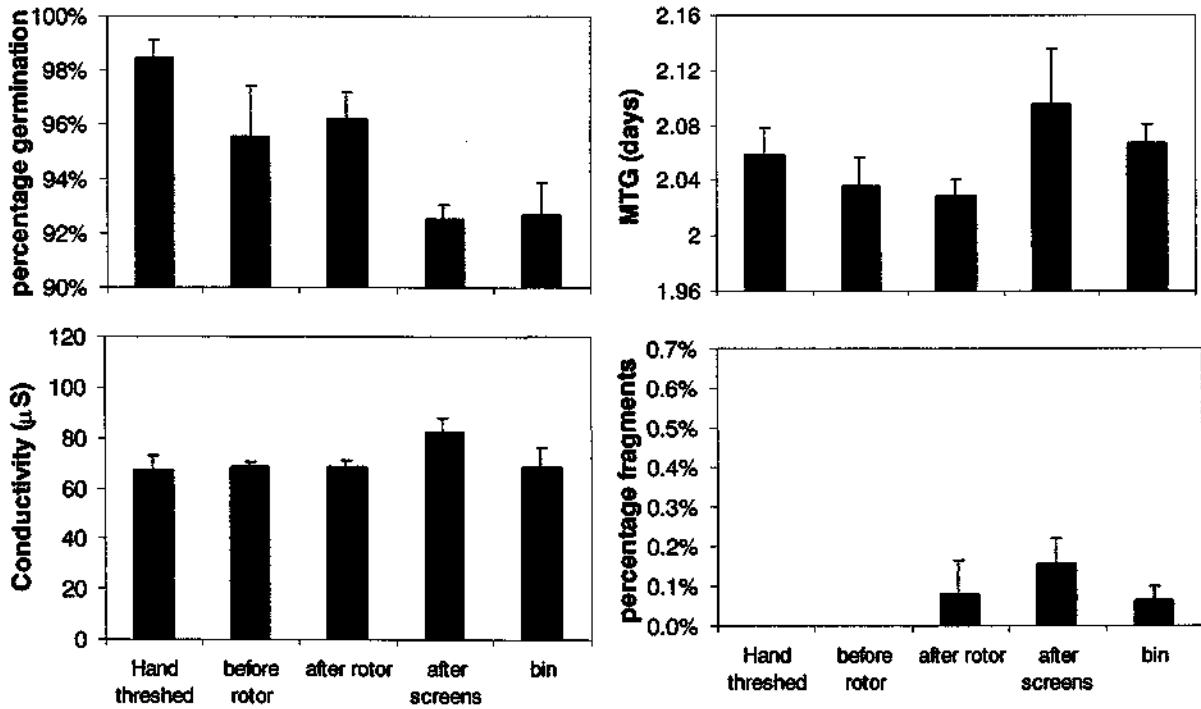


Figure 4.3 Seeds were collected from different positions within the International 1420 during harvesting. Seed quality was recorded as (a) percentage germination, (b) mean time to complete germination, (c) leachate conductivity, and (d) percentage (by weight) of fragmented seeds. Mean values \pm standard error bars ($n=4$) shown.

Harvester Settings

The effect of altering rotor speed and concave gap of the combine harvesters on seed quality was examined. A rotor speed of 500 rpm and concave gap of 3.5 units represented the current commercial operation setting on the International 1420. Both a decrease in concave gap to 2.5 units and an increase in rotor speed to 600 rpm resulted in a higher germination percentage (Figure 4.4a). However, this result contrasts with increased leachate conductivity (Figure 4.4c) and increased fragmented seeds (Figure 4.4d), which both indicate greater physical damage as a result of these altered settings. A possible explanation is that the higher rotor speed and decreased concave gap increased the likelihood of splitting of weak or misshapen seed in the sample, resulting in a higher percentage of good seed in the samples following cleaning (but slightly lower seed yield), along with a higher percentage of seed fragments. The results indicate that small improvements in seed quality may be possible through changes in harvester settings, but the limited replication used in the trial warrant that caution be taken when interpreting the data. Each setting was replicated 4 times at one site. Further replication at a number of sites would be required to confirm the findings of this preliminary study and to determine to extent of the changes in seed quality associated with altering rotor speed and concave gap on the International 1420.

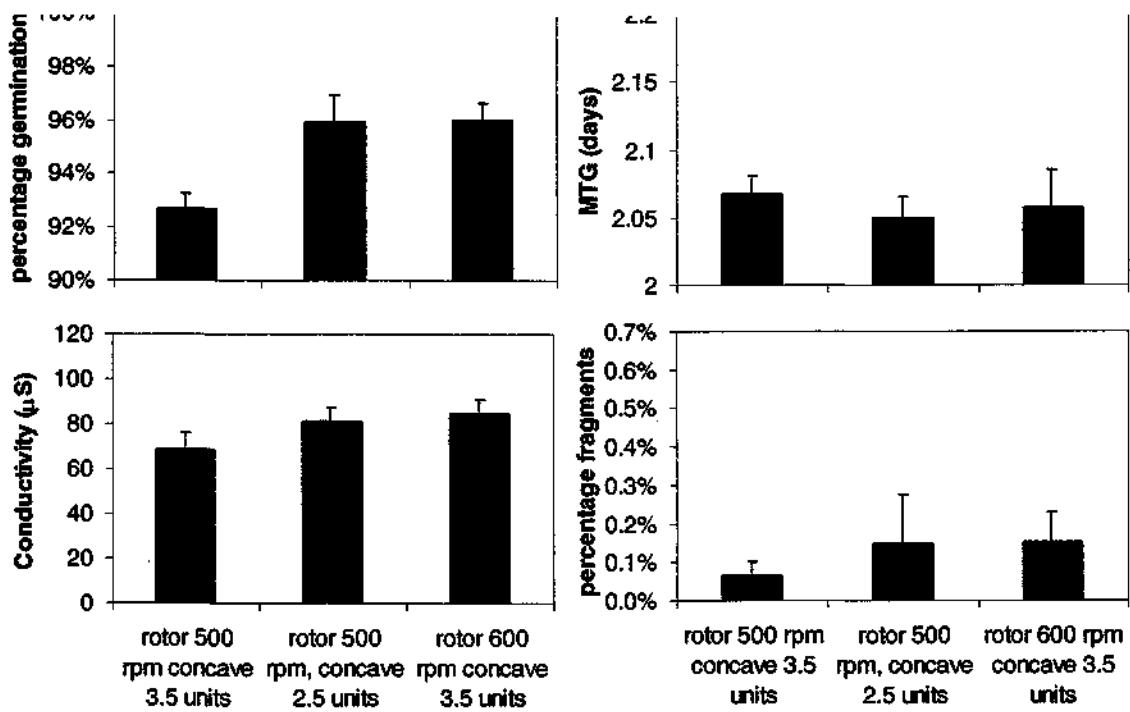


Figure 4.4 Cabbage seed samples were collected from the bin after modification of settings (rotor speed and concave gap) to the International 1420 during harvest. Seed quality was recorded as (a) percentage germination, (b) mean time to complete germination, (c) leachate conductivity and (d) percentage (by weight) of fragmented seeds. Mean values \pm standard error bars ($n=4$) shown.

A rotor speed of approximately 680 rpm represented the current commercial setting on the Allcrop combine harvester. The current commercial setting of the concave was not recorded. No significant difference in percentage germination was recorded with either an increase or a decrease in concave gap of 1mm from the commercial setting, or an increase (810 rpm) or a decrease (600 rpm) in rotor speed (Figure 4.5a). The increase in proportion of fragmented seeds with increasing the rotor speed suggests an increase in seed damage associated with higher rotor speed (Figure 4.5d). A decrease in level of fragmented seeds was recorded when the concave gap was increased by 1mm. However, neither of these two results was reflected in the level of leachate conductivity or MGT. Given the inherent variability in seed quality within commercial crops further replication of the different settings would also be required.

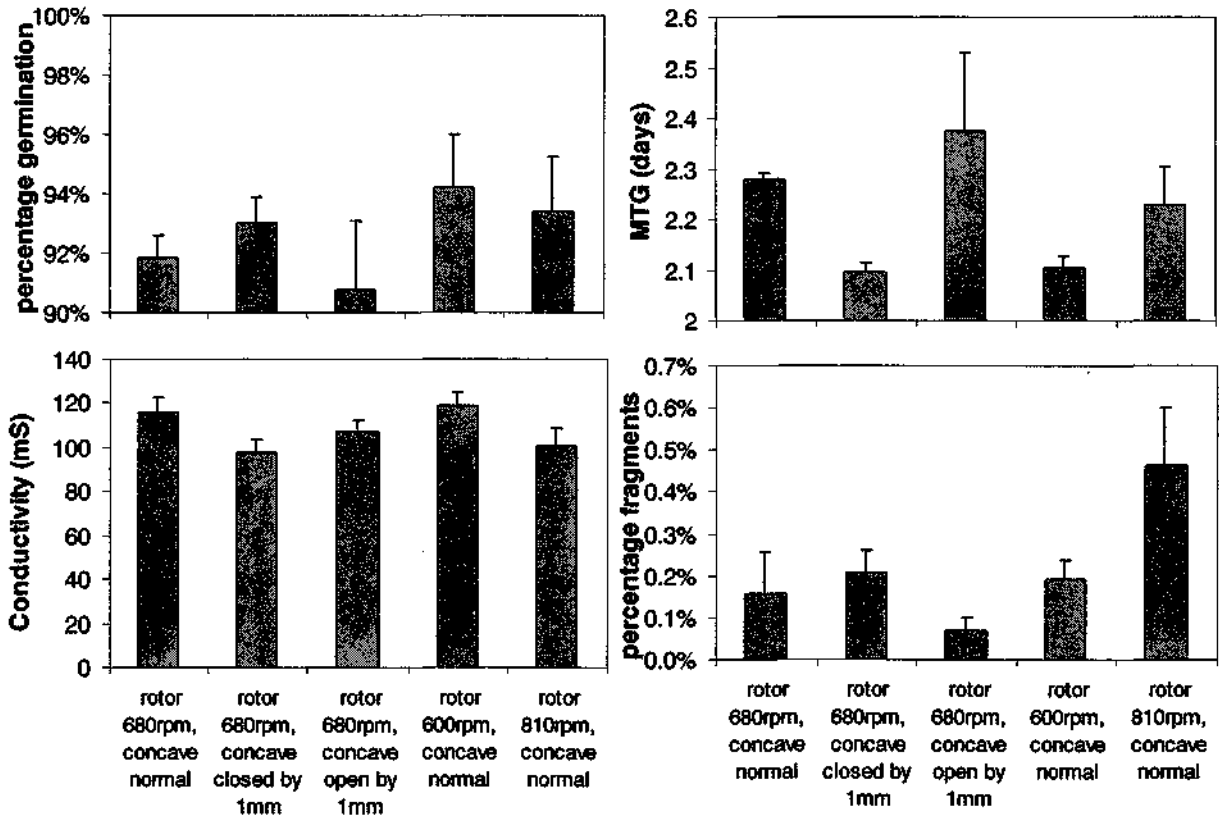


Figure 4.5 Cabbage seedlots were collected from the bin after modification of settings (rotor speed and concave gap) to the Allis Chalmers Allcrop during harvest. Seed quality was recorded as (a) percentage germination, (b) mean time to complete germination, (c) leachate conductivity, and (d) percentage (by weight) of fragmented seeds. Mean values \pm standard error bars (n=4) shown.

The results from the preliminary studies of levels of seed damage in different positions in the International 1420 harvester and effects of harvester settings on levels of seed damage provide useful data for defining areas for further investigation. Small improvements in seed quality and reduction in level of seed damage may be possible through changes in harvester settings. Replication of the experimental designs used in this preliminary study across a number of crops would allow the identification of the major damage point in the harvester to be confirmed. This information, along with the preliminary data presented above on different harvester settings, would allow harvester settings likely to cause less damage to seed to be identified and tested.

4.4 Conclusions

The results from this study indicate that the rotary combine harvester (International 1420) was superior to the conventional combine harvester (Allcrop) in terms of harvested seed quality and damage levels. Seedlots harvested with the Allcrop had a significantly lower germination level than hand harvested seedlots. While the germination rate of seed harvested with the Allcrop was lower than that harvested with the International 1420, the difference was not statistically significant. The low level of replication within crops, combined with sampling from only three crops, lead to a low likelihood of statistically significant differences between harvesters being detected. Further studies with increased replication are recommended if statistical significance in differences between harvesters needs to be proven. The study generated strong evidence of lower seed quality and increased seed damage in seedlots harvested with the Allcrop. The higher leachate conductivity, lower mean time to complete germination and higher level of fragmented seed pieces found in seedlots harvested with the Allcrop indicate greater physical damage and

lower seed quality. When these results are considered in combination with the germination percentages, sufficient evidence exists to conclude that performance of the International 1420 was superior to that of the Allcrop.

Preliminary investigation of position within the International 1420 harvester where seed may be damaged and effects of altered rotor speeds and concave gap on seed quality were also undertaken. The major site of the loss of germination within the harvester was between rotor and the screens. Small improvements in seed quality were obtained through changes in harvester settings, possibly as a result of splitting of weak seed which was subsequently removed during cleaning, but the limited replication used in the trial warrant that caution be taken when interpreting the data. Replication of the experimental designs used in these preliminary studies across a number of crops would allow the identification of the major damage point in the harvester to be confirmed. This information, along with the preliminary data presented on different harvester settings, would allow harvester settings likely to cause less damage to seed to be identified and tested.

In this study seed was tested within one month following harvesting. Seedlots from the trials undertaken are being stored to assess the impact of physical damage during harvest on the rate of seed deterioration.

Chapter 5. Radiata pine seed quality

5.1 Introduction

Demand for pulp and timber has led to the development of large areas of plantation forest across Australia. The North American species *Pinus radiata* (radiata pine) is one of the major plantation species. Plantations are established from nursery transplants usually produced from seeds. Breeding programs for improved growth form, timber qualities and disease resistance have been developed. There is growing interest in using vegetative propagation to produce nursery transplants, but at present and into the immediate future seeds remain the most common method of propagating improved material. The seeds are generally produced in open- or wind-pollinated (OP) seed orchards. However, control-pollinated (CP) seeds, with known parentage, are increasingly being used.

Control-pollinated seedlots are expensive to produce due to the labour-intensive nature of the process. There are concerns that the genetic gain, in terms of wood production, achieved through breeding may be obtained at the expense of quality germination characteristics. In addition to CP seedlots, some open-pollinated seedlots have shown low and variable germination in field nurseries. Generally, laboratory germination tests have indicated that seedlots in current use in nurseries exhibit high viability. However, as the species is a temperate conifer, there is the distinct possibility that dormancy will be exhibited by some seedlots.

Good nursery management results in 60 to 70 % of the seeds sown producing useable trees in the nursery (Boomsma, pers. comm.). There exists, therefore, the potential to substantially improve production. This is particularly a concern with increasing seed cost. The traditional approach to overcome dormancy and seedlot germination deficiencies in temperate conifer species is to apply a pretreatment such as stratification to the seed prior to sowing in the nursery. Stratification has been applied to radiata pine seeds in nurseries in Australia in the past. However, due to difficulties in applying the treatment and concerns regarding effectiveness, the practice of presoaking has become more prevalent in preference to stratification. At present the most commonly used seed pretreatment is to soak seeds for a short period (48 hours or less) prior to sowing. There is currently a lack of knowledge about how pretreatments influence the success of germination and early growth in the field nursery.

Uncertainty exists over the dormancy status of radiata pine seeds, but Grose (1958) observed that radiata pine seeds did not germinate readily and concluded that a degree of dormancy was present. Krugman and Jenkinson (1974) recommended stratifying radiata pine seeds for 35 to 45 days, and Minko and Craig (1976) reported that stratification was the standard pre-sowing treatment for radiata pine seeds in North-Eastern Victoria. In spite of evidence favouring stratification, Rimbawanto et al. (1988, 1989) reported that stratification did not significantly affect the germination of radiata pine seed, but it should be noted that these authors only stratified for one week.

Moist radiata pine seeds stratified for sufficiently long periods of time will eventually germinate at stratification temperature, as the seeds move from phase II to phase III of imbibition. During phase II, at low temperature, germination processes are able to proceed, leading to hastened radicle emergence once seeds are transferred to conditions more suitable for germination (Downie et al. 1997b). Levitt and Hamm (1943) speculated that it may be possible to supply seeds with sufficient moisture to enable them to undergo the processes associated with germination occurring in phase II but insufficient to permit germination. Wilson (1971, 1973)

applied this principle by imbibing crested wheatgrass (*Agropyron desertorm* Fisch. ex Link) seeds in vapour over solutions and observed that the seeds germinated more rapidly than untreated seeds. Downie and Bergsten (1991) and Jones and Gosling (1994) took a different approach and supplied eastern white pine; and Douglas-fir, lodgepole pine and sitka spruce seeds, respectively, with a known quantity of water to bring them to the required moisture content. The treated seeds germinated more rapidly than did the controls. The desired seed moisture content for chilling is at, or near, the plateau moisture content of phase II.

Pretreatments that limit moisture availability in this way share the principle with priming, which is often applied to vegetable seeds to improve germination. Simak (1985) stated that pretreating Scots and lodgepole pine seeds by controlling moisture content during incubation was at least as efficient as priming in PEG, and was easier to apply and less risky to the seeds as extra aeration need not be supplied. The optimal moisture content range for incubating Scots and lodgepole pine was found to be slightly less than the moisture content obtained by priming seed in PEG.

The objectives of the present study were to investigate pretreatment effects on the germination and imbibition of radiata pine seeds.

5.2 Improvement of germination by stratification

5.2.1 Introduction

The objectives of this study were to examine the effect of stratifying for up to 12 weeks using two stratification methods on germination of a range of seedlots in the incubator and glasshouse. Seedlings were grown in the glasshouse to determine the effect of stratification on seedling growth rates.

5.2.2 Materials and Methods

Four seedlots were selected for use in the stratification trial (Table 5.1). The seedlots showed a range of germination behaviour and were selected so that the effect of stratification treatment on a range of germination characteristics could be examined.

Table 5.1 Seedlots selected from preliminary germination test for use in the stratification trial.

	Seedlot	Pollination
<i>a</i>	STBA Level 1, 4 - 5 mm, 1995	OP
<i>b</i>	STBA Level 1, 4 - 5 mm, 1996, heat damaged	OP
<i>c</i>	51246 x 51201	CP
<i>d</i>	51231 x 51235	CP

Stratification

The experiment was designed as a 2 x 4 x 5 factorial with 4 replicates, where the 3 factors were the method of stratification, seedlot and the duration of stratification respectively. The treatments are detailed in Table 5.2 below. Stratification was carried out on samples of 50 seeds.

Table 5.2 Stratification treatments applied to seeds in the present study

<i>Laboratory stratification method</i>	<i>Bulk stratification method</i>
1. 50 seeds placed in each petri dish containing 3 filter papers and 5 ml distilled water	1. 50 seeds soaked in 20 ml distilled water in plastic bags for 48 hours at 5 ° C
2. Chilled at 5 ° C for 0 (control A), 2, 4, 8, or 12 weeks	2. After 48 hours, soak water drained and seed sealed in fresh plastic bags
3. Petri dishes weighed periodically and water replaced where necessary	3. Chilled at 5 ° C for 0 (control B), 2, 4, 8 or 12 weeks

Germination

Seeds were germinated at 20 ° C under standard conditions (ISTA 1999). Treatments were compared using FG and MGT (Appendix 1). Selected treatments were also sown in potting mix in seed trays in the glasshouse. The selected treatments were seedlots *a* and *b* stratified using both methods for 0, 2 or 4 weeks. Seedlings were considered emerged once the seedcoat had been lifted above the soil surface. Emergence counts were made daily for a 3 week period starting with the emergence of the first seedling for each plot. Plots were harvested individually, 3 weeks after the first seedling of that plot emerged. The harvested seedlings were dried in a drying oven at 70 ° C and weighed daily until constant weight was achieved. Comparisons between treatments were made using mean dry weight.

Seed water potential

Water potential (Ψ) of the seed at the completion of treatment was measured with a thermocouple psychrometer. As Ψ measurement is non-destructive, the seeds were returned to their respective samples after measurement.

Statistical analysis

Results were analysed using ANOVA. The analysis was carried out using general linear models in the FASTAT package (Systat Corp.). Means were compared using Least Significant Difference (LSD) (Steele and Torrie 1980).

5.2.3 Results and Discussion

The stratification treatments applied in this study were found to benefit the germination of the radiata pine seedlots tested. Germination was enhanced by an increased rate of germination and higher germination percentage of some seedlots, including the deteriorated seedlot (Table 5.3). The longest duration of treatment (12 weeks) led to the most rapid germination. The improved germination of the deteriorated seedlot suggests either that repair processes proceeded within the seed, secondary dormancy was overcome during stratification, or more rapid germination enabled a greater proportion of the seedlot to germinate within the test period. Pre-soaking for 48 hours (control treatment B) reduced the germination percentage and time to germination of two seedlots. This soak injury was overcome when pre-soaking was followed by stratification.

Table 5.3 FG and MGT of seeds of four seedlots stratified using two methods for 0, 2, 4, 8 or 12 weeks. Each value is the mean of four replicates of 50 seeds. For comparisons within and between tables LSDs ($\alpha = 0.05$) are 14.2 and 2.6 for FG and MGT, respectively.

5.3(a) Bulk-stratified seed

Seedlot	Duration	FG (%)	MGT (days)
<i>a</i>	0	84.8	18.3
	2	86.1	12.1
	4	90.9	10.1
	8	79.7	9.8
	12	94.9	10.3
<i>b</i>	0	18.4	22.8
	2	51.5	18.0
	4	49.5	18.1
	8	58.7	15.6
	12	73.4	12.3
<i>c</i>	0	53.5	20.4
	2	68.6	15.6
	4	77.9	12.8
	8	72.8	9.9
	12	88.0	8.0
<i>d</i>	0	87.6	20.5
	2	94.4	12.2
	4	95.8	10.8
	8	85.7	12.6
	12	91.3	8.8

5.3(b) Lab-stratified seeds

Seedlot	Duration	FG (%)	MGT (days)
<i>a</i>	0	87.6	18.1
	2	94.9	14.4
	4	90.9	13.8
	8	95.0	10.1
	12	95.4	8.6
<i>b</i>	0	42.8	19.2
	2	65.4	17.2
	4	42.7	19.6
	8	61.2	15.0
	12	63.9	14.2
<i>c</i>	0	73.3	17.7
	2	83.1	13.4
	4	82.8	11.1
	8	79.2	9.3
	12	88.2	7.4
<i>d</i>	0	97.4	17.5
	2	97.4	13.0
	4	97.2	15.1
	8	95.4	10.0
	12	96.9	7.9

Mean seedling dry weight after 3 weeks of growth was not significantly affected by stratification despite the decreased time to germination. This suggests seedling growth rates were not improved by treatment. This result is consistent with that reported by Barnett and McLemore (1984) who found that although growth rates were not improved, pine seedlings grown from stratified seeds emerged earlier and hence had a longer growing season before harvest date than later-emerging unstratified seedlings. Consequently, the seedlings from stratified seeds were larger at harvest.

Generally, Ψ_s was higher after longer periods of stratification (Table 5.4). However, in some cases seeds stratified for 4 weeks had significantly lower Ψ_s than those stratified for 2 weeks, after which Ψ_s began to rise. The Ψ_s of seedlots changed little between weeks 8 and 12 when bulk stratified, but the drop was more substantial when lab stratified. These results suggest further investigations into seed water potential during stratification are required.

Split seeds were observed in the samples stratified for 12 weeks, particularly those stratified using the laboratory method. However, the method of stratification had little effect on Ψ_s or germination. This suggests that the seeds were able to absorb sufficient water during the soak to enable the embryo to reach positive turgor during stratification, given enough time. It is possible that the seedcoat became hydrated very quickly, and then acted as a reservoir for the tissues contained within, as Schneider and Renault (1997) reported in soybean seeds. Seedlot *c* had the greatest percentage of split seeds at the end of treatment.

Table 5.4 Ψ_s (MPa) of seeds of four seedlots after stratification using two methods for 0, 2, 4, 8 or 12 weeks. Each value is the mean of four replicates of 15 seeds. LSD is 4.5 ($\alpha = 0.05$).

Seedlot	Duration	Lab stratified	Bulk stratified
A	2	-13.4	-9.3
	4	-24.8	-17.6
	8	-14.9	-2.5
	12	-4.0	-2.3
B	2	-4.6	-5.3
	4	-6.2	-20.7
	8	-6.9	-2.6
	12	-2.0	-2.3
C	2	-3.9	-4.1
	4	-3.8	-3.0
	8	-5.7	-1.4
	12	-0.5	-2.1
D	2	-9.0	-9.4
	4	-13.8	-13.2
	8	-8.5	-2.7
	12	-1.2	-2.7

Rising Ψ_s and seed splitting suggests that the hydration of the seeds increased during stratification. Stanley (1958) and Schneider and Gifford (1994) found that the water content of sugar and loblolly pine seeds, respectively, increased during stratification and the increase in water content correlated well with increased germinability. Germination is thought to occur in the range of approximately -2 to 0 MPa (Bradford 1995). It is clear that some of the seeds in the samples stratified for 12 weeks were within this range as split seed coats were observed. The reduced MGT of stratified seeds may have been the result of the increased level of hydration, as less time was required for imbibition once the seeds were placed in conditions suitable for germination.

Although stratification proved to be a beneficial treatment by decreasing the time to germination and increasing the percentage germination of seedlots with relatively poor germination, this experiment highlighted the problems long associated with stratification, in particular premature germination. The splitting of the seed coat seen in the longer stratification treatments may present difficulties if stratified seeds were to be sown in a nursery. While radicles did not emerge, the Ψ_s readings and observation of split seed coats suggests that this would have occurred had stratification been prolonged. This presents a problem if, for example, sowing must be delayed due to bad weather. Seed coat splitting also increases the susceptibility of the embryo to drying out while sowing.

5.3 Cold hydropriming to improve germination

6.3.1 Introduction

The present study combined chilling and hydropriming treatments to limit water uptake by radiata pine seeds held at low temperature. The objective was to allow seeds to benefit from pretreatment but to prevent germination by controlling Ψ of the atmosphere in which the seeds were held, in order to avoid soak injury and premature germination associated with prolonged stratification.

5.3.2 Materials and Methods

Experimental procedure and results are reported fully in Donovan (2001).

The experiment was a 4 by 5 factorial with 4 replicates. Seeds (seedlot *STBA Level 1, 4 - 5 mm*) were chilled for 4, 8, 12 or 16 weeks at 5 °C over NaCl solutions with water potentials of 0, -3, -6, -9 and -12 MPa at 5 °C. Samples of 100 seeds were chilled in 150 ml sealed plastic containers. The seeds were held on a mesh shelf above 50 ml of the appropriate solution.

The containers were held at 5 °C for 4, 8, 12 or 16 weeks. Three additional containers of each treatment were placed in the cold room with the 16 week treatment. Seed water potential (Ψ_s) and moisture content were measured on these seeds. Ten seeds were sampled from each of the 3 replicates after 1, 3, and 7 days of chilling and thereafter at weekly or fortnightly intervals. Water potential was measured using the psychrometer. Moisture content was determined following Ψ_s measurement using ISTA (1999) guidelines, and expressed as a percentage of the dry weight of the ten seeds.

Following chilling, the seeds were removed from containers and the number of split and germinated seeds recorded. Seeds with fungal contamination were discarded. Seeds were germinated according to the rules of the ISTA (1999).

Treatments were compared using FG and MGT (Appendix 1). Results were analysed using ANOVA. The analysis was carried out using general linear models in the FASTAT package (Systat Corp.). Means were compared using Least Significant Difference (LSD) (Steele and Torrie 1980).

5.3.3 Results and Discussion

Germination percentage was significantly affected by the length of stratification, seeds stratified for 12 weeks had significantly higher germination (96.4%) than those stratified for 4 (88.6%), 8 (91.6%) or 16 weeks (89.5%). Solution water potential had no effect on germination percentage.

Seeds treated with water potential of 0 or -3 MPa germinated much more rapidly than treatments with lower water potential, provided that the treatment was longer than 4 weeks (Figure 5.1). Twelve weeks of treatment led to earlier germination seeds treated for shorter periods of time. However, when chilling was extended to 16 weeks, the time to germination was increased except at Ψ_{sol} of 0. This suggests that seed quality began to deteriorate between 12 and 16 weeks of chilling, and is supported by the decrease in germination percentage observed after 16 weeks

of treatment. Hegarty (1978) discussed deterioration of seeds at low Ψ , and speculated that below Ψ of about -5 to -8 MPa seed activation and repair mechanisms cannot operate effectively and processes of deterioration dominate.

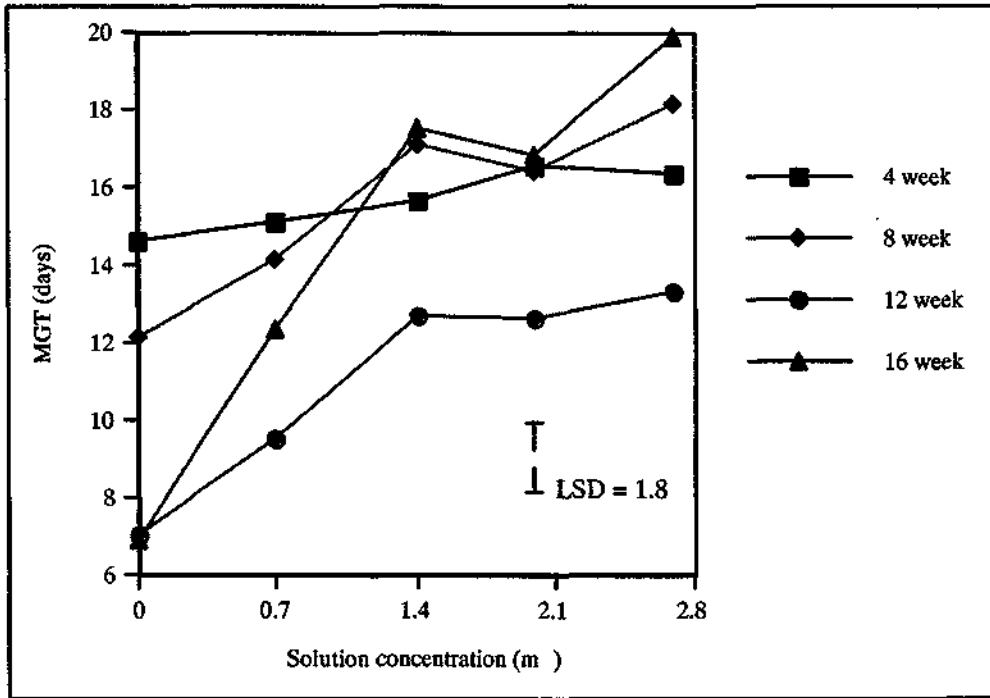


Figure 5.1 Mean germination time (MGT) of seeds chilled for 4, 8, 12 or 16 weeks over 0, 0.7, 1.4, 2.0 and 2.7 m NaCl solutions. Each point is the mean of 4 replicates of 50 seeds. The LSD is given at the lower right corner of the graph.

All seeds showed a rapid rise in water potential when placed in chilling containers (Figure 5.2). Solution water potential had little effect on the rate of increase until 7 days of chilling had passed. By the second week of chilling the rate of change had slowed substantially and any further rise in water potential occurred at a more gradual rate. Most treatments showed a small decline in Ψ_s between weeks 6 and 8. Seeds chilled over solutions of -6, -9 and -12 MPa showed a second rapid increase in Ψ_s between weeks 13 and 16. Moisture content, like Ψ_s , initially rose at a rapid rate until about 2 weeks of treatment at which time the rate of uptake slowed. Maximum moisture content during chilling was strongly influenced by the Ψ_{sol} . There was little difference in the moisture content of seeds chilled over solutions of -6 MPa or less, but those chilled over 0 or -3 MPa solutions showed a substantially higher moisture content which continued to rise during treatment. However, there is some indication of a decrease in moisture content in most treatments concurrent with the drop in Ψ_s between weeks 6 and 8. Hallgren and Wu (1995) discussed preliminary results that showed declining loblolly pine Ψ_s during priming. In the case of Hallgren and Wu (1995), the decline was from -1.0 to -2.0 MPa, and was explained as osmotic adjustment to maintain water flow into the embryo. Seed moisture content increased from 70 to 90% over the same period. However, in the present study moisture content declined during the period of decreasing Ψ_s . Dry weight did not decrease during treatment, which suggests that respiration rates were low enough to minimise utilisation of seed reserves.

Although water uptake had reached a plateau by 4 weeks, chilling for this length of time had little effect on subsequent germination. In contrast, the germination of seeds chilled for 12 weeks was substantially improved, even though the water potential of the seeds changed little during 8 weeks of further treatment. This suggests that improvements to germination are not the consequence simply of an advanced state of hydration. It suggests that when held at high

moisture content and low temperature for a prolonged period of time, the seeds are able to undergo processes that decrease the time to radicle emergence when the temperature is raised.

Seeds continued to take up water after 4 weeks of treatment, particularly those held over distilled water or a -3 MPa solution, even though there was relatively little change to seed Ψ after this time. The seeds chilled over distilled water or a -3 MPa solution reached a plateau at a Ψ_s below Ψ_{sol} . Continued water uptake can thus be explained by the presence of a Ψ gradient between the seed and the atmosphere. Seeds chilled over -6 and -9 MPa solutions reached a plateau at Ψ_s close to Ψ_{sol} . There was little further movement of moisture between the seed and the atmosphere once the plateau had been reached due to the absence of a Ψ gradient. The seeds chilled over -12 MPa solution reached a plateau at a Ψ_s above Ψ_{sol} . This suggests the presence of a Ψ_s gradient between the seed and its environment that would lead to the loss of moisture from the seed. There is little evidence of sustained moisture loss which suggests there are barriers to the movement of moisture out of the seed. Such barriers would be a selective advantage if periods of transient moisture stress were experienced during seed germination in the natural environment.

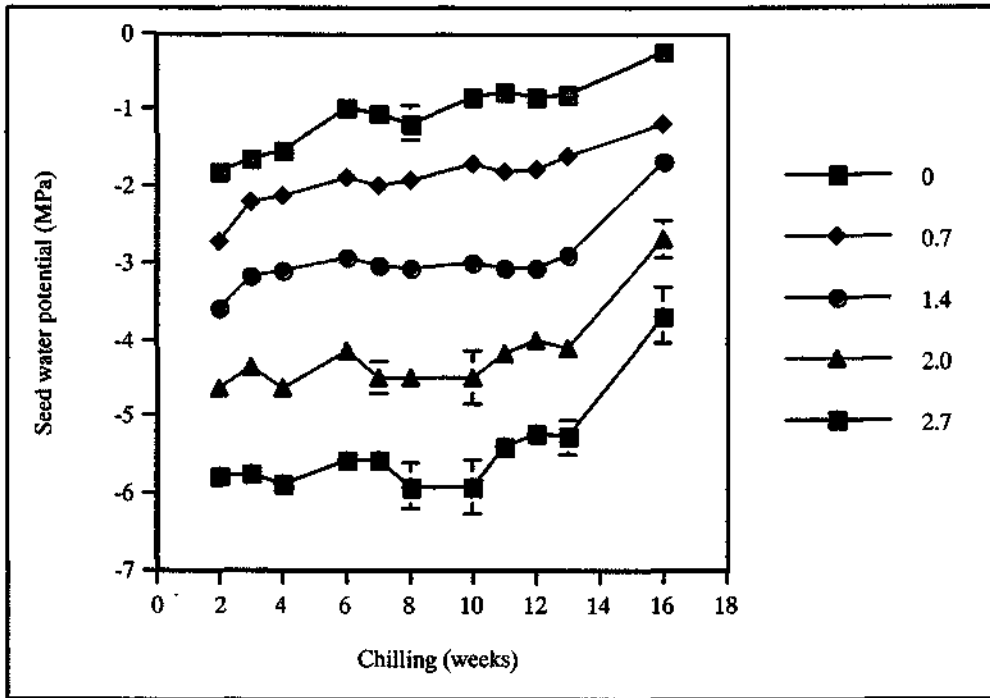


Figure 5.2 Ψ_s of seeds chilled over 0, 0.7, 1.4, 2.0 and 2.7 m NaCl solutions for 2 to 16 weeks. Each point is the mean of 3 replicates of 10 seeds. Standard error bars are shown where larger than the symbol.

The seeds chilled for 16 weeks over distilled water had a high percentage of split seeds present at the end of treatment ($12.5 \pm 2.10\%$). Some of the seeds of this treatment ($2 \pm 1.08\%$) had germinated after 16 weeks of treatment, but germination was prevented in all other treatments. Stratification at controlled high relative humidity therefore gave the seeds the benefits of stratification while preventing them from proceeding with germination to the point of embryo expansion. Only the seeds suspended over distilled water showed any signs of germination. It is concluded that seeds held over NaCl solutions -3 MPa and below equilibrated at moisture contents below that required for embryo expansion.

5.4 Hydropriming at 5 °C and 20 °C to improve laboratory germination and field emergence

5.4.1 Introduction

Earlier work demonstrated that stratification improved radiata pine seed germination, and that limiting moisture availability during treatment (combining hydropriming and chilling) prevented seeds from germinating until sown. However, it remains to be seen if the treatment is effective in improving germination in the field nursery, and in the laboratory at sub-optimal temperature. In this study optimal treatments from the previous trial (12 weeks chilling while hydropriming at 0 and -3 MPa) were compared with hydropriming at 20 °C for 1 or 2 weeks, to determine whether or not chilling contributes to the treatment effect. The effect of storing the treated seeds for 4 weeks with or without redrying was also examined.

A range of seedlots was used as conifer species show a strong genetic effect on germination. Seed quality varies with family, the effect of treatment would therefore be expected to vary with family. Consequently, CP seedlots were considered to be more useful than OP seedlots.

5.4.2 Materials and Methods

Three preliminary trials were carried out. Germination of 10 seedlots was tested at 20 °C to select 4 seedlots with different germination behaviour to test seed pre-treatments. The selected seedlots are given in Table 5.5. The second trial involved priming one seedlot in an atmosphere of 0 MPa (100% RH) at 20 °C. Moisture content was examined periodically to determine the time taken to reach phase II of water uptake. The two shorter treatments were set around this time. The third trial examined loss of moisture from fully imbibed seeds when dried in forced air at 20 °C. It was found that 24 hours drying time reduced seed moisture content to less than 10%, the moisture content at which radiata pine seeds are commonly stored.

Table 5.5. Seedlots selected from preliminary trial for use in main trial

Seedlot		FG	MGT
STBA Level 1 1996; heat affected	OP	83.0	15.6
STBA 38040	CP	95.7	11.9
STBA 99.02	CP	64.4	15.3
STBA 99.06	CP	94.1	11.6

The main trial was designed as an incomplete factorial experiment. The trial was a 4 (seedlot) by 2 (treatment temperature) by 2 (water potential) by 3 (times) factorial with 4 replicates. Treatments are given in Table 5.6. The 12 week/20 °C, and 1 week/5 °C treatments were excluded due to the likelihood of little benefit to seeds. An external untreated control for each seedlot was included (Treatment 9).

Table 5.6. Seed pre-treatments used in this study. The empty boxes indicate excluded treatments.

	5 degrees		20 degrees	
	0MPa	-3MPa	0MPa	-3MPa
1 week			Treatment 7	Treatment 8
2 weeks	Treatment 3	Treatment 4	Treatment 5	Treatment 6
12 weeks	Treatment 1	Treatment 2		

Samples of 225 seeds were set up in replicates of 250 ml screw top plastic containers containing 80 ml of the solution (distilled water or -3 MPa NaCl solution), with a plastic mesh shelf over it

holding the seeds. The seeds were held in a nylon mesh bag on the shelf above the solution. The containers were placed in a completely randomised design in 4 ° C or 20 ° C rooms for the required length of time.

Seeds were germinated in the laboratory in standard conditions (ISTA 1999) at 10 ° C and 20 ° C. Daily germination counts were carried out for 28 days. The germination test at 10 ° C was extended to 56 days as very little germination had occurred by 28 days. Treatments were compared using FG and MGT (Appendix 1).

Seeds were sown in the field nursery at Meadowbank in the Derwent Valley in southern Tasmania in 4 replicates, each consisting of 1m of row, with seedlings spaced 4 cm apart (25 seeds per experimental unit). The trial was hand sown in one bed divided into 6 rows. The outer 2 rows were sown with untreated seeds as buffers. The bed was covered to protect the seeds from birds. Emergence counts were made every second day until no new seedlings were counted on 3 consecutive dates. Cumulative percentage germination was plotted.

Samples of each seedlot and treatment, with or without redrying, were stored 4 weeks at 4 ° C after treatment. The re-dried seeds were dried at 20 ° C in forced air for 24 hours, then transferred to 4 ° C. The samples were germinated at 20 ° C under standard conditions.

5.4.3 Results and Discussion

The results suggest that the combination of cool temperatures and water uptake are required for successful pre-treatment of radiata pine seeds. Although the response varied between seedlots, the 12 week treatment at 5 ° C was the most beneficial to germination (Table 6.7). Seeds germinated more rapidly and, in some cases, to a greater percent when germinated at 20 ° C after this treatment. The effect was also apparent in the seeds sown in the nursery (Figures 5.3- 5.6). The treatment was substantially more beneficial when seeds were allowed unlimited water (0 MPa) compared to those with restricted moisture (-3 MPa). Two weeks treatment at 5 ° C had very little effect on germination, except in the case of seedlot 99.02, where germination was negatively affected by the treatment. The treatments carried out at 20 ° C generally slightly decreased the time to germination, but had little effect on germination percentage.

Table 5.7. Mean germination percentage and mean germination time of pre-treated seeds germinated at 20 ° C.

	99.02		Level 1 1996		38040		99.06	
	FG	MGT	FG	MGT	FG	MGT	FG	MGT
12 weeks, 5 °, 0 MPa	70.8	6.5	81.2	10.0	66.7	4.8	97.0	4.1
12 weeks, 5 °, -3 MPa	61.5	13.0	81.5	13.7	71.1	11.6	92.0	8.9
2 weeks, 5 °, 0 MPa	50.4	16.7	75.3	15.4	71.6	12.2	96.5	10.3
2 weeks, 5 °, -3 MPa	48.4	17.0	76.5	15.8	71.7	12.4	95.5	10.7
2 weeks, 20 °, 0 MPa	42.6	12.6	80.1	13.8	70.4	12.5	96.6	9.5
2 weeks, 20 °, -3 MPa	41.6	12.8	72.4	13.9	82.0	11.6	94.9	10.0
1 weeks, 20 °, 0 MPa	52.1	13.0	74.3	14.6	66.7	11.3	89.1	10.4
1 weeks, 20 °, -3 MPa	63.1	14.0	76.5	14.5	79.1	12.4	94.5	9.9
Control	56.1	14.7	76.8	15.2	64.7	13.0	94.9	11.4

The germination test at 10 ° C demonstrated that radiata pine seeds have a relatively narrow range of optimal germination temperatures. The time to germination was greatly increased by dropping the germination test temperature from 20 ° C to 10 ° C (Table 5.8). Percentage germination was also greatly reduced, and it was apparent from daily germination counts that this was due to a decrease in the number of seeds able to germinate rather than an increase in the time to germination. The 12 week treatment at 5 ° C greatly reduced the time to germination at

low temperature and increased the percentage germination. However, the improvement in germination, particularly in germination rate, was not as substantial for seeds with restricted moisture availability. Hydropriming at 20 °C had a substantial effect on the percentage of seeds able to germinate, but had very little effect on the time to germination.

Table 5.8. Mean germination percentage and mean germination time of pre-treated seeds germinated at 10 °C.

	99.02		Level 1 1996		38040		99.06	
	FG	MGT	FG	MGT	FG	MGT	FG	MGT
12 weeks, 5 °, 0 MPa	70.8	20.7	76.5	22.9	77.0	16.2	95.3	14.9
12 weeks, 5 °, -3 MPa	45.1	40.5	60.2	38.2	82.2	31.2	96.1	33.8
2 weeks, 5 °, 0 MPa	10.1	47.3	22.2	44.1	49.5	41.8	81.5	42.4
2 weeks, 5 °, -3 MPa	7.4	48.0	10.9	43.2	53.8	42.4	72.5	43.7
2 weeks, 20 °, 0 MPa	15.8	47.5	30.4	44.1	53.7	41.9	86.0	42.8
2 weeks, 20 °, -3 MPa	10.4	42.7	31.6	40.0	59.5	42.5	73.9	42.5
1 weeks, 20 °, 0 MPa	8.2	45.8	21.8	42.8	39.0	43.6	67.5	44.2
1 weeks, 20 °, -3 MPa	9.5	48.0	21.6	43.6	53.0	43.7	67.1	43.6
Control	4.5	46.6	10.3	44.2	44.2	45.1	44.4	45.5

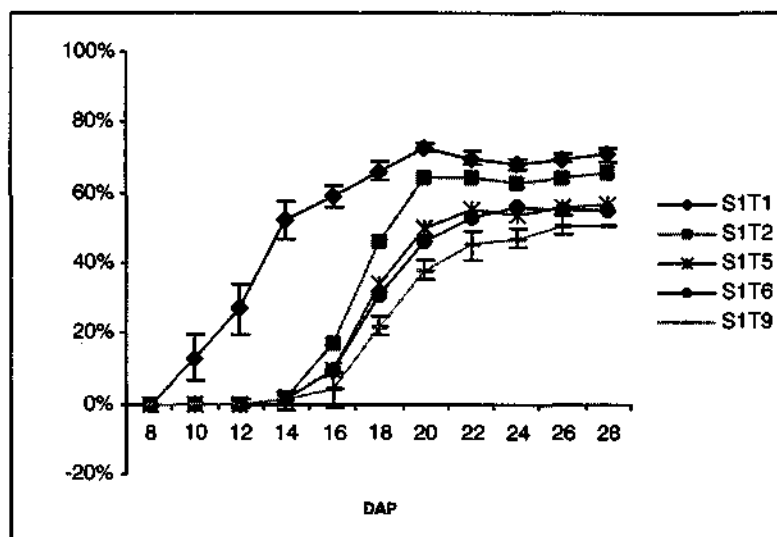


Figure 5.3. Percentage emergence of seedlings of pre-treated seedlot 99.02 in the nursery. Pre-treatments shown are: 12 weeks at 5 °C and 0 MPa (S1T1); 12 weeks at 5 °C and -3 MPa (S1T2); 2 weeks at 20 °C and 0 MPa (S1T5); 2 weeks at 20 °C and -3 MPa (S1T6); and untreated seeds (S1T9)

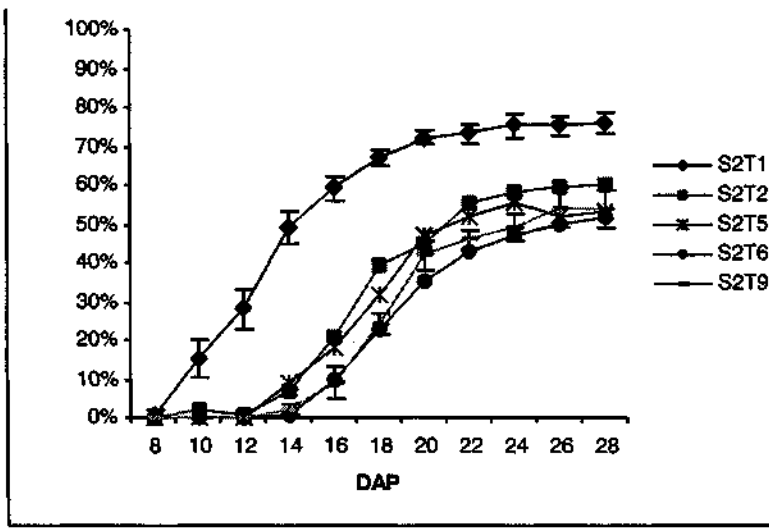


Figure 5.4. Percentage emergence of seedlings of pre-treated seedlot Level 1 1996 in the nursery. Pre-treatments shown are: 12 weeks at 5 °C and 0 MPa (S2T1); 12 weeks at 5 °C and -3 MPa (S2T2); 2 weeks at 20 °C and 0 MPa (S2T5); 2 weeks at 20 °C and -3 MPa (S2T6); and untreated seeds (S2T9)

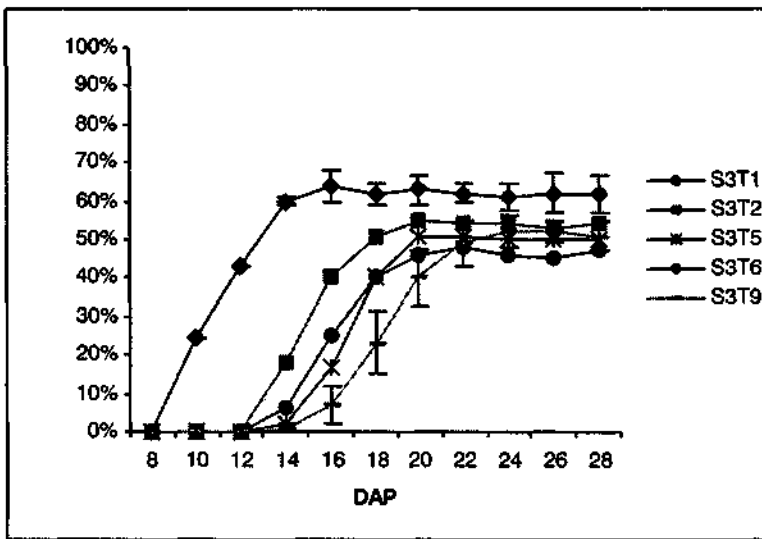


Figure 5.5. Percentage emergence of seedlings of pre-treated seedlot 38040 in the nursery. Pre-treatments shown are: 12 weeks at 5 °C and 0 MPa (S3T1); 12 weeks at 5 °C and -3 MPa (S3T2); 2 weeks at 20 °C and 0 MPa (S3T5); 2 weeks at 20 °C and -3 MPa (S3T6); and untreated seeds (S3T9)

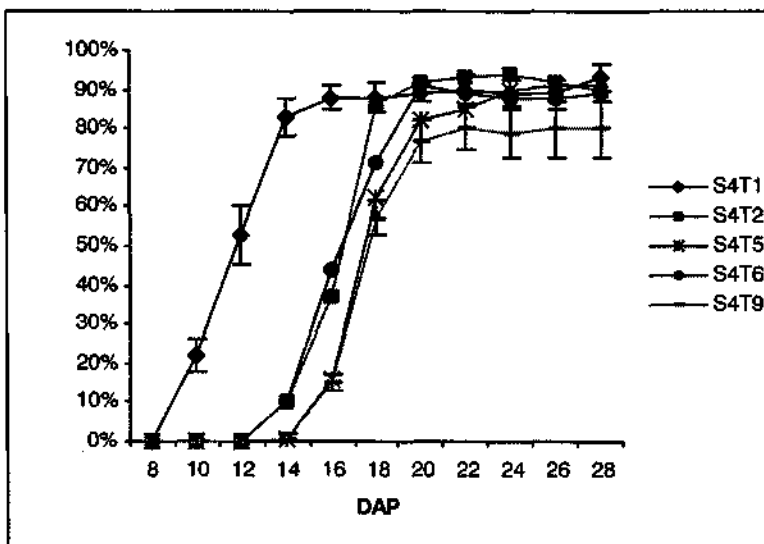


Figure 5.6. Percentage emergence of seedlings of pre-treated seedlot 99.06 in the nursery. Pre-treatments shown are: 12 weeks at 5 °C and 0 MPa (S4T1); 12 weeks at 5 °C and -3 MPa (S4T2); 2 weeks at 20 °C and 0 MPa (S4T5); 2 weeks at 20 °C and -3 MPa (S4T6); and untreated seeds (S4T9)

Re-drying after treatment

Storing seeds for 4 weeks at 4 °C had little effect on seed germination following pre-treatment (Table 5.7 and Table 5.9). Seeds stored after 12 weeks at 4 °C at 100% relative humidity (0 MPa) generally had higher germination percentage and a shorter time to germination following 4 weeks storage. However, seeds treated at 20 °C generally showed no benefit from 4 weeks storage, and in some cases (for example, seedlot 38040 treated for 1 week at 20 °C and -3 MPa) germination percentage was dramatically reduced when pre-treatment was followed by storage.

Table 5.9. Mean germination percentage and mean germination time of seeds removed from treatment and stored in sealed plastic bags at 4 °C for 4 weeks.

	99.02		Level 1 1996		38040		99.06	
	FG	MGT	FG	MGT	FG	MGT	FG	MGT
12 weeks, 5 °, 0 MPa	79.1	5.8	89.5	6.3	83.4	3.7	96.2	3.7
12 weeks, 5 °, -3 MPa	64.6	11.5	73.7	14.0	72.9	12.0	93.5	10.8
2 weeks, 5 °, 0 MPa	53.3	14.3	83.5	14.7	74.3	11.9	94.1	10.9
2 weeks, 5 °, -3 MPa	40.8	15.6	71.4	17.1	72.8	13.5	92.9	12.2
2 weeks, 20 °, 0 MPa	40.6	12.7	77.5	14.2	61.6	9.6	91.0	11.6
2 weeks, 20 °, -3 MPa	35.3	14.3	71.1	16.4	69.1	12.4	89.4	11.2
1 weeks, 20 °, 0 MPa	55.6	14.2	73.2	15.1	77.5	9.1	84.7	9.4
1 weeks, 20 °, -3 MPa	47.6	13.7	66.2	16.6	48.5	15.2	84.4	12.3

Seeds redried following pre-treatment and stored for 4 weeks (Table 5.10) showed relatively little loss of pre-treatment effect compared with seeds germinated at 20 °C immediately after treatment (Table 5.7). The time to germination was generally longer in seeds dried and stored after pre-treatment. This was particularly evident in the seeds treated for 12 weeks at 5 °C and 0 MPa.

The re-drying treatment had little effect on seed germination following storage. There was little or no benefit to seeds in re-drying before storage, apart from seedlot 99.02. Seeds of this seedlot pre-treated at 20 °C had substantially higher germination percentages, but slower germination rates, when dried before storage compared with those placed in storage when moist.

Table 5.10. Mean germination percentage and mean germination time of seeds removed from treatment, dried in forced air at 20 °C for 24 hours, then stored in sealed plastic bags at 4 °C for 4 weeks.

	99.02		Level 1 1996		38040		99.06	
	FG	MGT	FG	MGT	FG	MGT	FG	MGT
12 weeks, 5 °, 0 MPa	71.4	11.5	83.8	11.0	76.0	8.7	97.3	6.8
12 weeks, 5 °, -3 MPa	63.5	13.5	76.5	14.5	74.0	12.7	94.7	12.4
2 weeks, 5 °, 0 MPa	57.8	16.5	67.3	17.2	73.5	13.6	93.5	13.6
2 weeks, 5 °, -3 MPa	54.3	16.9	69.6	16.4	61.2	15.6	94.0	12.7
2 weeks, 20 °, 0 MPa	53.4	15.1	60.9	16.0	65.5	14.1	93.9	12.4
2 weeks, 20 °, -3 MPa	57.3	14.5	70.8	17.3	75.9	14.8	93.9	12.0
1 weeks, 20 °, 0 MPa	51.2	16.6	64.2	16.4	57.5	16.2	89.2	13.5
1 weeks, 20 °, -3 MPa	66.5	14.6	73.3	17.4	77.5	14.6	90.5	13.2

These results indicate that pre-treating seeds for 12 weeks at 5 °C and 100% relative humidity will substantially increase the germination rate of radiata pine seedlots, and is likely to increase the percentage germination of pine seedlots with poor initial percentage germination, when sown in field nurseries. Storing for 4 weeks prior to sowing is unlikely to negate the pre-treatment effects. Re-drying before sowing had little effect on pre-treatment effect, and should not be considered necessary unless it improves seed handling.

5.5 Conclusions

Radiata pine seedlots sown in commercial nurseries occasionally show low and variable germination. Where control-pollinated seeds have been used, there are concerns genetic gains have been achieved at the expense of good germination characteristics. The traditional approach to improving the germination of temperate conifer species has been to stratify the seeds.

The traditional stratification treatment applied in this study increased rate of germination of the four seedlots tested. Higher germination percentage of some seedlots was also achieved by applying a stratification treatment. In experiments 5.2 And 5.3, seeds stratified for 12 weeks performed better than untreated seeds and those stratified for a shorter or longer period of time. Stratification was also found to overcome soak injury. Seeds of two seedlots that were pre-soaked for 48 hours had reduced time to germination and germination percentage. This soak injury was overcome when pre-soaking was followed by stratification. The growth rate of seedlings in the glasshouse was not increased by stratification, however, as stratified seeds germinated earlier than untreated seeds, it would be expected that stratified seeds would produce larger seedlings at harvest. However, although stratification proved to be a beneficial treatment, signs of pregermination during treatment were observed. This presents a problem if, for example, sowing must be delayed due to bad weather. Seed coat splitting also increases the susceptibility of the embryo to drying out while sowing.

Restricting moisture availability during treatment allowed seeds to benefit from treatment without risking them by bringing them to the point of germination. Only seeds chilled for 16 weeks at 100% relative humidity were able to germinate during treatment. It can be concluded that seeds held over NaCl solutions -3 MPa and below equilibrated at moisture contents below that required for embryo expansion. However, the seeds treated with water potential of 0 or -3 MPa were still able to germinate more rapidly than treatments with lower water potential, provided that the treatment was longer than 4 weeks, or untreated seeds in the first experiment.

Although seeds performed relatively well with 12 weeks of treatment at 0 or -3 MPa, the third trial revealed that only seeds treated at 100% RH (0 MPa) for this length of time performed substantially better than untreated seeds when sown in the field nursery or at the sub-optimal temperature of 10 °C in the laboratory. Pre-treating seeds for 12 weeks at 5 °C and 100% relative humidity substantially increased the germination rate of radiata pine seedlots, and increased the percentage germination of pine seedlots with poor initial percentage germination, sown in the field nursery. When germinated at 20 °C the effect of this treatment was not lost following 4 weeks storage, and there was relatively little effect of redrying before storage. Redrying should not be considered necessary unless it improves seed handling.

The germination test at 10 °C demonstrated that radiata pine seeds have a relatively narrow range of optimal germination temperatures. The time to germination was greatly increased and percentage germination decreased by dropping the germination test temperature from 20 °C to 10 °C. Hydropriming at 5 °C for 12 weeks overcame the effect of reducing the temperature to some extent, particularly when treated at 0 MPa.

Chapter 6. Recommendations

A number of recommendations have arisen from this project. Recommendations relate to the adoption by industry of outcomes from research on specific crops, further research to clarify issues arising from trials undertaken, and maintenance of seed research within the School of Agricultural Science and TIAR.

The establishment of a Seed Research Group within the School of Agricultural Science and TIAR has delivered benefit to industry by building and coordinating a research effort in seed quality at the University of Tasmania which is responsive to industry identified problems and which undertakes industry specific projects. A recommendation of the project is that the Seed Research Group be maintained into the future to encourage quality research to be continued.

Onion

A major priority for the Tasmanian onion seed industry is further research investigating the cause of abnormal seedlings and to what extent the expression of abnormality is governed by the germination environment.

Studies on the effect of time of harvest on onion seed quality indicate there is an opportunity to improve seed yield and quality by delaying harvest until the seed moisture content is close to 30%, but findings need to be confirmed over a range of cultivars, seasons and locations. Delaying the harvest to this extent must be balanced against the increased risk of yield loss through unfavourable weather conditions.

Studies indicate that onion seedlot quality could be improved by reducing the variability of umbel maturity at harvest, or preventing the harvest of immature umbels. Further investigation into methods of reducing umbel variability need to consider the effect of these measures on yield. Seedlot quality could also be improved by optimising post-harvest drying conditions, results indicate that rapid drying at high temperature should be avoided.

Hydropriming effectively reduced the incidence of seedling abnormality in onion seedlots. The treatment also improved the rate and uniformity of germination. This suggests that the treatment may have potential as an application for the onion sprouting industry where rapid and even germination is critical. Further research into optimum priming conditions needs to be carried out before the technique can be extended to this industry.

Carrot

Carrot seed embryo size variability affected early seedling size variability, but the effect had diminished by 120 DAS. Further research into carrots size variability should consider: the interaction of seed quality, plant spacing arrangement and density; and the interaction between seed quality and field factors in obtaining the target stand density.

Extension of the carrot production season from the present system of planting mainly in spring through to early summer and harvesting from mid summer through to autumn may be possible with pretreatment. Further expansion of the industry to planting at other times of the year when conditions are less favorable for field establishment may benefit from seed priming in order to obtain target densities and uniform emergence. The effect of priming on seed establishment at less-than-optimal conditions has yet to be determined.

Brassica

Brassica seedcoat damage sustained during threshing reduces seedlot quality. Comparing the effects of two commercially used combine harvesters on seed damage suggested choice of harvester could impact on seed quality. Further studies with increased replication are recommended if statistical significance in differences between harvesters needs to be proven. These studies would also confirm the preliminary identification of the major damage point in the harvester and provide more information on harvester settings likely to cause less damage to seed.

Radiata pine

Results suggest that low and variable emergence of radiata pine seedlots in the field may be caused by variable seed quality and the effect of sub-optimal temperature on germination.

Current industry practice of presoaking can be maintained provided that attention is paid to aeration during treatment (Donovan 2001), or stratification or cold hydropriming treatment is applied after presoaking. The adoption of stratification or cold hydropriming by industry has the potential to increase emergence rate and percentage in field nurseries.

While it was determined that germination benefited from pretreatment (particularly pretreatment at low temperature), the mechanisms involved are still poorly understood. Further examination of: water relations; megagametophyte and seedcoat weakening and pathways for moisture to the embryo; an carbohydrate metabolism during treatment might improve our understanding of the processes involved.

Many of the outcomes of the project have been tested in commercial operation and found to be beneficial to crop establishment and yield. In some cases, these benefits need to be evaluated. There is also a need to communicate some findings to the broader industry to improve adoption.

Chapter 7. Technology Transfer

The science and technology outputs from this project have been significant in number and have been extended to a diverse audience. The successful completion of numerous honours and research higher degree training opportunities that have been either directly or indirectly associated with the project have resulted in increased researcher interest and capability as well as refereed scientific publication and examined/reviewed theses.

Industry partners have been involved at all stages of the project and have been exposed to the research outputs as they have arisen during the project. In many cases, the results that are presented in the previous chapters, have been fully incorporated into commercial production protocols by industry. Some of these significant technologies that have been adopted include;

- modified harvesting and post harvest handling of onion seed crops to overcome problems of abnormality
- modified seed treatment protocols for pine seed which has increased the germination capacity, vigour and uniformity of seedling emergence, especially in lower quality seedlots or more challenging controlled pollinated seed
- adoption of findings of investigations into pyrethrum seed germination
- recognition of the importance of seed quality in determining both yield and quality of fresh market carrots

The adoption of this technology has removed significant barriers previously existing in these cropping industries and is clear evidence of the extent and success of the technology transfer focus of this project.

Additional evidence that technology transfer occurred and the research outputs and the research capability developed through this project were valued by industry, lies in the willingness of industry partners to increase their investment in seed research and development. This ongoing research will ensure the continuation of the Seed Research Group at the University of Tasmania/Tasmanian Institute of Agricultural Research and the continued investment in personnel, supporting facilities and equipment to support the cropping industry.

Specific technology transfer activities associated with or directly arising from this project included;

Honours Thesis, School of Agricultural Science, University of Tasmania

Chapman, C. 2000. Improving the germination of Pyrethrum seed (*Asteraceae* (sic) *cinerariaefolium*). Honours Thesis, University of Tasmania

McKenzie, D.J. 1999. Umbel maturity and post-harvest drying effect on seed lot quality in onion (*Allium cepa* L.). Honours Thesis, University of Tasmania

Spurr, C.J. 1998. The changes in seed yield and quality with time of harvest in onion (*Allium cepa* L.). Honours Thesis, University of Tasmania

PhD Thesis, School of Agricultural Science, University of Tasmania

Donovan, N. 2001. The water relations of seed pretreatments and their effects on the germination of radiata pine seeds. PhD Thesis, University of Tasmania

Fulton, D. 1998. Agronomic and seed quality studies in pyrethrum (*Tanacetum cinerariaefolium*) Sch.Bip. PhD Thesis, University of Tasmania

Publications/Conference Presentations/Attendance

Brown, P.H. and Gracie, A.J. (2000). Managing Carrot Root Size. Proceedings of Carrot Conference Australia. pg 18-19

Brown, PH., Rithichai, P., Gracie, A.J. and Fulton, D.A. (2002) Fructans accumulation during onion seed germination. *Seed Science and Technology (submitted)*

Donovan, N., Wilson, S.J and Clark, R.J (2002) Water relations of *Pinus radiata* seed during imbibition *Tree Physiology (Accepted subject to minor revision)*

Fulton, D.A. Attended the 1999 World Seed Conference, Cambridge, UK

Gracie, A.J. (2002) Study of factors affecting the marketable yield of carrots (*Daucus carota* L.). PhD Thesis. University of Tasmania. Hobart, Australia.

Gracie, A.J. and Brown, P.H. (2001) Study of some factors affecting carrot taproot size uniformity. Proceedings of the 10th Australian Agronomy Conference.

Mehdi Tajbakhsh, Brown, P.H., Gracie, A.J., Spurr C, Donovan N and Clark, R.J. (2002) Mitigation of stunted root abnormality in onion (*Allium cepa* L.) using seed priming treatments. *(Prepared for submission to Seed Science and Technology)*

Mehdi Tajbakhsh, Brown, P.H., Gracie, A.J., Spurr C, Donovan N and Clark, R.J. (2002) The use of hydropriming to improve onion (*Allium cepa* L.) seed lot quality. *(Prepared for submission to Seed Science and Technology)*

Shabala.S.N, Newman, I.A., Wilson, S.A. and Clark, R.J. (2000) Nutrient uptake patterns over the surface of germinating wheat seed. *Australian Journal of Plant Physiology* 27 (2) 89-97

Spurr, C.J., Brown, P.H. and Mendham, N.J. (2000) Improving hybrid carrot seed production in Southern Australia. 8th International Carrot Conference, Pascoe, Washington State, USA

Spurr, C.J., Mendham, N.J. and Brown, P.H. (2001) The effect of Rutherglen Bug (*Nysius vinitor*) on yield and quality of hybrid carrot seed. Proceedings of the 10th Australian Agronomy Conference, Hobart, Tasmania

Spurr, C.J., Fulton, D.A., Brown, P.H. and Clark, R.J. (2002) Changes in seed yield and quality with maturity in onion *Allium cepa* L."Early Cream Gold". *Journal of Applied Seed Production (in press)*.

Visiting Fellows/Summer Scholarships and Sabbatical Visitors

Visiting Fellows

Dr Tony Haigh, Senior Lecturer in Plant Physiology, University of Western Sydney appointed as a Visiting Fellow

Professor Medhi Tajbakhsh, Iran Sabbatical Leave, Nov 2001 to April 2002. Abnormalities in Onion Seeds

Industry Presentations and Industry Meetings/Reviews

Targeted Technology Transfer Activities

- Pine Seed

- STBA Technical Committee Meetings, meetings Feb 98 in Hobart and Nov 99 in Melbourne

- Annual on site research presentations

- Industry partner – joint project supervisor (Sandra Hetherington)

- Field trials conducted on industry partner's nursery site

- Field nursery operation adopted recommended priming treatments

- **Onion Seed**
 - Field Fresh R&D Meeting 16 December, 1998 planning honours projects and reviewing research outputs
 - Annual research meetings with industry partner (Tasmanian Onion Seed Growers)
 - MKS (Lincoln Weddell) travelled to Hobart to attend R&D presentation, 3 December 1999
 - Incorporation of modified harvesting strategies and post harvest handling to improve quality and reduce abnormalities – resulting in all seed passing ISTA seed testing protocols 1999 through 2002.
- **Pyrethrum Seed**
 - Special R&D meeting with BRA, during TIAR Presentations Day, Launceston, June, 2000
 - Annual Presentations at Pyrethrum Growers Meeting, Ulverstone, Tasmania
 - Transfer of seed priming technology to BRA and successful field implementation and incorporation into commercial practice
- **Vegetable Seeds**
 - Presentation at Annual Vegetable ARAC Meetings
 - Presentation at Annual TIAR Presentation Days
 - Location of demonstration carrot and onion trials in growers/industry partner fields (e.g. Field Fresh/Harvest Moon)
 - Field trials with Bejo Seeds on brassica harvest and production of executive summaries for industry
 - Negotiation with Bejo Seeds and South Pacific Seed to develop an ARC Linkage Project to continue their investment in vegetable seed research – a result only possible due to industry partner’s valuing R&D outcomes
 - Gracie, A.J., Clark R.J. and Brown P.H. (Nov. 1996) Carrot Seed Tests. Industry report for Field Fresh Tasmania
 - Gracie, A, Brown P, Mendham, N, and Spurr, C (2002) Assessment of brassica seed damage - comparison between harvesters and harvester settings. Industry report for Bejo Seeds Inc (Tasmania).

References

- Adamson, R.M. 1960. The effect on germination of drying leek seed heads at different temperatures. *Canadian Journal of Plant Science*, 40: 666-671
- Ali, A., V.S. Machado and A.S. Hamill. 1990. Osmoconditioning of tomato and onion seeds. *Scientia Horticulturae*, 43: 213-224.
- Barton, L.V. 1966. Viability of pyrethrum seeds. *Contributions to the Boyce Thompson Institute*, 23:267-68
- Baskin, C.C. and J.M. Baskin. 1998. *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. Academic press, San Diego
- Benjamin, L.R. (1982) Some effects of differing times of seedling emergence, population density and seed size on root-size variation in carrot populations. *Journal of Agricultural Science* 98: 537-545.
- Benjamin, L.R. (1984) The relative importance of some sources of root-weight variation in a carrot crop. *Journal of Agricultural Science* 102: 69-77.
- Bewley, J.D., and M. Black. 1994. *Seeds: physiology of development and germination*. Second edition. Plenum press, New York
- Bodsworth, S. and Bewley, J.D. (1979) Osmotic priming of seeds of crop species with polyethylene glycol as a means of enhancing early and synchronous germination at cool temperatures. *Canadian Journal of Botany* 59: 672-676.
- Bradford, K.J. 1995. Water relations in seed germination. In: J. Kigel and G. Galili (eds) *Seed development and germination*. Marcel Dekker, New York, pp 351 - 396.
- Bratcher, C.B., J.M. Dole and J.C. Cole. 1993. Stratification improves seed germination of five native wildflower species. *Hortscience* 28(9):899-901
- Brewster, J.L. 1982. Flowering and seed production in overwintered cultivars of bulb onion. I. Effects of different raising environments, temperatures and daylengths. *Journal of Horticultural Science*, 57: 93-98
- Bujalski, W. and A.W. Nienow. 1991. Large-scale osmotic priming of onion seeds : a comparison of different strategies for oxygenation. *Scientia Horticulturae*, 46: 13-26
- Carpenter, W.J. and E.R. Ostmark. 1992. Growth regulators and storage temperature govern germination of *Coreopsis* seed. *HortScience*, 27(11): 1190-1193
- Chapman, C. 2000. Improving the germination of Pyrethrum seed (*Asteraceae* (sic) *cinerariaefolium*). Honours Thesis, University of Tasmania
- Currah, L. 1982. Onion flowering and seed production. *Scientific Horticulture*, 32: 26-46

- Currah, I.E., Gray, D. and Thomas, T.H. (1974) The sowing of germinating vegetable seeds using a fluid drill. *Annals of Applied Biology* 76: 311-318.
- Currah, L. and D.J. Ockendon. 1978. Protandry and the sequence of flower opening in the onion (*Allium cepa* L.). *New Phytologist*, 81: 419-428
- Currah, I.E. and Salter, P.J. (1973) Some combined effects of size grading and 'hardening' seed on the establishment, growth and yield of four varieties of carrots. *Annals of Botany* 37: 709-719.
- Dearman, J. P.S. Brocklehurst and R.L.K Drew. 1986. Effects of osmotic priming and ageing on onion seed germination. *Annals of Applied Biology*, 108: 639-648
- Donovan, N. 2001. The water relations of seed pretreatments and their effects on the germination of radiata pine seeds. PhD Thesis, University of Tasmania
- Downie, B. and U. Bergsten. 1991. An invigoration regime for *Pinus strobus* seeds. *Can. J. For. Res.*, 21: 1343 - 1348.
- Downie, B., H.W.M. Hilhorst, and J.D. Bewley. 1997. Endo-B-mannanase activity is associated with micropylar weakening during germination of white spruce seeds. *Physiol. Plant.* 101: 405 - 415
- Drew, R.L.K., L.J. Hands and D. Gray. 1997. Relating the effects of priming to germination of unprimed seeds. *Seed Science and Technology*, 25: 537-548
- Ellis, R. H. and P.D. Butcher. 1988. The effects of priming and 'natural' differences in quality amongst onion seed lots on the response of the rate of germination to temperature and the identification of the characteristics under genotypic control. *Journal of Experimental Botany*, 39: 935-950.
- Fang, J., F. Moore, E. Roos and C. Walters. 1998. Three-dimensional models represent seed moisture content as a function of relative humidity and temperature. *HortScience*, 33: 1207-1209
- Finch-Savage, W.E. 1986. A study of the relationships between seedling characters and rate of germination within a seed lot. *Annals of Applied Biology*, 108: 441-444
- Finch-Savage, W.E. (1990) The effects of osmotic seed priming and the timing of water availability in the seed bed on the predictability of carrot seedling establishment in the field. *Acta Horticulturae* 267: 209-216.
- Finch-Savage, W.E. and McQuistan, C.I. (1989) The use of abscisic acid to synchronize carrot seed germination prior to fluid drilling. *Annals of Botany* 63: 195-199.
- Finch-Savage, W.E. and Pill, W.G. (1990) Improvement of carrot crop establishment by combining seed treatments with increased seed-bed moisture availability. *Journal of Agricultural Science* 115: 75-81.

- Fulton, D. 1998. Agronomic and seed quality studies in pyrethrum (*Tanacetum cinerariaefolium*) Sch.Bip. PhD Thesis, University of Tasmania
- Globerson, D., A. Sharir and R. Eliasi. 1981. The nature of flowering and seed maturation of onions as a basis for mechanical harvesting of the seeds. *Acta Horticulturae*, 111: 99-108
- Gracie, A.J. (2002) Study of factors affecting the marketable yield of carrots (*Daucus carota* L.). PhD Thesis. University of Tasmania. Hobart, Australia.
- Gracie and Brown, 2001 Study of some factors affecting carrot taproot size uniformity. Proceedings of the 10th Australian Agronomy Conference.
- Gray, D. and Benjamin, L.R. (1993) Controlling the size of carrot - the roles of seed quality and plant density. *Acta Horticulturae* 354: 47-54.
- Gray, D. and Steckel, J.R.A. (1983a) A comparison of methods for evaluating seed quality in carrots (*Daucus carota* L.). *Annals of Applied Biology* 103: 327-334.
- Gray, D. and Steckel, J.R.A. (1983b) Seed quality in carrots: the effects of seed crop plant density, harvest date and seed grading on seed and seedling variability. *Journal of Horticultural Science* 58: 393-401.
- Gray, D., Steckel, J.R.A., Drew, R.L.K. and Keefe, P.D. (1991) The contribution of seed characters to carrot plant and root size variability. *Seed Science and Technology* 19: 655-664.
- Gray, D., J.A. Steckel and L.J. Hands. 1990. Response of vegetable seeds to controlled hydration. *Annals of Botany*, 66: 227-235
- Gray, D., Steckel, J.R.A., Jones, S.R. and Senior, D. (1986) Correlations between variability in carrot (*Daucus carota* L.) plant weight and variability in embryo length. *Journal of Horticultural Science* 61: 71-80.
- Gray, D., W.G. Tucker, L.J. Hands and J.R.A. Steckel. 1989. Some effects of drying leek (*Allium porrum* L.) seeds harvested at different stages of maturity on seed and seedling performance. *Journal of Horticultural Science*, 64(4): 455-465
- Grose, R.J. 1958. Notes on the influence of stratification at constant temperature on the germination of *Pinus radiata* D. Don. *Plantation Technical Papers*, 5: 8 - 10
- Haigh, A.M., E.W.R. Barlow, F.L. Milthorpe and P.J. Sinclair. 1986. Field emergence of tomato, carrot, onion, and sorghum seeds in a range of osmotica. *Journal of the American Society for Horticultural Science*, 111(5): 660-665
- Hallgren, S.W. and L. Wu. 1995. Water relations of seed germination in pine. Forests sustaining people: Proceedings of the 1995 Society of American Foresters Convention, pp 237-242
- Heather, D.W. and Siczka, J.B (1991). Effect of seed size and cultivar on emergence and stand establishment of broccoli in crusted soil. *Journal of the American Society for Horticultural Science*. 946-949.

Hegarty, T.W. (1971) A relation between field emergence and laboratory germination in carrots. *Journal of Horticultural Science* 46: 299-305.

Hegarty, T.W. (1978) The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. *Plant, Cell and Environment* 1: 101-119.

Hilhorst, H.W.M. and P.E. Toorop. 1997. Review on dormancy, germinability, and germination in crop and weed seeds. In: *Advances in Agronomy* V. 61, Academic press, Netherlands.

International Seed Testing Association (1995). *Handbook of Vigour Test Methods*. 3rd Edition. Zurich, Switzerland.

International Seed Testing Association (1999). *Seed Science and Technology* volume 27. Supplement Rules. Zurich, Switzerland

Jett, J.W., Welbaum, G.E. and Morse, R.D. (1996) Effects of matric and osmotic priming treatments on broccoli seed germination. *Journal of the American Society for Horticultural Science*. 121: 423-429.

Jones, S. K. and P.G. Gosling. 1994. "Target moisture content" prechill overcomes the dormancy of temperate conifer seeds. *New Forests*, 8: 309 - 321.

Kazinczi, G., K. Hunyadi and D. Lukacs. 1999. Data to the biology of cornflower; Germination biology and growth analysis. *Novenyvedelem* 35:2, 45-52

Khan, A.A., Maguire, J.D., Abawi, G.S. and Ilyas, S. (1992) Matricconditioning of vegetable seeds to improve stand establishment in early field plantings. *Journal of the American Society of Horticultural Science* 117: 41-47.

Krugman, S. L., and J.L. Jenkinson. 1974. *Pinus* L. Pine. In: *Seeds of woody plants in the United States*, Forest Service, U.S. Department of Agriculture, Agriculture Handbook No. 450, pp 598-638.

Letchamo, W. and A. Gosselin. 1996. Light, temperature and duration of storage govern the germination and emergence of *Taraxacum officinale* seed. *Journal of Horticultural Science*. 71:373-377

Levitt, J. and P.C. Hamm. 1943. A method of increasing the rate of seed germination of *Taraxacum kok-saghyz*. *Plant Physiology*, 18: 288 - 293.

Li, B., Watkinson, A.R. and Hara, T. (1996) Dynamics of competition in populations of carrots. *Annals of Botany* 78: 203-214.

Mathews, S. (1980) Controlled deterioration: a new vigour test for crops seeds. In *Seed production* pp 647-660. P.D. Hebblethwaite (ed.). London: Butterworths.

Mathews, S. and A.A. Powell. 1986. Environmental and physiological constraints on field performance of seeds. *Hortscience* 21(5):1125-1128

- McCormac, A.C. and Keefe, P.D. (1990) Cauliflower (*Brassica oleracea* L.) See Vigour: Imbibition Effects. *Journal of Experimental Botany*. 41: 893-899.
- McDonald, M.B. 1980. Assessment of seed quality. *HortScience*, 15: 784-788
- McDonald, M.B. 1999. Seed deterioration: physiology, repair and assessment. *Seed Science and Technology*, 27: 177-237
- McEvoy, P.B. 1984. Dormancy and dispersal in dimorphic achenes of tansy ragwort, *Senecio jacobaea* L. *Oecologia* 61:160-168
- McKenzie, D.J. 1999. Umbel maturity and post-harvest drying effect on seed lot quality in onion (*Allium cepa* L.). Honours Thesis, University of Tasmania
- Minko, G. and F.G. Craig. 1976. Radiata pine nursery research in north-eastern Victoria. Forests Commission, Victoria Bulletin No. 23. Melbourne. 24p.
- Mohamed-Yasseen, Y. and W.E. Splittstoesser. 1990. The role of the onion (*Allium cepa* L.) seed coat in aging and ultrastructural changes in root tips of low vigor seedlings. *Proceedings of the Plant Growth Regulator Society of America*. (17th) 10-15.
- Mohondass, S. and V. Sympath. 1988. Influence of gibberellic acid on pyrethrum seed germination. *Pyrethrum post*. 15:85-86
- Murray, G.A. (1989) Osmoconditioning carrot seed for improved emergence. *HortScience* 24: 701.
- Pandita, P.N. 1984. Effect of storage on seed germination in pyrethrum (*Chrysanthemum cinerariaefolium* Vis.). *Pyrethrum Post*. 16:91-92
- Peishi, Z., J.A. Plummer, D.W. Turner, Choengsaat and D.T. Bell. 1999. Low and high temperature effects on viability and germinability of seeds of three Australian Asteraceae. *Australian Journal of Botany*. 47:2, 265-275
- Perez, G.F., D. Come and F. Corbineau. 1993. Germination behaviour of *Onopordum acanthium* L.: effect of the origin of the cypsela. *Proceedings of the fourth international workshop on seeds: basic and applied aspects of seed biology*. Anger France. Vol2, 417-422
- Peterson, J.R. 1976. Osmotic priming of onion seeds - the possibility of commercial-scale treatment. *Scientia Horticulturae*, 5: 207-214
- Pill, W.G. (1995) Low water potential and presowing germination treatments to improve seed quality. *In* A.S. Basra (ed.). *Seed Quality: Basic Mechanisms and Agricultural Implications*. The Hawthorne Press, New York, US.
- Pill, W.G. and W.E. Finch-Savage. 1988. Effects of combining priming and plant growth regulator on the synchronisation of carrot seed germination. *Association of applied biologists p* 383-389

- Powell, A.A., Thornton, J.M. and Mitchell, J.A. (1991) Vigour differences in Brassica seed and their significance to emergence and seedling variability. *Journal of Agricultural Science, Cambridge* 116: 369-373.
- Rimbawanto, A., P. Coolbear, A.M. Dourado. and A. Firth. 1988. Seed maturation precedes cone ripening in New Zealand *Pinus radiata*. *New Zealand Journal of Forestry Science*, 18(2): 139 - 148.
- Rimbawanto, A., P. Coolbear and A. Firth. 1989. Morphological and physiological changes associated with the onset of germinability in *Pinus radiata* (D. Don) seed. *Seed Science and Technology*, 17: 399 - 411.
- Roos, E.E. 1980. Physiological, biochemical and genetic changes in seed quality during storage. *HortScience*, 15(6): 781-783
- Rubatzky, V.E., Quiros, C. F. and Simon, P. W. (1999) Carrots and related vegetable *Umbelliferae*, CABI, Wallingford, UK.
- Salter, P.J., Currah, I.E. and Fellows, J.R. (1979) The effects of plant density, spatial arrangement and time of harvest on yield and root size in carrots. *Journal of Agricultural Science* 93: 431-440.
- Salter, P.J., Currah, I.E. and Fellows, J.R. (1980) Further studies on the effects of plant density, spatial arrangement and time of harvest on yield and root size in carrots. *Journal of Agricultural Science* 94: 465-478.
- Salter, P.J., Currah, I.E. and Fellows, J.R. (1981) Studies on some sources of variation in carrot-root weight. *Journal of Agricultural Science* 96: 549-556.
- SAS Institute Inc. (1989) Users guide, version 6, 4th edition. SAS Institute, Cary. N.C., U.S.A.
- Schneider A. and P. Renault. 1997. Effects of coating on seed imbibition: I. Model estimates of water transport coefficient. *Crop Sci* 37: 1841 - 1849.
- Schneider, W.L. and D.J. Gifford. 1994. Loblolly pine seed dormancy. I. The relationship between protein synthesis and the loss of dormancy. *Physiologia Plantarum*, 90: 246 - 252.
- Shende, P.V., N.V. Sorte, A.D. Banginwar, B. Sunita and S. Bankar. 1998. Evaluation of various methods to improve seed quality in sunflower. *Journal of Soils and Crops*, 8(2): 201-203
- Simak, M. 1985. Seed germination after incubation at different moisture stress. "Seed Problems". Proceedings of the international symposium on seed problems under stressful conditions. International Union of Forest Research Organisations. Project Group: P2.04.00 Ed. J. Nather. Vienna and Gmunden, Austria.
- Spurr, C.J. 1998. The changes in seed yield and quality with time of harvest in onion (*Allium cepa* L.). Honours Thesis, University of Tasmania

Spurr, C.J., Fulton, D.A., Brown, P.H. and Clark, R.J. (2002) Changes in seed yield and quality with maturity in onion *Allium cepa* L."Early Cream Gold". *Journal of Applied Seed Production* (in press).

Stanley, R.G. 1958. Gross respiratory and water uptake patterns in germinating sugar pine seed. *Physiologia Plantarum*, 11: 503 - 515

Steiner, J. J. and D.C. Akintobi. 1986. Effect of harvest maturity on viability of onion seed. *Hortscience*, 21: 1220-1221.

Still, D.W. and Bradford K.J. (1998) Using Hydrotime and ABA-time models to quantify seed quality of Brassicas during development. *Journal of the American Society for Horticultural Science*. 123: 692-699.

Szafirowska, A., Anwar, A.A. and Peck, N.H. (1981) Osmoconditioning of Carrot Seeds to Improve Seedling Establishment. *Agronomy Journal* 73: 845-848.

Tajbaksh, M., Brown, P.H., Gracie, A.J., Spurr C, Donovan N and Clark, R.J. (2002a) Mitigation of stunted root abnormality in onion (*Allium cepa* L.) using seed priming treatments. (Prepared for submission to *Seed Science and Technology*)

Tajbaksh, M., Brown, P.H., Gracie, A.J., Spurr C, Donovan N and Clark, R.J. (2002b) The use of hydropriming to improve onion (*Allium cepa* L.) seed lot quality. (Prepared for submission to *Seed Science and Technology*)

Tamet, T., Boifin, J., Durr, C. and Souty, N. (1996) Emergence and early growth of an epigeal seedling (*Daucus carota* L.): influence of soil temperature, sowing depth, soil crusting and seed weight. *Soil and Tillage* 40: 25-38.

Taylor, A.G., Churchill, D.B., Lee, S.S., Bilsland, D.M. and Cooper, T.M. (1993) Color sorting of coated Brassica seeds by fluorescent sinapine leakage to improve germination. *Journal of the American Society for Horticultural Science*. 118: 551-556.

Thornton, J.M., Powell, A.A. and Matthews, S. (1990) Investigation of the relationship between seed leachate conductivity and the germination of Brassica seed. *Annals of Applied Biology* 117: 129-135.

Tyree, M.T. (1976) Negative turgor pressure in plant cells: fact or fallacy? *Canadian Journal of Botany* 54: 2738-2746.

Tyree, M.T. and Hammel, H.T. (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany* 23: 267-282.

Tyree, M.T. and Jarvis, P.G. (1982) Water in tissues and cells. *In*: O.L. Lange, P.S. Mokel, C.B. Osmond and Ziegler, H. (eds.). *Encyclopedia of Plant Physiology*, New series, vol 12B. Springer-Verlag, Berlin. Pp. 35-77.

Wartidiningsih, N., R.L. Geneve and S.T. Kester. 1994. Osmotic priming or chilling stratification improves seed germination of purple coneflower. *HortScience*, 29(12): 1445-1448

Weiner, J. and Thomas, S.C. (1986) Size variability and competition in plant monocultures. *Oikos* 47: 211-222.

Wheeler, T.R. and R.H. Ellis. 1994. Effects of seed quality and temperature on pre-emergence root growth of seedlings of onion (*Allium cepa*). *Seed Science and Technology*, 22: 141-148

Wilson, A.M. 1971. Amylase synthesis and stability in crested wheatgrass seeds at low water potentials. *Plant Physiology*, 48, 541 - 546.

Wilson, A.M. 1973. Responses of crested wheatgrass seeds to environment. *Journal of Range Management*, 26(1), 43 - 43.

Wilson, R.E., Jensen, E.H. and Fernandez, G.C.J. (1992) Seed germination response for eleven forage cultivars of Brassica to temperature. *Agronomy Journal* 84: 200-202.

Zheng, G, Wilen, R.W., Slinkard, A.E. and Gusta, L.V. (1994) Enhancement of canola germination and seedling emergence at low temperature by priming. *Crop Science* 34: 1589-1593.

Appendix 1. Germination parameters

Percentage germination (FG)

Final germination percentage (FG) is a measure of germination capacity. It is the seeds that germinated during the test as a percentage of the total number of seeds tested (except those that were empty).

$$FG = \frac{\text{number of seeds germinated} \times 100}{\text{total no. seeds incubated} - \text{empty seeds}}$$

Mean germination time (MGT)

Mean germination time (MGT) gives an average measure of the time taken by individual seeds to germinate. MGT is the reciprocal of the mean rate of complete germination

$$MGT = \frac{\sum (t_x \cdot n_x)}{\sum n}$$

Where

t_x = time in days starting from day zero as day of wetting, and
 n_x = number of seeds germinating on day x

Co-efficient of uniformity of germination (CUG)

Co-efficient of uniformity of germination (CUG) gives a measure of spread in germination and is expressed as a variance of individual times around the mean time to complete germination.

$$CUG = \frac{\sum n}{\sum [(MGT - t_x)^2 \cdot n]}$$

Appendix 2. Literature Review

Chapter 1. Seed quality	67
1.1 Introduction	67
1.2 Seed viability, germination and vigour	67
1.2.1 Definition	67
1.2.2 Seed vigour	67
1.3 Relationship of seed vigour to development, dormancy and deterioration	68
1.4 Pretreatments to improve seed quality	69
Chapter 2. Onion seed quality	70
2.1 Onions in Tasmania	70
2.2 Factors affecting seed quality: flowering time	70
2.2.1 Variation in flowering time between years, crops and cultivars	70
2.2.2 Variation in flowering time within a crop	72
2.3 Factors affecting seed quality: seed development, maturity and time of harvest	73
2.3.1 Seed development	73
2.3.2 Time of harvest	75
2.4 Treatment to improve seed quality	76
2.5 Seed quality measurement in onion	76
Chapter 3. Carrot seed quality	78
Chapter 4. Brassica seed quality	81
Chapter 5. Radiata pine seed quality	82
5.1 Introduction to radiata pine	82
5.2 Factors affecting seed quality: dormancy	83
5.3 Factors affecting seed quality: seed size	84
5.4 Factors affecting seed quality: cone development	84
5.5 Factors affecting seed quality: seed development	86
5.6 Factors affecting seed quality: seed orchard management	86
5.6.1 Fertiliser application	87
5.6.2 Girdling, banding and strangulation	87
5.6.3 Moisture stress	87
5.6.4 Thinning/tree density	88
5.6.5 Light	88
5.6.6 Growth regulators	88
5.6.7 Miscellaneous treatments	88
5.7 Factors affecting seed quality: handling and storage	88
5.7.1 Harvest time	88
5.7.2 Seed extraction	89
5.7.3 Cleaning and grading	89
5.7.4 IDS	90
5.7.5 Storage	90
5.8 Treatment to improve seed quality	90
5.8.1 Presoaking	90
5.8.2 Priming	91
5.8.3 Stratification	91
5.9 Nursery management and seed quality	94
5.9.1 Sowing time	94
5.9.2 Sowing depth	95
5.9.3 Density	95
Chapter 6. References	96

1.1 Introduction

The objective of this review is to identify areas critical to the production of a reasonable quantity of high quality radiata pine seed. Literature relating to the growth, development and management of seed is discussed. The review firstly discusses the concept of seed quality and then details aspects of seed quality of species examined in this project. Detailed reviews of literature associated with radiata pine, pyrethrum and onion seed quality have been conducted. Preliminary reviews of green bean and brassica seed quality are also included.

1.2 Seed viability, germination and vigour

1.2.1 Definition

A viable seed is defined as a living seed that has the capacity to produce enzymes capable of catalysing the metabolic reactions necessary for germination and seedling growth (Hampton 1995). Germination, in contrast, is defined as the protrusion of the radicle tip from the seedcoat (Bewley and Black 1994). Viable seed may be prevented from germinating by unfavourable conditions or dormancy. Confusion exists however due to definitions used by seed technologists in contrast to seed physiologists. Germination in ISTA germination tests is defined as the emergence and development from the seed embryo of those essential structures, which are indicative of the ability to produce a normal plant under optimal conditions (ISTA 1999). That is, the term germination in the ISTA germination test is used to incorporate seedling properties. The approach taken throughout this report is the physiological definition not the seed technologists perspective.

Seed vigour is a concept that took over 27 years to define (Hampton and Coolbear 1990). Ferguson Spears (1995) reported the current ISTA definition as the sum total of those properties of the seed that determine the level of activity and performance of the seed or seedlot during germination and seedling emergence. Seed vigour refers to the potential for rapid, uniform emergence and development of a normal seedling over a wide range of environmental conditions (Perry 1982; Dornbos 1995a). It is not a definition of a specific property it is rather a concept, in which a range of seed properties determine the performance of a seed in the field and in storage. Seed viability, germination and vigour each describe a different aspect of seed quality, but when understood collectively enable an accurate description of the physiological quality of a seedlot (Dornbos 1995a). It can be visualised as a sequence as follows: a seed needs to be viable to germinate, of which the rate, uniformity and subsequent growth can be described by its vigour.

Generally, high quality seed will germinate quickly and evenly into normal seedlings while poor quality seed germinates slowly and less uniformly. In a wide range of species, a seedlot is considered to be higher quality if it has the capacity to germinate (and does so rapidly) in stressful environments such as high or low temperatures, drought or waterlogging. In addition to capacity to germinate, larger seed is considered to be of higher quality than smaller seed. Often desirable germination behaviours and large-seededness are found to be correlated.

1.2.2 Seed vigour

The concept of seed vigour has evolved out of the experience that high germination capacity does not guarantee the field performance of a seedlot (Perry 1982). If sowing conditions are near optimal, then emergence percentage in the field may correlate well with germination

percentage (Hampton and TeKrony 1995). These conditions are rarely encountered in the field, which leads to variable performance of the seedlot (Hampton 1995). Emergence level and rate may be affected which has important implications for the performance of the crop. Benjamin (1990) has shown that plant density has a large effect on economic yield in many vegetable crops. Seed vigour has also been shown to have a direct effect on marketable yield of many crops harvested during vegetative growth and early reproductive growth in the absence of any population difference such as plant density (TeKrony and Egli 1991). The storage potential of a seedlot is related to their vigour status on entering storage (Hampton 1992 cited in Hampton and TeKrony 1995). Therefore seed vigour can be used to measure the potential of the seedlot to survive storage and be of high planting value at the end of storage.

1.3 Relationship of seed vigour to development, dormancy and deterioration

Maximum seed vigour is acquired at physiological maturity (Dombos 1995a). This represents the time when an individual seed has the greatest capacity to germinate and produce a normal seedling. In many seeds physiological maturity coincides with maximum seed dry weight. However according to Coolbear (1995), most seeds continue to undergo physiological changes after maximum dry weight has been achieved. Prior to physiological maturity, the seed is in the developmental phase. Deterioration begins immediately after the acquisition of physiological maturity. The processes of deterioration reduce the capacity of a seedlot to germinate quickly and uniformly. Changes in seed vigour generally occur before any noticeable change in germination or the occurrence of normal seedlings. Seed vigour is thus a finer measure of seed quality than either germination capacity or viability. Seeds harvested prior to physiological maturity are likely to have poor vigour and storage life (Coolbear 1995). Conversely seed harvested after physiological maturity will be exposed to weathering and pathogen attack prior to harvest. High quality seed may never be achieved due to a range of environmental and plant factors. Seed quality may also be lost due to threshing and storage practices. Seed quality may vary considerably within a population.

Hilhorst and Toorop (1997) developed a link between seed dormancy and seed vigour. The authors proposed that there is an overlap of factors that determine both seed vigour and seed dormancy. The proposal does not imply that there are two definitions to describe the same process. However these processes are closely linked, with dormancy modifying the observed vigour response.

Seed vigour is a measure of seed performance that may be altered by numerous factors. These include seed deterioration, production environment, seed dormancy and seed treatments. Further work is required to develop the concept of seed vigour and realise the potential benefits in seed production that seed vigour can accomplish.

Seed vigour has traditionally been investigated through studies involving deterioration of seedlots. Species, storage temperature and relative humidity are the major factors that determine the degree of deterioration during storage. Seeds are generally stored at low moisture content and low temperature. At low seed moisture content metabolic activity is very low but some potentially damaging activity continues. Certain hydrolytic enzymes are active, membranes may undergo phase transitions, and free radicals may be produced (Hilhorst and Toorop 1997). Free radicals can damage membranes by lipid peroxidation (Roos 1980; Coolbear 1995). Free radicals can also damage other biomolecules including enzymes and DNA.

It has been suggested that the slow germination rate of deteriorated seed is a consequence of the time taken for self-repair when imbibition begins (Coolbear 1995). Repair processes may be able to proceed under treatments such as priming. Where deterioration has proceeded beyond affecting vigour, and viability is reduced, there may be damage to repair mechanisms.

1.4 Pretreatments to improve seed quality

Germination performance can often be improved by various seed treatments. Seed treatments can be used to overcome dormancy mechanisms, assist in the process of cell repair in deteriorated seed, bring seeds to the same development stage before germination, or simply hasten the process of germination. Application of a pretreatment to overcome dormancy widens the germination temperature range, improves germination capacity at some temperatures and increases germination rate. A wide range of treatments can be applied including priming, presoaking, hardening, humidifying, chitting, aeration, irradiation, growth regulator application, stratification, thermal shock, scarification, and coating with pesticides, nutrients or microbes (Khan 1992). Two of the most commonly used pretreatments are priming and stratification (moist chilling).

Priming involves incubating seeds in a solution of known osmotic potential at a specific temperature for a given period of time (Heydecker et al. 1973). The solution osmotic potential is usually between -1.0 and -1.5 MPa. Water potentials (Ψ) in this range are generally lower than the water potential at which the radicle begins to elongate. Holding seeds in solutions of this Ψ prolongs phase II of imbibition and prevents the embryo taking up sufficient water for radicle protrusion (Karssen et al. 1989). When primed seeds are transferred to conditions suitable for germination, phase II of imbibition may be reduced.

There is some uncertainty about the mechanisms by which priming acts. Karssen et al. (1989) reported that the endosperm of celery (*Apium graveolens* L. var. *dulce* (Miller) Pers.), tomato and lettuce seeds was hydrolysed at the micropylar end of the seed, opposite the radicle tip, during priming. This enabled the radicle to expand rapidly when imbibition began. It was found that celery seeds would not germinate until the embryo attained a critical length, which was achieved by cell division during priming (Karssen et al. 1989).

Stratification has long been used to pretreat seeds of temperate conifer species to overcome dormancy. The treatment aims to replicate the conditions the seeds experience during winter in their natural environment. Many of these species shed their seed in autumn or early winter (Krugman and Jenkinson 1974). Once shed the seeds are able to take up water, but cold winter temperatures and dormancy prevent germination. Over time dormancy is overcome, and as temperatures rise in spring the seeds germinate.

2.1 Onions in Tasmania

The Tasmanian onion seed industry produces about ten tonnes of onion seed per annum. Production is predominantly located in the Derwent Valley of southern Tasmania. Two varieties of onion seed (early cream gold and cream gold) are produced. The seed is primarily supplied to the local vegetable onion industry, although some is also exported.

Factors affecting the quality of onion seedlots include low seed weight (Ogawa 1961; Voss 1979; Gamiely et al 1990); immaturity (Sandhu et al 1972; Neal and Ellerbrock 1986; Steiner and Akintobi 1986; and Gray and Ward 1987); deterioration (Ellis and Roberts 1977; Ellis and Roberts 1981; Ward and Powell 1983; and Salama and Pearce 1993). Seedlot performance is also affected by field factors including temperature (Gray and Steckel 1984) and pathogen attack (Wu 1979). Priming has been used to improve seedlot quality (Ellis and Butcher 1988). The onion seed produced in the state is generally perceived to be of good quality but it does contain high percentages of seed that generate abnormal seedlings. The Tasmanian industry produces seedlots that contain in the order of 14-18% abnormal seedlings. The highest acceptable level of abnormal seedlings is less than 5%. Seedlings with stunted root growth account for the majority of abnormal in Tasmanian seedlots. The percentage of abnormal seedlings is perceived by industry as an important seedlot quality determinant. Identifying why abnormal onion seedlings are found at high levels in Tasmanian seedlots is critical for the industry.

The implication of seedling abnormality on seed performance has not been determined. Likewise, the effects of other seed and germination characteristics on field performance have not been determined. The physiological basis and the field practices responsible for the production of abnormal onion seedlings remain unclear. Abnormal onion seedlings are identified as part of the routine germination test required for the sale of seedlots. The majority of abnormal onion seedlings are characterised by a poorly developed root system in the seedlings shortly after germination.

2.2 Factors affecting seedlot quality: flowering time

Onion is a biennial plant that forms a bulb before flowering. Some cultivars will flower prior to bulbing in inductive conditions (Brewster 1987). The entire flowering structure in onion is a terminal spherical umbel on a scape produced from the bulb or seedling. It consists of a large number of cymes, producing an umbel with 50 to over 1000 individual flowers (Currah 1982). Flowering time in onion is not uniform and variation exists between years, crops and cultivars, and within crops.

Investigations into onion flowering have generally been studied at the level of the whole umbel. This has led to the development of models to predict flowering time in onion based on the effects of temperature and photoperiod (Brewster 1997b) However, the causes and effects of variation in flowering time are less well understood. Variation in flowering time may exist between cultivars, years, individual plants, within plants and within the umbel.

2.2.1 Variation in flowering time between years, crops and cultivars

Brewster (1997a) reported that flowering in onion is controlled by the interaction of temperature and photoperiod. Bulbing in onion is also controlled by these environmental factors. The two stages of the flowering process are flower initiation and flower development.

Initiation

Onions require a period of vernalisation to initiate floral development in the vegetative apex. Optimal temperatures for vernalisation have been shown to occur in the range of 5 - 12 °C (Rabinowitch 1985). However, temperatures as high as 21 °C have been reported to vernalise African cultivars (Rabinowitch 1985). Duration of the vernalisation treatment has also been shown to be cultivar dependent (Brewster 1997a). Similar values of optimal temperatures and duration of treatments have been reported for leek (*Allium porrum* L.) (Wiebe 1994). The plants response to vernalisation is modified by several factors. A juvenile phase is reported to exist in onion (Rabinowitch 1990a) which is commonly measured by plant size. The juvenile phase is of critical importance in both seedlings and bulbs (Brewster 1987). The rate of inflorescence initiation increases with bulb size over a twenty-fold range, with 4g appearing to be the minimum size for initiation in bulbs (Brewster 1987). Brewster (1997a) limited this statement by reporting that the minimum weight distinction is not this clear. For seedlings the minimum size for initiation was cultivar dependent, with an eight-fold range in weight. The ambiguity of these findings is a reflection of the measurement of plant size. No authors report the juvenile phase in terms of physiological age such as day degrees. Such representation of results may reduce the variation.

Photoperiod has been shown to have no effect on initiation of onion inflorescences (Holdsworth and Heath 1950; Rabinowitch 1985). In contrast, Brewster (1983) reported that initiation was favoured by long days in onion seedlings. The author expected that the response was cultivar dependent, as different cultivars were used by Holdsworth and Heath (1950). Flower initiation and bulbing interact under a combination of long days and warmer temperatures. Unlike onions, flower initiation in leek is favoured by short days (Wiebe 1994). Brewster (1987) reported that low photon flux density caused reduced structural carbohydrate concentrations within the plant. This influenced floral evocation in onion. Low nitrogen status can increased the rate of initiation in growing seedlings (Brewster 1983). Bulb dormancy delayed inflorescence initiation (Brewster 1977).

In summary, initiation of flowering in onion is controlled primarily by temperature and the length of the juvenile phase. Other factors that modify flower initiation include photoperiod, nitrogen status, photon flux density and dormancy. The quantitative effects of these factors are unknown and the implications for seedlot quality require further investigation.

Development

In contrast to initiation, flower development responds to both temperature and photoperiod. This response has been quantified to about 311 day-degrees above 10 °C, from appearance of the elongated scape to flower opening (Brewster 1982b). Holdsworth and Heath (1950) established that long days favour elongation and development of the flower. Brewster (1982a) confirmed these results and modelled development showing that temperature and photoperiod accounted for all but 17% of the variation associated with different rates of development. Wiebe (1994) reported that leek also responds to long days during flower development over a similar range of temperatures.

De-vernalisation can occur in onions at temperatures above 28 °C in most cultivars (Brewster 1997a). It is a complex interaction involving the direct suppression of the initiated flowers and competition from bulbing. Vegetative buds usually exist laterally to the floral bud. Vegetative

bud development is favoured by long days and warm temperatures, which leads to competition between the buds for resources (Rabinowitch 1985). Swelling of the lateral bud will also physically impair the floral bud (Rabinowitch 1985). This was termed the competitive phase of development by van Kampen (1970, cited by Brewster 1997a). De-vernalisation has also been reported to occur during the completion phase (the period from appearance of the scape to flowering) by high temperatures (Brewster 1997a). The critical temperature and time of exposure are cultivar dependent.

In summary, flower development is controlled by both temperature and photoperiod. High temperatures can cause de-vernalisation in the developing flower, significantly reducing the rate of development.

Genotype and environment interact to affect floral initiation and rate of development, thereby causing variation in flowering time. Brewster (1997b) reported that a few weeks difference in sowing had a large effect on onion flowering time. The author also reported that year to year weather variation caused large differences in the date of flowering. These differences will also be evident between crops in different locations. Variation in cultivar response to environmental conditions has also been reported (Holdsworth and Heath 1950; Rabinowitch 1985; Brewster 1997a). Wiebe (1994) reported similar differences in cultivar response to environment in leek.

2.2.2 Variation in flowering time within a crop

Variation within the umbel

Currah (1982) reported that the flowering of a single umbel might last from three to five weeks. Currah and Ockendon (1978) studied the sequence of flower opening in onion, and reported that 50% of the flowers of an umbel opened during a period that lasted for 4-10 days. The mean total flowering period ranged from 20 to 29 days, and depended on cultivar. The sequence of flower-opening across the umbel showed no obvious pattern. This is in contrast with *Allium fistulosum* L., where the sequence of flower opening is regular from the top downward (Currah and Ockendon 1978). Variation in flowering time within an umbel results in a variation in maturation of fertilised flowers. The factors associated with the process of flower opening within the onion umbel have not been investigated thoroughly. The qualitative effects of temperature are well recognised, however, the effect of factors such as flower nutrition, sink strength, vernalisation, cultivar, and hormone levels are not known.

Variation within the plant

Currah (1982) reported that the variation in flowering time in a whole plant may be longer than five weeks. The variation is caused by the development and flowering of secondary and higher order umbels. A week or more may elapse between the start of flowering of the first and last umbel (Currah and Ockendon 1978). The number of umbels per plant varies with cultivar, and is related to the number of centres in the onion bulb (Currah and Ockendon 1978). Harvey (1993) reported that without selection, bulbs have a tendency to develop multiple centres. The cause of variation in flowering time may be the result of competition between umbels for resources, competition from bulbing and de-vernalisation.

Variation between plants

Investigations into variation of flowering time between plants have been limited to the effect of parent in hybrid seed production (Currah 1982). However, within-cultivar differences across a field are likely to be affected by the factors that control flowering in onion, if these vary spatially across a field. These factors have already been described. Time of flowering would be expected to follow microclimatic variation, particularly in temperature. Differences in response to temperature between plants might be attributed to differences in size at the time of induction, in addition to differences in genetic background (even within a cultivar). Low nitrogen status has been shown to effect the rate of initiation in seedlings (Brewster 1983). Spatial variation of nitrogen supply may therefore result in variation in flowering time. Rabinowitch (1990a) reported that genetic variation exists within cultivars, but the effect of this genetic variation on flowering time has not been thoroughly investigated.

2.3 Factors affecting seedlot quality: seed development, maturation and time of harvest

2.3.1 Seed development

Fertilisation of the ovule occurs within twelve hours of pollination and is completed in three to four days (Chang and Struckmeyer 1976). Initially temperature has little effect on ovule or endosperm development (Rabinowitch 1990b). Pollination itself stimulates initial development (Brewster 1997a). The embryo of the developing ovule only becomes evident six days after pollination, from which time non-fertilised ovules abort (Chang and Struckmeyer 1976). The critical temperature for high temperature-induced abortion is not well reported, but temperatures of 40 ° C may cause up to 50% abortion of fertilised ovules (Rabinowitch 1990b). This response would be expected to be modified by many factors including genetic variation, environmental differences and spatial distribution in umbel, but these were not reported. Localised heating on the side of the onion umbel facing the sun causes a condition known as dollar spot, in which flower and ovule abortion occur. The severity is determined by the angle of the sun, air temperature, wind speed and direction, and the nature of the umbel (Tanner and Goltz 1972). Chang and Struckmeyer (1976) reported that high temperatures can cause either an imbalance between embryo growth and/or an early dissociation of nucellus cells from the ovule, resulting in seed abortion.

After the initial development of the globular, liquid pro-embryo, growth is controlled by temperature. Traditionally investigation reported development of the ovule in terms of days after flowering and noted that this was affected by temperature (Chang and Struckmeyer 1976; Gray and Ward 1987; Rabinowitch 1990b). However, a more useful quantitative description of the system in terms of day-degrees has been reported by Brewster (1997a). Cell walls began to develop in the endosperm 330 day-degrees above 0 ° C after flowering. This was equivalent to fourteen days after pollination reported by Chang and Struckmeyer (1976), or nine to ten days after pollination reported by Rabinowitch (1990b).

Maximum fresh mass occurred after 570 day-degrees above 0 ° C after flowering, and after 810 day-degrees the seeds matured, capsules began to split and seeds were shed (Brewster 1997a). Both environment and genotype greatly affected the total duration of growth of developing seeds, with cultivars ranging from 31 to 76 days from full bloom to physiological maturity (Rabinowitch 1990b).

Acquisition of desiccation tolerance

Desiccation tolerance is the ability to withstand drying and maintain viability and the capacity to germinate. Gray and Ward (1987) reported that desiccation tolerance occurred approximately 46 days after flowering in the cultivar Rijnsburger Robusta. This coincided with the attainment of maximum dry mass. This is in contrast to leek where desiccation tolerance occurred 70 to 80 days after flowering (Gray and Ward 1987). This is indicative of the slower development of leek seeds despite similar flowering times. Hilhorst and Toorop (1997) reported that desiccation tolerance is controlled by and is extremely sensitive to changes in ABA concentration. Other changes induced by ABA include the modification of carbohydrate metabolism and the slow desiccation of intolerant seeds. Slow drying promotes the production of late embryogenesis abundant (LEA) proteins that are associated with the acquisition of desiccation tolerance (Hilhorst and Toorop 1997). However, the work done in the elucidation of induction of desiccation tolerance has been done in model species such as *Arabidopsis thaliana*, and has not been shown to apply in *Allium* species.

Pollock and Lloyd (1994) showed a change in carbohydrate metabolism during seed development in the cultivar Rijnsburger. Fructans formed a transitory store in developing seeds, peaking at 23 days after flowering before returning to low levels at 40 days after flowering. This change in carbohydrate metabolism did not correspond with maturity, as indicated by seed mass, or imbibition. It remains to be seen if a link exists between the change in carbohydrate metabolism and desiccation tolerance. The timing of events correlates with Gray and Ward (1987) and is seen in model species (Bewley and Black 1994; Hilhorst and Toorop 1997).

Physiological maturity

Physiological maturity is the attainment of maximum germination percentage in the seedlot. The time to physiological maturity is highly dependent on genotype and environment (Rabinowitch 1990b). Steiner and Akintobi (1986) observed that physiological maturity coincided with seed shattering in onion. Brewster (1997a) found that seed shattering occurred after 810 day-degrees above 0 °C. Gray and Ward (1987) showed that physiological maturity occurred approximately 10 days later in leek than in onion, when grown under identical conditions. Prior to the attainment of physiological maturity, both leek and onion germinated to varying percentages from 24 to 31 days after flowering (Gray and Ward 1987) or after 570 day-degrees above 0 °C (Brewster 1997a). ABA concentration and the osmotic environment of the seed are responsible for the prevention of precocious germination in model species (Bewley and Black 1994). Hilhorst (1995) concluded that the effects of ABA and the osmotic environment are related and not readily distinguishable from each other. The role of these inhibitory factors is to maintain the embryos in a developmental state, when conditions are suitable for germination, until maturation is attained.

Mass maturity

Mass maturity is the attainment of maximum dry mass per seed. Steiner and Akintobi (1986) reported in the cultivar Sweet Spanish, that maximum dry mass per seed occurs 35 days after flowering, and then remains constant. Gray and Ward (1987) studied the growth of the seed in greater detail and found that during the first 31 days of growth, seed dry mass increased exponentially, with maximum mass occurring 45 days after flowering in the cultivar Rijnsburger Robusta. Brewster (1997a) observed that mass maturity occurred at the beginning of capsule splitting, 810 day-degrees above 0 °C after flowering. This corresponds with the findings of Steiner and Akintobi (1986).

Maximum seed fresh mass in onion occurs prior to maximum seed dry mass. The general theoretical model presented by Bewley and Black (1994) indicates that fresh mass peaks before dry mass as histodifferentiation and cell expansion cause a rapid rise in fresh mass. Dry mass peaks when reserve materials accumulate after histodifferentiation. Gray and Ward (1987) reported that protein and oil reserves are not observable in the seed until cell walls form in the endosperm 45 days after flowering. Embryo growth differentiation ceased 30 days after flowering.

Dormancy

Dormancy has not been reported in onions. Numerous studies have investigated the effects of the maternal environment on subsequent seed performance (eg. Gray and Steckel 1984) and have reported that primary dormancy was absent. However, Gray and Steckel (1984) reported that onion seeds grown at high temperature, had a lower maximum temperature at which the seeds germinated, than seeds grown at low temperature. Therefore, according to the definition of Vleeshouwers et al (1995), the seeds grown at high temperature exhibited a degree of primary dormancy. The level of dormancy may not only act to inhibit germination, but may affect the rate of growth and lead to abnormal seedlings (Hilhorst and Toorop 1997). Thus dormancy may be associated with seed vigour. In contrast to onion, dormancy in leek has been recognised to occur occasionally due to high temperature induction during seed drying (Gray et al 1989).

Deterioration

Seed deterioration usually begins at physiological maturity and continues throughout the life of the seed until death or germination (Hampton and TeKrony 1995). The rate of deterioration is greatly influenced by genetic, production and environmental factors, but is primarily controlled by moisture content and temperature. Onion seeds can retain high physiological quality for up to ten years in storage (Stumpf et al 1997). Deterioration changes shown to occur in seeds include: membrane damage; genetic damage; changes in respiratory activity; enzyme and protein changes; hormonal changes; and accumulation of toxic metabolites (Coolbear 1995). Powell and Matthews (1981) demonstrated a relationship between deterioration and reduced field emergence.

Onion is an orthodox species, that is, the effects of deterioration can be minimised when the seeds are stored at a low moisture content (Bewley and Black 1994). Ward and Powell (1983) have shown that onion seeds deteriorate less at high seed moisture content, contrary to most orthodox seeds. The authors proposed that the raised moisture content enabled repair processes to be activated which counteracted the normal deterioration observed during storage. This response appears to be similar to that achieved via osmoconditioning in orthodox species.

Variation in deterioration has been proposed as a source of variation in germination within a population of onion seeds (Ellis and Butcher 1988). These authors proposed that the last seeds to germinate were the most deteriorated. These seeds tended to produce weaker seedlings. Finch-Savage (1986) reported that slow germinating seeds of three species (including onion) produced fewer normal healthy seedlings. Mohamed-Yasseen and Splittstoesser (1990) reported that deterioration led to the death of root tip cells and stunted root systems.

2.3.2 Time of harvest

Several studies have been conducted to determine the optimum time for harvesting onion seedlots in a range of production environments. Steiner and Akintobi (1986) found a close correlation between umbel and seed moisture content and recommended harvesting when the umbels had a moisture content between 66 and 52%. Globerson et al. (1981) found optimum harvesting conditions in several cultivars at seed moisture contents between 30 and 40%. Vik (1992) recommended harvesting when the seed moisture content was between 53 and 16%. Neal and Ellerbrock (1986) concluded that harvesting should be conducted when 25% of the capsules have dehisced, but found no effect of timing of harvest on seed quality over a period of 16 days.

In these studies optimum times for harvest with respect to seed quality were largely determined by seed weight and germination percentage. Little information was reported on the effects of timing of harvest of onion seed crops on seed quality characteristics including, rate and uniformity of germination, germination at sub optimal temperatures and seedling quality.

2.4 Treatment to improve seed quality

A number of studies have examined the effects of priming on onion seedlot quality. Priming has generally been carried out in polyethylene glycol (PEG) and has been found to decrease the time to germination and increase the uniformity of germination (Ellis and Butcher 1988; Gray et al. 1990). Murray et al. (1992) reported that the results found in the laboratory were significantly correlated with field emergence. Priming was found to be of particular benefit to seeds sown in cold soils during spring (15 ° C) rather than summer when soil temperatures were higher (24 ° C).

Ellis and Butcher (1988) reported that priming at -1.4 MPa at 20 ° C for seven days increased the rate of germination of a number of seedlots at a range of temperatures. However, the base temperature of germination was found to be a genotypic characteristic that was unaffected by priming.

The optimum priming water potential was reported to be -1.19 MPa (at 15 ° C) (Ali et al. 1990). Gray et al. (1990) found that at water potentials higher than -1.0 MPa, some seeds germinated during treatment.

Bujalski et al. (1989) and Bujalski and Nienow (1991) reported that germination could be further improved by aerating the PEG solution with air enriched with oxygen. The latter study also examined the effect of redrying after treatment, and found that although the time to germination was increased compared with primed seeds that weren't dried back, the seeds primed in solutions with enriched air still germinated more rapidly than untreated seeds.

Gray et al. (1990) reported that there was no increase in the cell volume or number of cells per embryo after priming.

2.5 Seed quality measurement in onion

Recent work in this area has so far not been able to provide an accurate and precise method for the determination of seed vigour in onion. Kretschmer (1996) reported a relationship between germination in soil and field emergence. The test ranked seedlots in terms of potential performance, but was of little predictive value. Problems with such methods have been recognised by the ISTA, which has led to the recommendation of two methods of comparing seedlot quality - the conductivity test and accelerated aging test (Hampton and TeKrony 1995).

The conductivity test has been shown to be of limited use in onion as the seeds contain a semi-permeable layer composed of cutin that restricts the leakage of solutes, thereby producing variable results (Taylor 1997). Lee et al (1995) showed that the leakage of sugars could be developed into a test to measure seed germinability.

Finch-Savage (1986) observed that late-to-germinate onion seeds produced fewer normal healthy seedlings. The author also found that the rate of germination within a seedlot is related to seed vigour. Wheeler and Ellis (1994) also reported that the rate of germination was a key determinant of seed quality. These observations, however, have not led to the development of a seed vigour test. Dornbos (1995a) noted that this type of seed vigour test has the potential to be influenced by seed size. Small seeds with a large surface area to volume ratio imbibe more rapidly and germinate earlier than large seeds. The results may be biased towards seedlots of small seeds. Size variation could be overcome by measuring both germination time and seed size. Current commercial practice evaluates seed quality only by germination testing as it is the only standardised methodology available.

3. Carrot seed quality

The production of carrot taproots for international markets is an expanding industry. The fresh carrot market pays a premium for high quality taproots falling within stringent size ranges. Taproots falling outside the specified size requirements represent a potential loss of income. Carrots like many other vegetable crops are established from seed. The quality of the seed sown has been reported as having an impact on the uniformity of seedling emergence (Gray and Steckel, 1983), stand density (Benjamin, 1982), and in some cases marketable yield of taproots (Currah and Salter, 1973). In trials akin to commercial practice from 3 to 72% of the variability in taproot size at harvest can be accounted for by differences in time of emergence of individual seedlings depending on the time of year trials were undertaken (Benjamin, 1984). Values ranging from *ca.* 20 to 50% tend to be the most common (Salter *et al.*, 1981; Benjamin, 1982; Benjamin, 1984). Therefore the establishment phase can be considered one of the most important factors governing the uniformity of mature taproots (Benjamin, 1984 and Salter *et al.*, 1981).

The spread in time of seedling emergence can be attributed to the quality of the seed (Gray *et al.*, 1991) and environmental conditions. The environmental conditions that the seed germinate under are commonly referred to as 'field factors' and encompass variables such as temperature, soil moisture and physical impedance (Finch-Savage and Pill, 1990; Tamet *et al.*, 1996; Hegarty, 1978). Seed quality refers to the performance of seed under a range of environmental conditions.

The internationally recognised method of evaluating carrot seed quality is to germinate seeds at 20°C and after 14 days record the proportion of seedlings with the necessary structural components for development as a normal plant (ISTA, 1999). This test provides a measure of the level of seed viability, however, given that the viability test is done under ideal germination conditions it does not always equate to the performance of the seed under sub optimal conditions for germination (Hegarty, 1971; Matthews, 1980). Therefore alternative methods to the ISTA viability test have been examined to assess seed performance under field conditions. Some researchers have shown that germinating carrots seeds under a sub-optimal temperature of 10°C, or a controlled deterioration test to provide a better measure of seed vigour than the ISTA test (Hegarty, 1971; Matthews, 1980).

Gray and Steckel (1983a) undertook an extensive comparison of methods for evaluating seed vigour on 18 separate carrot seedlots displaying a similar range of viability to that used commercially in the United Kingdom. They found that the results from the ISTA test were equivalent and sometimes better than other common methods of assessing seed vigour (i.e., cold test (10°C), slope test, controlled deterioration test). Of particular note was the finding that the variability of individual seedling weights at establishment was strongly correlated with the coefficient of variation (CV) of embryo length within the seed. Additional work by Gray and colleagues indicated that a measure of CV of embryo length combined with a level of seed viability could be used to predict seedling size variability and stand density under a wide range of conditions (Gray and Steckel, 1983b; Gray *et al.*, 1986; Gray *et al.*, 1991). From their assessment of commercially available seed they proposed a universal classification of carrot seed with low, medium and high size variability as follows: CV of embryo length less than 20% 'low'; 21 to 30% 'medium'; and above 30% 'high' (Gray *et al.*, 1986). Approximately 5% of commercially available seed in the UK were categorised as low, 70% as medium and 25% as high (Gray *et al.*, 1986).

Further field trials assessing the effect of variability of embryo size on the variability of mature taproots showed that the CV of seedling sizes 20 days after 50% emergence was positively correlated with CV of embryo size and spread in emergence regardless of stand density (Gray *et al*, 1991). While there have been some reports of a positive correlation between CV of plant sizes soon after emergence and the CV of taproot sizes at maturity (Salter *et al*, 1981) this has not always been found to be the case (Gray *et al*, 1986). Further evidence suggests that this relationship is more likely to exist at high densities (>200 plants.m⁻²) (Gray *et al*, 1991). These findings indicate that competition *per se* is not a primary source of variation in taproot size but magnifies any initial variation within the crop at the time of seedling emergence (Salter *et al*, 1981; Li *et al*, 1996; Weiner and Thomas, 1986; Benjamin, 1982). Figure 3.1 illustrates the relationship between variability of taproots at harvest and variability of embryo length within planted seed at a high and low density.

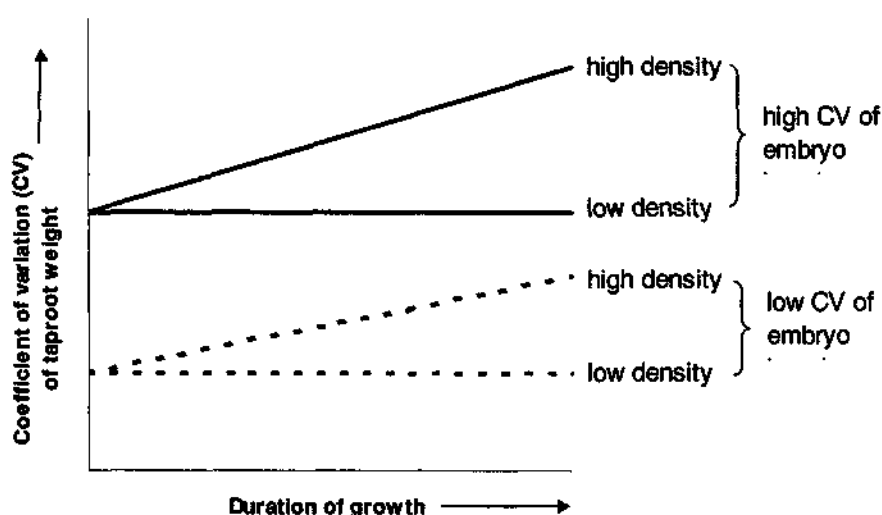


Figure 3.1 Illustration of the variation in taproot size with crop growth, associated with low (<20%) or high (>30%) CV of embryo size at a low (50 plants.m⁻²) or high (>250 plants.m⁻²) stand density. Adapted from Gray and Benjamin (1993).

Physiological treatments to enhance the performance of vegetable seed in the field have been widely trialed. The seed treatments trialed to enhance carrot seed performance have primarily been based on hydration and include; osmo-conditioning, matri-conditioning and hardening. All three seed treatments generally improve the rate of seedling development, synchronicity of emergence and the proportion emerging in the field (Currah and Salter, 1973; Finch-Savage and Pill, 1990; Khan *et al*, 1992), sometimes resulting in higher yields of carrot (Szafirowska *et al*, 1981; Currah and Salter, 1973). However, in some trials osmo-conditioning seeds has increased seedling size variability (Finch-Savage, 1990). This was thought to be due to the germination of seed that developed into small seedlings following treatment whereas similar seed in the control (untreated) group failed to germinate. Further, any advantage gained from pre-conditioning seeds often diminishes when either ambient air temperature increases (above approximately 10°C), field conditions become more favourable to germination (Currah *et al*, 1974; Szafirowska *et al* 1981; Finch-Savage and McQuistan, 1989; Bodsworth and Bewley, 1979), or seed is dried back to original water potential to plant through conventional precision air seeders (Murray, 1989).

An alternative planting technique has been developed that enables sowing of pre-germinated seed suspended in a gel medium to protect the emerging radicles - this is referred to as fluid drilling. While a more rapid emergence and predictable stand density can be achieved (Pill, 1995), the lack of precision in spacing and short amount of time to plant seeds have proved to be two obstacles to the wider use of fluid drilling in vegetable production (Rubatzky *et al*, 1999). The latter problem can be partially overcome by the addition of the plant hormone abscisic acid to pre-germinated seeds to inhibit further development prior to planting (Finch-Savage and McQuistan, 1989). However, this technique has not been proven to be economically advantageous in commercial production of carrots and is therefore not widely used.

In summary, factors influencing the variation in carrot taproot sizes have received some attention in the literature, particularly in the past 20 years. The most comprehensive studies have been undertaken in the UK on soils of a sandy loam or light sandy texture. Outcomes of these studies have shown that the variation in taproot size at harvest is largely influenced by the initial variability in plant size at emergence and the magnitude of competition between plants, i.e., stand density. While field factors have a large impact on the rate and spread in seedling emergence, seed quality is also important. The variation in embryo size within a seedlot has been shown to provide a reliable measure of the potential variation in seedling size.

4. Brassica seed quality

The need for quality seed for vegetable brassica industries has been recognised both for production systems relying on modular transplants and those using direct seeding (McCormac and Keefe, 1990; Jett *et al.*, 1996). The importance of various germination characteristics varies depending on the conditions under which the seed is expected to germinate, but generally, high quality seeds germinate quickly and evenly into normal seedlings while poor quality seeds germinate slowly and less uniformly. Seeds are considered to be of high quality if they have the capacity to germinate rapidly in stressful environments such as high or low temperatures, drought or waterlogging. In addition to capacity to germinate, larger seeds are considered to be of higher quality than smaller seeds. Often germination and seed size are found to be correlated (Heather and Siczka, 1991). Many of the production and harvesting practices and technologies used by brassica seed producers are specifically targeted at achieving the highest possible seed quality in addition to high crop yield.

Until recently, assessment of brassica seed quality has been by determination of germination capacity, that is, germination of seeds under ideal temperature and moisture conditions. However, this measure fails to provide any information on the ability of seeds to perform under less than ideal conditions. Workers have investigated alternative ways of assessing seed quality in brassica species, for example, high quality brassica seedlots germinate rapidly over a range of temperatures (Wilson *et al.* 1992) and water potentials (Still and Bradford, 1998), have slow solute leakage during imbibition (Thornton *et al.*, 1990), and, when coated, the absence of fluorescence due to sinapine leakage (Taylor *et al.*, 1993). Seed companies have developed individual methods of seed quality assessment. One such method is the soil germination test, where seed is sown in soil in a glasshouse and emergence recorded after a period of time. It can be seen, therefore, that although the importance of seed quality has been recognised, there are a range of methods of brassica seed quality assessment that are currently in use, and there is no single measure of seed quality that is widely accepted.

There are many aspects of seed crop management, production environment and harvesting methods that influence the quality of seed produced. Factors identified from the literature as important determinants of seed quality in vegetable brassica crops include: seed size and cultivar (Heather and Siczka, 1991); seed maturity at harvest (Still and Bradford, 1998); seed deterioration or aging (Powell *et al.*, 1991); and seed coat integrity (McCormac and Keefe, 1990). While priming has been found to overcome poor seed quality in brassica species including broccoli (Jett *et al.*, 1996) and a range of canola cultivars (Zheng *et al.*, 1994), the objective of seed producers is to obtain high quality seed. Of the factors affecting brassica seed quality, anecdotal evidence supplied by local seed companies indicated that seed coat damage during threshing is an area of particular concern. Damaged seed coats cause increased rates of water uptake in cauliflower seeds (McCormac and Keefe, 1990). More rapid imbibition led to decreased growth rates and reduced emergence in soil. Therefore seed coat integrity is likely to be an important determinant of seed performance in locally produced brassica crops.

5. Radiata pine seed quality

5.1 Introduction to Radiata pine

Pinus radiata D. Don (radiata pine, Monterey pine) is an evergreen tree belonging to the family Pinaceae of the Gymnosperms. Radiata pine has a natural distribution of about 6700 hectares in coastal central California in the U.S.A. and about 200 hectares on Guadalupe and Cedros Islands off the coast of Mexico. As it is a rapidly growing species it has potential for shelter belts and a source of softwood for construction and pulpwood. The species has been introduced and is now grown extensively in Australia, New Zealand and South Africa. Breeding programs for improved growth form are under way (Fielding 1961; Lee 1994).

Demand for the species has led to the development of large areas of plantation forest in Australia. Plantations are established from nursery transplants, usually produced from seed orchard seeds grown into seedlings in nurseries across south-eastern Australia. The seeds are generally produced in open- or wind-pollinated (OP) seed orchards. Increasingly control-pollinated (CP) seed with known parentage is being used, as it offers better genetic gain (Arnold 1990).

Radiata pine seeds presently being planted in commercial nurseries generally demonstrates relatively high germination capacity. However, the seed is slow to germinate which is suggested to indicate relative dormancy (Gosling 1988). The slow germination rate increases vulnerability to predation and adverse environmental conditions in the nursery. Other seed quality parameters including seed size and the range of conditions over which the seed will germinate have been recognised but relatively little research has been conducted with this species.

Mature conifer seed consists of tissue of three different genetic types. The seedcoat and remnants of the nucellus (the integument layers) are derived from maternal tissue. The megagametophyte differs from the endosperm of angiosperms although it performs the same nutritional function. The endosperm is triploid and arises when a sperm nucleus unites with the two polar nuclei of the embryo sac, whereas the megagametophyte is haploid and develops from the megaspore which is the maternal gamete. The maternal contribution to the seed anatomy (2n seedcoat, 1n megagametophyte, 1n embryo) is thus more dominant compared with the male contribution (1n embryo). This explains the strong genetic control on germination parameters (Edwards and El-Kassaby 1996), particularly considering the role of the maternal tissues in dormancy (Barnett 1996), discussed in more detail below.

The majority of the reserve foods of pine seeds are stored in the megagametophyte, only small quantities are present in the embryo. The reserves are primarily lipids and proteins (Stone and Gifford 1997). The embryo lies inside a cavity in the megagametophyte. The embryo is thus dependent on the megagametophyte for food and water as absorbed moisture passes through the megagametophyte to the embryo (Baron 1978). The characteristics of the seedcoat, integument layers and megagametophyte play a role in regulating water uptake by the embryo, and hence germination rate. Tillman-Sutela and Kauppi (1995a, 1995b) reported that water uptake by Scots pine and Norway spruce was regulated by the megaspore membranes between the seed coat and megagametophyte.

5.2 Factors affecting seed quality: dormancy

Many conifer seeds, including radiata pine, benefit from a period of moist chilling and are thus considered dormant. It is not clear what mechanisms are involved in dormancy in conifer seeds, however, the role of seed coat constraints and germination inhibitors have been examined to some extent.

It has been suggested that dormancy in some conifer seeds is controlled by the seedcoat and other tissues surrounding the embryo. Barnett (1972, 1976, 1996, 1997) found that the ratio of seedcoat weight to total seed weight gave an estimate of the relative dormancy of seeds of the southern pine species. Compromising these tissues led to improved germination of loblolly (Barnett 1972, 1976; Carpita et al. 1983), longleaf, slash, shortleaf (Barnett 1976), sugar (Baron 1978), eastern white (Kozłowski and Gentile 1959) and western white pine (Hoff 1987) and white spruce (Downie and Bewley 1996) seeds. Improved germination could be due to improved permeability, or the removal of the barrier to expansion of the embryo.

Tillman-Sutela and Kauppi (1995a, 1995b) found that the nucellar layers rather than the seedcoat itself were the major barrier to water uptake in Scots pine and Norway spruce. This tissue has also been found to be an important factor in regulating the water uptake in loblolly, sugar and western white pine seeds (Barnett 1976; Baron 1978; Hoff 1987).

As dormancy is overcome by stratification, this suggests that the barrier to water uptake and radicle expansion is removed or weakened during treatment. Downie et al. (1997) reported that there was a decrease in the force needed to puncture the tissues surrounding the embryo during stratification of dormant white spruce seed. An increase in the activity of the enzyme endo- β -mannanase was also observed. This enzyme has been found to be involved in cell wall weakening in the endosperm of tomato seeds (Downie et al 1997).

It has been suggested that coat impermeability to oxygen may play a role in seed dormancy in some pine species including radiata pine (Rimbawanto et al. 1989). Higher respiration rates have been recorded in decoated radiata (Rimbawanto et al. 1989), loblolly (Barnett 1972), and eastern white (*Pinus strobus* L.) (Kozłowski and Gentile 1959) pine seeds compared with intact seeds. Kozłowski and Gentile (1959) also found that seeds had increased oxygen uptake in high oxygen environments and suggested that this evidence indicated that the seedcoat inhibits germination by restricting oxygen and water uptake. Barnett (1972) suggested that oxygen uptake is not a controlling factor in loblolly seed germination as stratified or clipped seeds had similar uptake rates as intact and unstratified seeds. The seedcoat and probably also the dense megagametophyte present barriers to oxygen diffusion, but from the evidence it is difficult to say to what extent this controls conifer seed germination.

Dormancy of loblolly pine seeds varies between parent trees but was found to be constant for individual trees over 4 years of seed production (McLemore and Barnett 1966). Germination of Virginia pine seeds has also been found to be significantly influenced by maternal effects (Bramlett et al. 1983). Given that the seedcoats of pine seeds are of the same genotype as the parent plant, maternal effects on germination may therefore be a consequence of dormancy imposed by the seedcoat or other maternal tissues. Barnett (1996) suggested that if seeds are not pretreated to overcome maternal seedcoat effects on germination, then the contribution of the embryo to growth processes might be obscured.

In some species, germination of decoated or clipped seed was further improved by a period of stratification. Ocala sand pine seed germination was not improved by removing the seedcoat prior to sowing in soil (Outcalt 1991). This suggests that there may be more than one mechanism involved in conifer seed germination. Noland and Murphy (1986) suggested that a germination inhibitor might be involved in dormancy of sugar pine seeds. Schneider and Gifford (1994) observed a decrease in the synthesis of specific proteins when loblolly pine seeds was overcome and suggested that these proteins may be germination inhibitors.

5.3 Factors affecting seed quality: seed size

Studies on seed size and weight frequently report correlations between seed size and germination capacity, growth rate, and resistance to stress. Reich et al. (1994) found that among 24 Scots pine populations, seed weight was not related to growth rate but was positively related to hypocotyl height and cotyledon number. However, the effect of seed weight had become less apparent or disappeared after five to seven years of growth. In contrast, Ching (1963) reported that the heaviest Douglas-fir seedlots contained more nitrogenous compounds and lipids and had a higher rate of lipid reserve breakdown and a faster growth rate than lighter seedlots. Dunlap and Barnett (1983) also reported that larger loblolly pine seeds germinated more rapidly and produced larger germinants after 28 days of growth compared with smaller seeds.

Donald (1968) found that grading seeds from bulked radiata pine seedlots had no effect on germination, except for the smallest seed (less than 2.83mm) which had significantly lower germination capacity and rate. In contrast, Menzies et al. (1985) reported that smaller radiata pine seeds had poorer germination capacity and produced taller seedlings after one year of growth compared with larger seeds. The seed was graded according to weight, however. Donovan (1996, 2001) reported that the smallest seeds (less than 4mm) of a bulked radiata pine seedlot showed more rapid germination and were less susceptible to soak injury than larger seeds of the same seedlot. However, as this was a bulked seedlot the smaller seeds may have represented families with better germination characteristics.

Seed size varies between seeds produced by a single parent plant, and between parent plants. Seed size variation in coniferous species has been attributed to the effect of the position of the seed in the cone, the height of the cone on the parent tree, and the aspect of the cone in the crown (Edwards and El-Kassaby 1996). Adams and Kunze (1996) reported a positive correlation between the number of white spruce cones per tree and the number of seeds. White spruce and black spruce trees that produced more cones tended to have smaller seeds than those with fewer cones.

Environmental conditions also influence seed weight. Generally, water availability, temperature, soil nutrients, photoperiod and light quality have the greatest effect (Hilhorst and Toorop 1997).

5.4 Factors affecting seed quality: cone development

Radiata pine trees have a juvenile phase of about nine years, during which no reproductive structures are produced (Fielding 1960). The production of cones is sometimes referred to as flowering. Female radiata pine cones (or seed cones) are produced several years prior to male (pollen) cones. Female cones develop in the place of lateral shoots; male cones replace short shoots.

Most species of *Pinus* display a three year reproductive cycle. Male and female cones are initiated in the first year when apical meristems irreversibly develop into strobili (cone) initials

(Owens and Blake 1985). Sweet and Bollmann (1970) reported that radiata pine development in New Zealand takes about 30 months from strobili initiation to cone maturity. Development ceases over the winter period then resumes with the onset of spring (Owens and Molder 1984). Seasonal growth appears to coincide with vegetative growth rather than aspects of seed development (Sweet and Bollmann 1970). However, Cremer (1992) reported that most of the growth of male radiata pine cones in Canberra (Australia) occurred between July and September at a time when vegetative growth was slow due to low temperatures. In the same study, female radiata pine cones were found to grow mainly between October and January, about 13 months after pollen shed.

Owens and Blake (1985) suggested that the relatively long reproductive cycle of conifers resulted in increased susceptibility to poor seedlot quality. Environmental conditions and seed orchard cultural practices may adversely affect the quality of seeds developing on the tree over this period. Stoehr et al. (1998) reported that the germination capacity and rate of white spruce seeds was affected by the seed orchard environment (particularly temperature).

Cone initiation is affected by a number of environmental factors including temperature, light intensity, photoperiod, moisture and nutrition. However, independent of environmental conditions, conifers do not produce similar quantities of cones in consecutive years. Trees often demonstrate biennial bearing, where an abundant crop is followed by a small crop. Resources may be directed to the developing cone crop at the expense of cone initiation and vegetative growth (Owens and Blake 1985). Significant energy goes into the reproductive growth, reproductive structures make up 16% of the total dry weight of mature *Pinus radiata* trees (Fielding 1960).

Complicating any investigation of factors influencing flowering is the interdependence of many of the determining factors. Reviews on flowering in pines include Matthews 1963; Jackson and Sweet 1972; Lee 1979; Lavender and Zaerr 1985; and Pharis and Ross 1995.

Positive correlations between higher summer temperatures and floral initiation have been reported in several conifer species (Daubenmire 1960). Fober (1976) found that high temperature increased reproductive bud differentiation in *Pinus sylvestris*. Warmer spring conditions in the year before reproductive bud differentiation was also important as it allowed greater accumulation of storage substances. Owens and Blake (1985) suggested that high temperature during bud development was beneficial because of the increased rate of photosynthesis, carbohydrate metabolism, water and nutrient uptake. However, in most studies it is difficult to separate the effects of increased temperature from those of light intensity and moisture.

Most studies investigating the influence of light intensity on floral initiation have been indirect. They relate flowering to crown exposure, slope, shading, and floral distribution within the crown. Generally, branches exposed to higher light intensity tend to flower more than shaded branches. Thinning, which increases light exposure, has been a successful method of floral induction in many conifer species including radiata pine (Eldridge 1966). Simpson and Powell (1981) noted greater bud production of trees on slopes facing the sun compared with other aspects. Flowering has also been found to be greater on the side of the crown most exposed to the sun (Smith and Stanley 1969).

Photoperiod has been demonstrated to have no effect on floral initiation in *Pinus* species (Mirov 1956; Waring 1958; Lanner 1963) but there is some evidence indicating that photoperiod may

affect the sex of the reproductive buds in *Pinus contorta* (Longman 1961). Giertych (1967) suggested that pollen cone buds were initiated during longer photoperiods than seed cone buds. Owens and Blake (1985) suggested that any photoperiodic effect on cone production may be due to a complex interaction between decreased shoot elongation and subsequent reproductive bud development, since a reduction in vegetative growth rate appeared to be a requirement for cone initiation.

Studies investigating the effect of moisture availability on flowering suggested that bud production improved with increasing moisture stress (Owens and Blake 1985). Conifer trees on grown on more fertile soils produced more seeds than those grown on less fertile soils (Matthews 1963). Sarvas (1962) reported that *Pinus sylvestris* trees grown on less fertile sites produced fewer pollen cones and seeds than trees on more fertile sites. The nutrients most important in flowering are nitrogen and phosphorus (Jackson and Sweet 1972).

5.5 Factors affecting seed quality: seed development

Fertilisation in radiata pine seeds in New Zealand takes place about 14 months after pollination (Rimbawanto et al. 1989). During this time the seed may be affected by both environmental conditions and the physiology of the parent tree. There has been little work examining the effect of these factors on seed quality.

Rimbawanto et al. (1989) reported that in New Zealand, radiata pine embryos ceased growth by about late April, but germination was poor at this stage. Over the following month seed moisture content fell to about 20% (fresh weight) and germinability increased to 30%. One month later germinability had increased to 90%, and continued to improve over the next three months (Rimbawanto et al. 1988, 1989). Kong et al. (1997) suggested that germinability of white spruce is acquired with desiccation, but germination itself is suppressed by ABA. Tissue sensitivity to ABA subsequently declines, and seed germination is controlled by environmental factors such as water availability.

5.6 Factors affecting seed quality: seed orchard management

Cultural practices applied to the seed orchard may affect seed quality. Seed orchard management aims to produce a sufficient quantity of high quality seeds of desirable genotypes within the shortest possible time. The effects of seed orchard culture on seed quality in the southern pines was reviewed by Barnett (1996). Generally, however, studies examining cultural practices in seed orchards have been limited to the effects on cone production. Rarely have studies been expanded to determine the effects of cultural practices on seed quality.

A range of cultural techniques have been found to affect cone production by conifers. There is interest in promoting flowering to overcome the relatively long generation interval in conifers (Ross 1977) and to ensure all clones in an orchard contribute to a seed crop (Fries 1994). These management techniques generally affect cone production by controlling the environmental factors that influence cone production (discussed above). The effect of cultural practices on seed quality varies between species (Barnett 1996) and between families (Edwards and El-Kassaby 1996). Generally, it is considered that practices that improve tree vigour, such as fertilisation, insecticide application, reducing competition, irrigation, thinning and pruning will improve cone production (Barnett 1996). These practices were also found to increase the number of seeds per loblolly pine cone. Cremer (1992) reported that there was a positive relationship between the

vegetative growth in one season, and cone crop the following season, and concluded that this was due in part to tree vigour and in part to an increased number of cone sites on bigger trees.

An area of growing concern is the effect of control pollination techniques on seed quality. Control pollination requires greater handling of the developing cones and limits the range of pollen genotypes. Industry results suggest that less than 50% of hand pollinated cones in commercial seed orchards are retained through to maturity. There is no clear relationship between seed number and development and retention of cone.

5.6.1 Fertiliser application

The effect of fertilisers on flowering in woody plants (Jackson and Sweet 1972; Brazeau and Veilleux 1976) and specifically in pines (Lee 1979; Owens and Blake 1985) has been reviewed. The success of fertiliser application in promoting flowering varies with nutrient type and time of application. Sweet and Will (1965) reported that adequate levels of phosphorus may be necessary for male flowering in *Pinus radiata* and Sweet and Hong (1978) found no relationship between cone production and nitrogen fertilisation. Since many factors may interact with the treatment, the effect of fertiliser application is difficult to determine.

Barnett (1996) reported that fertilisation increased the size of longleaf pine seeds. Larger seeds tend to germinate more rapidly, the treatment is thus considered to reduced dormancy.

5.6.2 Girdling, banding and strangulation

Such techniques have been used in a wide range of *Pinus* species with significant success (Owens and Blake 1985). However, although flowering is generally increased, there is often a variable impact on cone set and the damage to the tree can be significant. Ross (1977) reported that girdling 2 year old grafts and 4 year old douglas-fir seedlings did not improve flowering, however, treatment of mature trees promotes flowering.

5.6.3 Moisture stress

Controlled irrigation, drought treatments and root pruning have all been used successfully to increase flowering in *Pinus* (Owens and Blake 1985). Trials using containerised trees demonstrated that moisture stress enhanced flowering in many species. Sweet and Will (1965) induced male cone production by radiata pine trees by limiting moisture and nutrient availability. Richie and Hinkley (1975) suggested that pre-dawn moisture stresses of -1.2 to -2.0 MPa were required to induce flowering but field moisture stress conditions rarely exceed -0.7 to -0.8 MPa (Cade and Jackson 1976). Ross (1977) found that although water stress promotes flowering of mature trees, the treatment did not improve cone production by 2 year old douglas-fir grafts and 4 year old seedlings. Cremer (1992) found that the production of both male and female cones by radiata pine trees in Canberra was related to rainfall in the period between September and January, but reported that the effect of irrigation was puzzling. However, reviews of seed quality (Delouche 1980; Fenner 1992) reported that moisture stress during flowering reduced the number of seeds produced, while moisture stress during seed development reduced seed weight and increased the concentration of storage proteins.

5.6.4 Thinning/Tree density

Eldridge (1966) reported that thinning radiata pines led to increased seed production per acre for five years after treatment. Although significant increases in cone number per tree can be obtained through thinning, the decreased number of trees will also affect yield per hectare.

5.6.5 Light

Thinning also reduces competition for light between individual trees. Wheeler et al. (1982) reported that the vegetative phase of *Pinus contorta* could be shortened with increased light exposure. Cone yields in subsequent years also increased. Owens and Blake (1985) suggested that early onset of maturity of the trees resulted from attainment of a certain size rather than a given number of growth cycles.

5.6.6 Growth regulators

Although a range of growth regulating substances have been investigated for their possible influence on flowering behaviour in conifers, only GA treatments have been found to have significant benefits (Philipson 1990). Application of GAs has increased cone production by douglas-fir (Ross 1977), Norway spruce (Johnsen et al 1994), and loblolly pine (Greenwood 1977) trees, among other conifer species. The effect of GA is often increased when combined with another treatment such as girdling, water stress, fertilisers or heat. The treatment generally has little effect on pollen cone production.

5.6.7 Miscellaneous treatments

Other techniques including pruning, topping, hedging (Copes 1973; Sweet and Krugman 1978), covering with plastic (Tompsett and Fletcher 1977), grafting (Kruche and Melchior 1978) and the use of cover crops (Schultz 1971) have been used to stimulate flowering. The imposed treatments may influence temperature, light, carbohydrate partitioning, growth regulator production and receptivity, nutrition and water relations.

5.7 Factors affecting seed quality: handling and storage

Management of the seed crop from a seed orchard has the potential to affect seed quality. Harvest time, extraction from cones, cleaning, grading and subsequent storage may affect quality.

5.7.1 Harvest time

Cones within a seed orchard ripen at different rates. Hence it could be expected that, at a given harvest date, maturity will vary within a cone crop. Wakely (1954) reported that the germination of southern pines was directly related to cone maturity at the time of seed extraction. However, cone maturity at harvest was found to have little effect on the germination of radiata pine seeds. Rimbawanto et al. (1988, 1989) reported that radiata pine seeds were fully mature with high vigour at a time when cones were still green and had a high moisture content. Wilcox and Firth (1980) harvested two successive cone crops from 15 radiata pine clones. At harvest, the older crop had ripened on the tree but the younger crop was green and immature. The green cones were stored at 20 to 24 ° C for 10 weeks. Germination and growth rates were the same for all seeds.

Donald (1968) reported that opening of radiata pine cones depends on their moisture content. Cones began opening at 13.7% moisture content, and were fully opened at 13.0%. Cones are considered ripe once they have become brown and desiccated. In the natural range, radiata pine seed is shed in winter. However, as radiata pine cones are serotinous they may remain on the parent tree unopened for many years (Krugman and Jenkinson 1974). Consequently, cones are usually harvested before fully ripened and seeds extracted. Sahlen and Abbing (1995) found that a period of artificial ripening improved the vigour of Scots pine seeds from early harvested cones containing immature seeds. The cones were collected from parts of northern Sweden where frosts late in the ripening period prevent cones and seeds reaching maturity. Barnett (1996) suggested that if the clonal cone ripening sequence within a seed orchard is known, then cones can be harvested when mature to minimise the reduction in seed quality associated with harvest of immature cones and seeds.

5.7.2 Seed extraction

Cones harvested before fully ripened need to be kilned to extract the seed. Many conifer species have an optimal temperature range and duration of kilning above which seed quality is reduced, and below which cones will not open. The germination of longleaf pine seeds is reduced at kiln temperatures above 46 ° C (Barnett 1996) while loblolly pine cones kilned at about 38 ° C for 48 hours showed improved germination (McLemore and Barnett 1966). Wang et al. (1992) suggested that prolonged exposure to heat could result in a loss of membrane integrity. The germination of lodgepole pine seeds was least affected if seeds were able to escape from the kiln once the cones had opened.

5.7.3 Cleaning and grading

Following extraction, the seedlot is cleaned, seeds are dewinged and dead seeds removed. The seedlot is graded and dried to storage moisture content. Specialist seed cleaning companies are generally used for this work. Injury caused by de-winging is a common cause of poor seed quality in the southern pine species (Barnett 1996).

Seedlots are usually graded by size to improve uniformity of germination and remove the smallest seeds. Menzies et al. (1985) reported that radiata pine seeds produced in New Zealand are graded into 4 sizes. 95% of the seeds fall into the middle two grades. Small seeds had lower germination capacity and slower seedling growth than larger seeds.

When seeds from a seed orchard are bulked together, management practices have the potential to remove families from the crop. As seed size varies among pine clones, clones producing small seeds may be lost from a seedlot if the smallest seed is discarded (Edwards and El-Kassaby 1996). Radiata pine seed quality varies with family, and bulked seedlots have been found to contain small seeds that perform better than larger seeds (Donovan 2001). Loss of families from a seedlot may also occur if bulk seedlots are thinned after sowing, as small seeds produce smaller seedlings. There is further potential for loss of families if late germinating seeds are thinned due to smaller size, as it has been demonstrated that time to germination is under genetic control (Bramlett et al. 1983). However, Menzies et al. (1985) reported that when sown in a mixture, there was no evidence of radiata pine seedlings from smaller seeds being suppressed by seedlings from larger seeds.

5.7.4 IDS

Conventional seedlot grading is generally unable to remove filled-dead seeds from the seedlot. These are seeds that possess both an embryo and megagametophyte but are unviable. It is difficult to separate filled-dead seeds from viable seeds due to similarities in density. Simak (1984) developed the IDS (incubation, desiccation, separation) technique to improve separation. The technique involves imbibing the seeds, drying, and then separating in a water column. Seeds that float are considered dead while those that sink are considered viable. Viable seeds retain more water during the desiccation stage which allows the seeds to be separated. The technique has been used on a number of conifer species including eastern white pine (Downie and Bergsten 1991b), white spruce (Downie and Bergsten 1991b; Downie and Wang 1992), Caribbean pine (Poulsen 1995), lodgepole pine and jack pine (Downie and Wang 1992). The technique is unnecessary where there is a relatively low proportion of filled-dead seeds in the seedlot, and is likely to be unsuccessful if there is an overlap in the density of viable and filled-dead seed density after desiccation.

Commercial radiata pine seedlots presently being sown in nurseries generally possess high germination capacity which suggests few filled-dead seeds are present. Consequently at this time there is probably little need for the IDS technique. With increased use of CP seedlots the need for the technique may arise.

5.7.5 Storage

Pine species, including radiata pine, have been found to deteriorate very slowly under storage conditions (Donald and Jacobs 1990; Barnett 1996). However, Edwards and El-Kassaby (1996) suggested that generalisations across species may be misleading as the rate of seed viability decline in stored seed varied between conifer families.

5.8 Pretreatment to improve seed quality

Presoaking, stratification, and priming are treatments commonly applied to conifer seeds prior to sowing. These treatments are discussed in more detail below.

5.8.1 Presoaking

Presoaking may be carried out as part of a treatment such as stratification (Tanaka and Edwards 1986; Mason et al. 1995), or as a seed pretreatment in itself (Rudolf 1950; Barnett 1971; Wilcox and Firth 1980; Kyauk et al. 1995). Although they reported no soak effects, Wilcox and Firth (1980) presoaked radiata pine seeds for 24 hours in cold water prior to sowing in a nursery in an experiment comparing cone-ripening treatments. Rudolf (1950) found soaking at 5 ° C for 40 days to be as effective as stratification when applied to eastern white pine, white spruce, black spruce (*Picea mariana* (Mill.) BSP), balsam fir (*Abies balsamea* (L.) Miller) and tamarack (*Larix laricina* (DuRoi) K. Koch). Soaking for this period of time reduced the time taken to treat and germinate the seeds by 25 to 60 % compared with stratification. Barnett (1971) also found soaking loblolly pine seeds to be as effective as stratification in overcoming dormancy. When seeds were soaked at temperatures less than 25 ° C, soaked seedlots had a higher germination capacity than those given an equivalent period of stratification (7, 14, 21 or 28 days). Where soaks were carried out at temperatures above 25 ° C soaked seeds had a lower germination capacity than stratified seeds. This was attributed to insufficient aeration of the water at higher temperature. The supplied air was only able to maintain dissolved oxygen at about half the

saturation level. The solubility of oxygen in water decreases as temperature rises and oxygen-consuming respiration increases with increasing temperature. Both factors contribute to the development of hypoxic conditions when seeds are soaked at elevated temperatures.

The link between reduced oxygen levels and poor subsequent germination suggests that the seeds of some species are susceptible to soak injury as a consequence of hypoxic effects on respiration. There have been few studies on seed anaerobiosis. Plant cells are thought to contain relatively low stores of ATP. In order to survive anoxia for any length of time, anaerobic metabolism must take place. Seed injury under anoxic or hypoxic conditions has variously been attributed to reduced ATP production, the accumulation of toxic end-products, and lack of substrates for respiration (Drew 1997). Ethanol accumulation was initially believed to be responsible for injury (Crawford 1977). Attention has increasingly been focused on acidification of the cytoplasm as a cause of injury (Kennedy et al. 1992).

5.8.2 Priming

Haridi (1985) reported that the germination rate of slash pine seeds was improved by priming at -1.5 MPa and 25 ° C for 5 days. Bourgeois and Malek (1991) reported that jack pine (*Pinus banksiana* Lamb.) seeds primed at -1.7 MPa for 6 days germinated 2 days earlier than unprimed seeds. Hallgren (1989) reported that loblolly and shortleaf pine germination rate was increased by priming. Huang (1989) reported that germination capacity of lodgepole pine (*Pinus contorta* Dougl. ex Loud.) and white spruce was reduced by priming, but uniformity of germination was increased and the number of abnormal seedlings was reduced. In contrast, Hallgren (1989) found that the germination capacity of loblolly pine and some slash pine seeds was increased by priming.

Some species, including lettuce (Valdes and Bradford 1987), lodgepole pine, white spruce (Huang 1989), loblolly, shortleaf and slash pine (Hallgren 1989) germinated over a wider range of temperature following priming. Huang (1989) also found that primed lodgepole pine and white spruce seeds were less affected by deterioration than untreated seeds, and also that germination of deteriorated seeds could be improved by priming.

Increased embryo length during priming has been reported in Scots pine (Sahlen and Wiklund 1995) seeds. However, in spite of an increase in embryo size, only immature embryos showed improved germination (Sahlen and Wiklund 1995). Huang and Zou (1989) suggested that priming may improve membrane integrity in *Pinus sylvestris* var. *mongolica* (Rupr.) Kuzen and *Larix gmelinii* (Dahurian larch) seeds as primed seeds lost fewer cell contents to the imbibition solution than control seeds.

5.8.3 Stratification

The dormancy status of radiata pine seeds is not known, but Grose (1958) found that radiata pine seeds do not germinate readily and concluded that a degree of dormancy was present. Krugman and Jenkinson (1974) recommended stratifying radiata pine seeds for 35 to 45 days and Minko and Craig (1976) reported that stratification was the standard pre-sowing treatment for radiata pine seeds in north-eastern Victoria.

Stratification has been reported to improve seed quality, germination capacity and rate. Germination has been improved under difficult conditions, including dry soils (Outcalt 1991), the absence of light (Li et al. 1994) and temperatures outside the optimal range (Allen 1962b;

Gosling 1988; Jones and Gosling 1994). Skordilis and Thanos (1995) reported that three provenances of East Mediterranean pine with varying degrees of dormancy responded differently to treatment. After treatment, one provenance had an increased germination rate, the second had a broadened temperature range for germination, and the third provenance had an absolute requirement for stratification.

Stratification has also been reported to improve the diameter and height of loblolly pine seedlings at harvest, as a result of an increased rate of germination (Barnett and McLemore 1984; Boyer et al. 1985). Stratification was found to increase germination rate and capacity, decrease the time to achieve seedlings of a given size, and widen the range of temperatures over which radiata pine seeds germinated (Grose 1958; Minko and Craig 1976). In contrast, Rimbawanto et al. (1988, 1989) found that stratification did not significantly affect the germination of radiata pine seeds, but it should be noted that these authors stratified for only one week.

The requirements for successful stratification are sufficient moisture, low temperature, and adequate ventilation (Bonner et al 1974). Stratification traditionally involved burying seeds in layers of moist sand. The seeds were exposed to winter temperatures. In more recent times this has been replaced by a method sometimes called naked stratification or moist prechilling. Seeds are either soaked for a short period of time, drained and chilled in plastic bags or containers, or placed on moist filter papers and held at low temperature (Bonner et al. 1974; Jones and Gosling 1990; Aldhous and Mason 1994).

Some seedlots may be damaged by stratification. Barnett and McGilvray (1971) reported that germination of two out of 16 shortleaf pine seedlots was decreased by stratification.

Treatment method

The treatment factors: duration of stratification; the amount of moisture available to the seed during treatment; and the post-stratification treatment vary substantially between studies.

Generally, increasing the duration of stratification increases the effects on germination (Allen 1962b; Barnett and McGilvray 1971; Baron 1978; Jones and Gosling 1994; Mason et al. 1995). The duration of stratification required to achieve maximum germination can vary between seedlots of a species (Hoff 1987). The rate and capacity of germination of radiata pine (Grose 1958) and sugar pine (Stanley 1958; Baron 1978) were correlated with the length of the stratification period. It appears that treatment has a greater effect on germination rate than germination capacity. For sitka spruce, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and lodgepole pine seeds it was found that the shortest length of prechilling to achieve the fastest germination rate was 36 weeks, which is longer than the prechill required to achieve the maximum germination percent (Jones and Gosling 1994).

After a certain length of time, seeds begin to germinate at the stratification temperature (Grose 1958; Stanley 1958). Aldhous and Mason (1994) recommend stratifying for no longer than 10 weeks to prevent seed germination during treatment. Germinated seeds can be damaged during sowing (Gosling and Rigg 1990) and the seeds are susceptible to desiccation once removed from treatment.

Limiting the amount of water available to the embryo during stratification theoretically allows the seeds to benefit from the treatment without imbibing enough water to allow the radicle to

elongate sufficiently to germinate. A number of studies have examined the effect of stratification with limited water on species including eastern white pine (Downie and Bergsten 1991a), sitka spruce (Gosling and Rigg 1990; Jones and Gosling 1994; Jinks et al. 1994) Douglas-fir, lodgepole pine (Jones and Gosling 1994) noble fir (*Abies procera* Rehd.) (Tanaka and Edwards 1986), Japanese larch (*Larix kaempferi* (Lamb.) Carr.) (Jinks et al 1994) and European ash (*Fraxinus excelsior* L.) (Van de Walle 1987).

Some studies allowed seeds to take up enough water to reach a given moisture content, sometimes called a target moisture content (TMC). Target moisture contents are usually achieved either by restricting the amount of water available to the seed from the beginning of the chilling treatment (Gosling and Rigg 1990; Downie and Bergsten 1991a; Jones and Gosling 1994), or partially stratifying the seed, drying to obtain a particular moisture content and continuing chilling (Tanaka and Edwards 1986; Van de Walle 1987). To inhibit germination of deeply dormant European ash seeds during stratification Van de Walle (1987) interrupted stratification after 3 months, and continued stratifying at low water potential (-1.5 MPa) for another 4 months. Jinks et al. (1994) reported a technique called soak and spin dry which involved soaking the seed, then spin drying, and repeating until the seeds reach the desired moisture content.

Post-stratification treatment

There has been much interest in drying seeds after treatment. If seeds can be dried back without losing the benefits of stratification they may be stored if conditions are unsuitable for sowing immediately after treatment. Allen (1962b) and Danielson and Tanaka (1978) reported that post-stratification drying of Douglas-fir seeds reduced the effect of stratification as seed moisture content dropped below about 40 %. However, the effects of stratification were not completely lost, even when dried back to 10 % moisture content and stored for a year at 0 - 2 ° C (Allen 1962b). Douglas-fir seeds stratified at high moisture content (70%) were less affected by post-stratification storage than those stratified at low moisture content (40%) (Allen 1962a).

In contrast, Danielson and Tanaka (1978) reported that ponderosa pine seeds could be held at 26 % moisture content for up to 9 months without any reduction of germination capacity. Jones and Gosling (1990) reported that sitka spruce seeds can be dried back to their original moisture content (about 7 %) following stratification without reducing germination capacity. However, drying to increasingly lower moisture contents progressively reduced the germination rate. Stratified Ocala sand pine seeds also retained their germination capacity when dried back to moisture contents less than 10%, but it was not reported whether or not the treatment affected the rate of germination (Outcalt 1991).

The mechanisms of action

Although a number of studies have examined imbibition and reserve mobilisation in conifer seeds during stratification, there is little understanding of the mechanisms by which stratification acts to improve germination. In sugar pine and loblolly pine seedcoat removal is as effective in improving germination capacity as stratification (Baron 1978; Carpita et al. 1983). This suggests that during stratification the seedcoat barrier to germination is weakened. Downie and Bewley (1996) and Downie et al (1997) reported that the force required to puncture the megagametophyte and nucellus of white spruce seeds declined during stratification. The tissues may be weakened by mannanase, the activity of which increased during stratification (Downie et al. 1997). However, when the seedcoat of western white pine seed was removed, only 50 % of

the seeds germinated (Hoff 1987) and the germination rate of uncoated loblolly and western white pine seeds was further improved by stratification (Carpita et al 1983; Hoff 1987). This suggests that in at least some pine species the seedcoat barrier is not wholly responsible for dormancy.

Schneider and Gifford (1994) found that increased germinability of loblolly pine seeds correlated with increased water content during stratification. Baron (1978) reported that seeds of this species show much the same pattern of imbibition when held at 5 ° C as at 20 ° C, but the phases were lengthened under stratification. This suggests that the level of seed imbibition achieved during stratification determines the time taken to germinate. Longer periods of stratification allow the seed to reach a higher moisture content. The seed is able to germinate more rapidly as there is a small difference between the seed moisture content and the threshold moisture content it must reach for the radicle to expand. However, it is still unclear if releasing seeds from dormancy during stratification controls seed imbibition.

Other mechanisms suggested include reserve mobilisation and overcoming germination inhibitors. Baron (1978) suggested that sugars accumulate in the embryo during treatment. The sugars provide a substrate for rapid seedling growth. There is evidence that reserves are mobilised during stratification. Murphy and Hammer (1993) reviewed metabolic activities in pine seeds during stratification. Carbohydrate metabolism in the embryo and lipid metabolism in the megagametophyte is induced by stratification. Ching (1963, 1966) found that during stratification carbohydrates accumulated in both the embryo and megagametophyte of Douglas-fir seeds. Lipid concentration in the megagametophyte was reduced and the activity of lipases increased during stratification (Ching 1968). In contrast, Schneider and Gifford (1994) reported that the total lipid content of loblolly pine megagametophyte did not change during stratification, and Carpita et al. (1983) found that the effect of stratification on radicle elongation was not altered if seeds were stratified without the megagametophyte. The extent to which radicle elongation depends on reserves from the megagametophyte is therefore difficult to determine from this evidence.

Schneider and Gifford (1994) found that synthesis of some proteins in the megagametophyte and embryo of loblolly pine seeds decreased during stratification and suggested this was associated with the loss of dormancy. The decline of this set of proteins coincided with an increase in germinability.

5.9 Nursery management and seed quality

Donald (1968) and Menzies et al. (1985) presented studies that examined aspects of nursery production of radiata pine seedlings in South Africa and New Zealand, respectively. Menzies et al. (1985) stated that greater than 85% survival of radiata pine seedlings is generally considered satisfactory. Seedling quality is determined by both the quality of the seed and the management applied in the nursery. Seeds were sown directly into the nursery in the New Zealand study, but for the South African work seedlings were raised in containers and pricked out when sufficiently large. Nurseries in Australia raise seedlings by sowing seeds directly into beds.

5.9.1 Sowing time

Sowing time affects radiata pine seedling height at harvest. Radiata pine seeds were sown in spring in New Zealand (September to November), shortly after the previous crop had been lifted (Menzies et al. 1985). Seeds sown later produced smaller seedlings. The height increment after

transplanting was greater when larger seedlings were used (Menzies et al. 1985). Menzies et al. (1985) suggested that there is an optimal height, however, beyond which little further benefit is achieved.

5.9.2 Sowing depth

Depth of sowing affects the germination capacity and uniformity of emergence of radiata pine seeds (Donald 1968; Menzies et al. 1985). Menzies et al. (1985) reported the optimum depth for radiata pine seeds sown in New Zealand nurseries was 6 mm. Donald (1968) reported a similar depth. Seeds sown shallower were subject to bird predation and drying. When sown too deep, emergence was decreased as the seeds had insufficient energy for the seedling to reach the surface, particularly in the case of small seeds. Donald (1968) found that damping-off damage to seedlings also became more severe with increasing depth of sowing.

5.9.3 Density

Menzies et al. (1985) reported that seeds were sown in New Zealand nurseries at a spacing of 5 cm x 12.5 cm for 1/0 seedlings and 7 cm x 15 cm for 1.5/0 seedlings. Lower densities produced better seedling growth, but this was not necessarily reflected in later field performance. Menzies et al. (1985) also found that the optimum density varied with nursery climate. Seedlings from nurseries with a higher elevation or with heavier soils benefited from widening of spacing as this led to improved robustness.

6. References

- Adams, G.W., and H.A. Kunze. 1996. Clonal variation in cone and seed production in black and white spruce seed orchards and management implications. *The Forestry Chronicle*, 72: 475-480
- Aldhous, J.R. and W.L. Mason (eds). 1994. *Forestry Nursery Practice*. Forestry Commission Bulletin 111, London
- Ali, A., V. Souza Machado and A.S. Hamill. 1990. Osmoconditioning of tomato and onion seeds. *Scientia Horticulturae*, 43: 213-224
- Allen, G.S. 1962a. Factors affecting the viability and germination behavior of coniferous seed. V. Seed moisture content during stratification and secondary storage, *Pseudotsuga menziesii* (Mirb.) Franco. *The Forestry Chronicle*, 38: 303-308
- Allen, G.S. 1962b. Factors affecting the viability and germination behavior of coniferous seed. VI. Stratification and subsequent treatment, *Pseudotsuga menziesii* (Mirb.) Franco. *The Forestry Chronicle*, 38: 485-496
- Arnold, R.J. 1990. Control pollinated radiata pine seed - A comparison of seedling and cutting options for large-scale deployment. *New Zealand Forestry*, November: 12-17.
- Barnett, J.P. 1971. Aerated water soaks stimulate germination of southern pine seeds. *USDA Forest Service Research Paper SO-67*, 9p.
- Barnett, J.P. 1972. Seedcoat influences dormancy of loblolly pine seeds. *Canadian Journal of Forest Research*, 2: 7-10
- Barnett, J.P. 1976. Delayed germination of southern pine seeds related to seed coat constraint. *Canadian Journal of Forest Research*, 6: 504-510
- Barnett, J.P. 1996. How seed orchard culture affects seed quality: Experience with the southern pines. *The Forestry Chronicle*, 72: 469-473
- Barnett, J.P. 1997. Relating pine seed coat characteristics to speed of germination, geographic variation, and seedling development. *Tree Planter's Notes*, 48: 38-42
- Barnett, J.P., and J.M. McGilvray. 1971. Stratification of shortleaf pine seeds. *Tree Planters Notes*, 22: 19-21
- Barnett, J.P., and B.F. McLemore. 1984. Germination speed as a predictor of nursery seedling performance. *Southern Journal of Applied Forestry*, 8: 157-162
- Baron, F.J. 1978. Moisture and temperature in relation to seed structure and germination of sugar pine (*Pinus lambertiana* Dougl.). *American Journal of Botany*, 65: 804-810
- Benjamin, L.R. 1990. Variation in the time of seedling emergence within populations: A feature that determines individual growth and development. *Advances in Agronomy*, 44: 1-25
- Bewley, J.D. and M. Black. 1994. *Seeds: physiology of development and germination*. (2nd ed). Plenum Press, New York/London
- Bonner, F.T., B.F. McLemore and J.P. Barnett. 1974. Presowing treatment of seed to speed germination. *In* *Seeds of woody plants in the United States*, Forest Service, U.S. Department of Agriculture, Agriculture Handbook No. 450.
- Bourgeois, J. and L. Malek. 1991. Metabolic changes related to the acceleration of jack pine germination by osmotic priming. *Trees*, 8: 407-413
- Boyer, J.N., D.B. South, C. Muller, H. Vanderveer, W. Chapman and W. Rayfield. 1985. Speed of germination affects diameter at lifting of nursery-grown loblolly pine seedlings. *Southern Journal of Applied Forestry*, 9: 243-247

- Bramlett, D.L., T.R. Dell, and W.D. Pepper. 1983. Genetic and maternal influences of virginia pine seed germination. *Silvae Genetica*, 32: 1-4
- Bratcher, C.B., Dole, J.M., and Cole, J.C. (1993). Stratification improves seed germination of five native wildflower species. *Hortscience* 28(9):899-901
- Brazeau, M. and Veilleux, J. M. (1976). Bibliographie annotée sur les effets de la fertilisation sur la production de cônes et de semences. Quebec, Ministère des Terres et Forêts, Service de la Recherche, Memoir 25. 26p.
- Brewster, J.L. 1977. The physiology of the onion. *Horticultural Abstracts*, 47: 103-111
- Brewster, J.L. 1982a. Flowering and seed production in overwintered cultivars of bulb onions. I. Effects of different raising environments, temperatures and daylengths. *Journal of Horticultural Science*, 57: 93-101
- Brewster, J.L. 1982b. Flowering and seed production in overwintered cultivars of bulb onions. II. Quantitative relationships between mean temperatures and daylengths and the rate of inflorescence development. *Journal of Horticultural Science*, 57: 103-108
- Brewster, J.L. 1983. Effects of photoperiod, nitrogen nutrition and temperature on inflorescence initiation and development in onion (*Allium cepa* L.) *Annals of Botany*, 51: 429-440
- Brewster, J.L. 1987. Vernalisation in the onion - a quantitative approach. In: *Manipulation of Flowering*, J.G. Atherton (ed). Butterworths
- Brewster, J.L. 1997a. Onions and garlic. In: *The physiology of vegetable crops*. H.C. Wein (ed). CAB International
- Brewster, J.L. 1997b. Environmental physiology of the onion: towards quantitative models for the effects of photoperiod, temperature and irradiance on bulbing, flowering and growth. In: *Proceedings of the first international symposium on edible Alliaceae*. J.L. Burba and C.R. Galmarini (eds). *Acta Horticulturae*, 433: 347-373
- Bujalski, W., A.W. Nienow and D. Gray. 1989. Establishing the large scale osmotic priming on onion seeds by using enriched air. *Annals of Applied Biology*. 115: 171-176
- Bujalski, W. and A.W. Nienow. 1991. Large-scale osmotic priming of onion seeds: a comparison of different strategies for oxygenation. *Scientia Horticulturae*, 46: 13-24
- Cade, S. C. and Jackson, K. 1976. Cone bud production of potted Douglas-fir grafts as influenced by cultural practices. *For. Res. Tech. Rep.*, Weyerhaeuser Corp. 27p.
- Carpita, N.C., A. Skaria, J.P. Barnett, and J.R. Dunlap. 1983. Cold stratification and growth of radicles of loblolly pine (*Pinus taeda*) embryos. *Physiologia Plantarum*, 59: 601-606
- Chang, W.N. and B.E. Struckmeyer. 1976. Influence of temperature on seed development of *Allium cepa* L. *Journal of the American Society for Horticultural Science*, 101: 296-298
- Ching, T.M. 1963. Change of chemical reserves in germinating Douglas fir seed. *Forest Science*, 9: 226-231
- Ching, T.M. 1966. Compositional changes of Douglas fir seeds during germination. *Plant Physiology*, 41: 1313-1319
- Ching, T.M. 1968. Intracellular distribution of lipolytic activity in the female gametophyte of germinating Douglas fir seeds. *Lipids*, 3: 482-488
- Coolbear, P. 1995. Mechanisms of seed deterioration. In A.S. Basra (ed) *Seed Quality. Basic mechanisms and agricultural implications*, pp 223-277. Food Products Press, New York/London/Norwood
- Copes, D. L. 1973. Effects of annual leader pruning on cone production and crown development of grafted Douglas-fir. *Silvae Genetica*. 22, 167-173.

- Crawford, R.M.M. 1977. Tolerance of anoxia and ethanol metabolism in germinating seeds. *New Phytologist*, 79: 511-517
- Cremer, K.W. 1992. Relations between reproductive growth and vegetative growth of *Pinus radiata*. *Forest Ecology and Management*, 52: 179-199
- Currah, L. 1982. Onion flowering and seed production. *Scientific Horticulture*, 32: 26-46
- Currah, L. and D.J. Ockendon. 1978. Protandry and the sequence of flower opening in the onion (*Allium cepa* L.). *New Phytologist*, 81: 419-428
- Danielson, H. R. and Y. Tanaka. 1978. Drying and storing stratified Ponderosa Pine and Douglas-Fir seeds. *Forest Science*, 24: 11-16
- Daubenmire, R. 1960. A seven year study of cone production as related to xylem layers and temperature in *Pinus ponderosa*. *American Midland Naturalist*. 64, 187-193.
- Delouche, J.C. 1980. Environmental effects on seed development and seed quality. *HortScience*, 15: 13-18
- Donald, D.G.M. 1968. Fundamental studies to improve nursery production of *Pinus radiata* and other pines. *Ann. Univ. Stell. Vol. 43, Ser. A, No. 1. Univ. of Stellenbosch*
- Donald, D.G.M. and C.B. Jacobs. 1990. The effect of storage time, temperature and container on the viability of the seed of four pine species. *South African Forestry Journal*, 154: 41-46
- Donovan, N. 1996. Factors affecting the germination of radiata pine seeds. Honours thesis, University of Tasmania
- Donovan, N. 2001. The water relations of seed pretreatments and their effects on the germination of radiata pine seeds. PhD thesis, University of Tasmania
- Dornbos, D.L. Jr. 1995. Seed vigour. In: A.S. Basra (ed) *Seed Quality. Basic mechanisms and agricultural implications*, pp 223-277. Food Products Press, New York/London/Norwood
- Downie, B. and U. Bergsten. 1991a. An invigoration regime for *Pinus strobus* seeds. *Canadian Journal of Forest Research*, 21: 1343-1348
- Downie, B. and U. Bergsten. 1991b. Separating germinable and non-germinable seeds of eastern white pine (*Pinus strobus* L.) and white spruce (*Picea glauca* [Moench] Voss) by the IDS technique. *The Forestry Chronicle*, 67(4), 393 - 396.
- Downie, B. and J.D. Bewley. 1996. Dormancy in white spruce (*Picea glauca* [Moench.] Voss.) seeds is imposed by tissues surrounding the embryo. *Seed Science Research*, 6: 9-15
- Downie, B., H.W.M. Hilhorst and J.D. Bewley. 1997. Endo- β -mannanase activity is associated with micropylar weakening during germination of white spruce seeds. *Physiologia Plantarum*, 101: 405-415
- Downie, B. and B.S.P. Wang. 1992. Upgrading germinability and vigour of jack pine, lodgepole pine, and white spruce by the IDS technique. *Can. J. For. Res.*, 22, 1124 - 1131.
- Drew, M.C. 1997. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology*, 48: 223-250
- Dunlap, J.R. and J.P. Barnett. 1983. Influence of seed size on germination and early development of loblolly pine (*Pinus taeda* L.) germinants. *Can. J. For. Res.* 13: 40 - 44
- Edwards, D.G.W., and Y.A. El-Kassaby. 1996. The biology and management of coniferous forest seeds: Genetic perspectives. *The Forestry Chronicle* 72(5): 481 - 484
- Eldridge (1966). A seed production experiment with *Pinus radiata*. *Australian Forester*. 30, 43-50.

- Ellis, R.H. and P.D. Butcher. 1988. The effects of priming and "natural" differences in quality amongst onion seed lots on the response of the rate of germination to temperature and the identification of the characteristics under genotypic control. *Journal of Experimental Botany*, 39: 935-950
- Ellis, R.H. and E.H. Roberts. 1981. The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology*, 9: 373-409
- Fenner, M. 1992. Environmental influences of seed size and composition. *Horticultural Reviews*, 13: 183-213
- Ferguson Spears, J. 1995. An introduction to seed vigour testing. In: Contributions to seed vigour testing seminar, Copenhagen, Denmark, June 7, 1995. H.A. Venter (ed). International Seed Testing Association
- Fielding, J. M. 1960. Branching and flowering characteristics of Monterey pine. *Aus. For. Timber Bur. Bull.* 37, 59.
- Fielding, J.M. 1961. Provenances of Monterey and bishop pines. *Forestry and Timber Bureau, Bulletin No. 38.* Canberra.
- Finch-Savage, W.E. 1986. A study of the relationships between seedling characters and rate of germination within a seed lot. *Annals of Applied Botany*, 108: 441-444
- Fober, H. (1976). Relation between climatic factors and Scots pine *Pinus sylvestris* L. cone crops in Poland. *Arboretum Kornickie*. 21, 367-374.
- Fries, A. 1994. Development of flowering and the effect of pruning in a clonal seed orchard of lodgepole pine. *Canadian Journal of Forest Research*, 24: 71-76
- Gamiely, S. Smittle, D.A., Mills, H. A. and Banna, G. I. (1990). Onion seed size, weight, and elemental content affect germination and bulb yield. *HortScience*. 25, 522-523.
- Giertych, M. M. (1967). Analogy of the differences between male and female strobiles in *Pinus* to the differences between long- and short-day plants. *Canadian Journal of Botany*. 45, 1907-1910.
- Globerson, D., A. Sharir and R. Eliasi. 1981. The nature of flowering and seed maturation of onions as a basis for mechanical harvesting of the seeds. *Acta Horticulturae*, 111: 99-108
- Gosling, P.G. 1988. The effect of moist chilling on the subsequent germination of some temperate conifer seeds over a range of temperatures. *Journal of Seed Technology*, 12: 90-98
- Gosling, P. G. and P. Rigg. 1990. The effect of moisture content and prechill duration on the efficiency of dormancy breakage in Sitka Spruce (*Picea sitchensis*) seed. *Seed Science and Technology*, 18: 337-343
- Gray, D. and J.R.A. Steckel. 1985. Variation in flowering time as a factor influencing variability in seedling size in the subsequent carrot (*Daucus carota* L.) crop. *Journal of Horticultural Science*, 60: 77-81
- Gray, D., J.R.A. Steckel and L.J. Hands. 1990. Responses of vegetable seeds to controlled hydration. *Annals of Botany*, 66: 227-235
- Gray, D., W.G. Tucker, L.J. Hands and J.R.A. Steckel. 1989. Some effects of drying leek (*Allium porrum* L.) seeds harvested at different stages of maturity on seed and seedling performance. *Journal of Horticultural Science*, 64: 455-465
- Gray, D. and J.A. Ward. 1987. A comparison of leek (*Allium porrum*) and onion (*Allium cepa*) seed development. *Annals of Botany*, 60: 181-187
- Greenwood, M.S. 1977. Flower stimulation techniques for loblolly pine (*Pinus taeda* L.). Third World Consultation on Forest Tree Breeding. Session 4: Constraints on progress. Canberra, pp 1031-1042
- Grose, R.J. 1958. Notes on the influence of stratification at constant temperature on the germination of *Pinus radiata* D. Don. *Plantation Technical Papers*, 5: 8-10

- Hallgren, S.W. 1989. Effects of osmotic priming using aerated solutions of polyethylene glycol on germination of pine seeds. *Annales des Sciences Forestières*, 46: 31-37
- Hampton, J.G. 1995. Methods of seed viability and vigour testing: a critical appraisal. In: A.S. Basra (ed) *Seed Quality. Basic mechanisms and agricultural implications*, pp 223-277. Food Products Press, New York/London/Norwood
- Hampton, J.G. and P. Coolbear. 1990. Potential versus actual seed performance - can vigour testing provide an answer? *Seed Science and Technology*, 18: 215-228
- Hampton, J.G. and D.M. TeKrony (eds). 1995. *Handbook of vigour testing methods*. 3rd edn. International Seed Testing Association
- Haridi, M.B. 1985. Effect of osmotic priming with polyethylene glycol on germination of *Pinus elliotti* seeds. *Seed Science and Technology*, 13: 669-674
- Harvey, M.J. 1993. Onion: *Allium cepa* L. In: *Genetic improvement of vegetable crops*. G. Kalloo and B.O. Bergh (eds). Pergamon Press.
- Heather, D.W. and J.B. Siczka. 1991. Effect of seed size and cultivar on emergence and stand establishment of broccoli in crusted soil. *Journal of the American Society of Horticultural Science*, 116: 946-949
- Heydecker, W., J. Higgins and R.L. Gulliver. 1973. Accelerated germination by osmotic seed treatment. *Nature*, 246: 42-44
- Hilhorst, H.W.M. 1995. A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research*, 5: 61-73
- Hilhorst, H.W.M. and P.E. Toorop. 1997. Review on dormancy, germinability, and germination in crop and weed seeds. *Advances in Agronomy*, 61: 111-165
- Hoff, R.J. 1987. Dormancy in *Pinus monticola* seed related to stratification time, seed coat, and genetics. *Canadian Journal of Forest Research*, 17: 294-298
- Holdsworth, M. and O.V.S. Heath. 1950. Studies in the physiology of the onion plant. IV. The influence of daylength and temperature of the flowering of the onion plant. *Journal of Experimental Botany*, 1: 353-375
- Huang, Y.G. 1989. Enhancing tolerance of lodgepole pine and white spruce seeds to thermo-hygro-stresses by osmoconditioning. *Seed Science and Technology*, 17: 341-353
- Huang, Y.G. and Q. Zou. 1989. Effects of osmoconditioning and drying on germination of *Pinus sylvestris* var. *mongolica* and *Larix gmelinii* seeds. *Seed Science and Technology*, 17: 235-242
- International Seed Testing Association (1999). *International rules for seed testing*. *Seed Science and Technology* 27, supplement.
- Jackson, D. I. and Sweet, G. B. (1972). Flower initiation in temperate woody plants. A review based largely on the literature of conifers and deciduous fruit trees. *Horticultural Abstracts*. 42, 9-24.
- Jinks, R., S. Jones and P. Gosling. 1994. Improving germination of conifer seed. *Forestry and British Timber*, December: 24-27
- Johnsen, O., G. Haug, O.G. Daehlen, B.S. Gronstad and A.T. Rognstad. 1994. Effects of heat treatment, timing of heat treatment, and gibberellin A_{4/7} on flowering in potted *Picea abies* grafts. *Scand. J. For. Res.*, 9: 333-340
- Jones, S. K. and P.G. Gosling. 1990. The successful redrying of imbibed, or imbibed plus prechilled Sitka spruce seed. *Seed Science and Technology*, 13: 299-355
- Jones, S. K. and P.G. Gosling. 1994. "Target moisture content" prechill overcomes the dormancy of temperate conifer seeds. *New Forests*, 8: 309-321

- Karssen, C.M., A. Haigh, P. van der Toorn and R. Weges. 1989. Physiological mechanisms involved in seed priming. In: R.B. Taylorson (ed) Recent advances in the development and germination of seeds, pp 269-280. Plenum Press, New York
- Kazinczi, G., Hunyadi, K., and Lukacs, D. (1999). Data to the biology of cornflower; Germination biology and growth analysis. *Novenyvedelem* 35:2, 45-52
- Kennedy, R.A., M.E. Rumpho and T.C. Fox. 1992. Anaerobic metabolism in plants. *Plant Physiology*, 100: 1-6
- Khan, A. A. 1992. Preplant physiological seed conditioning. *Horticultural Reviews*, 13: 131-181
- Kong, L., S.M. Attree and L.C. Fowke. 1997. Changes of endogenous hormone levels in developing seeds, zygotic embryos and megagametophytes in *Picea glauca*. *Physiologia Plantarum*, 101: 23-30
- Kozlowski, T.T. and A.C. Gentile. 1959. Influence of the seed coat on germination, water absorption, and oxygen uptake of eastern white pine seed. *Forest Science*, 5: 389-395
- Kretschmer, M. 1996. A vigour test for onion seeds. *Gartenbauwissenschaft*, 61: 184-187
- Krugman, S. L. and J.L. Jenkinson. 1974. *Pinus* L. Pine. In *Seeds of woody plants in the United States*, Forest Service, U.S. Department of Agriculture, Agriculture Handbook No. 450.
- Krusche, D. and Melchior, G. H. (1978). The choice of rootstock as a means to stimulate flowering and increase the cone yield in Norway spruce grafts. Pages 1079-1089. In: *Proceedings Third World Consult Forest Tree Breeding, 1977*. Vol. 2. CSIRO, Canberra, Australia.
- Kyauk, H., N.W. Hopper and R.D. Brigham. 1995. Effects of temperature and presoaking on germination, root length and shoot length of sesame (*Sesamum indicum* L.). *Environmental and Experimental Botany*, 35: 345-351
- Lanner, R. M. (1963). Growth and cone production of Knobcone pine under interrupted nights. USDA Forest Serv. Res. Paper No. PSW-38. 16p.
- Lavender, D. P. and Zaerr, J. B. (1985). Temperate conifer forest trees. Pages 1-13 in Halvey, A. h., ed. *Handbook of Flowering*. CRC Press Inc. Boca Raton, Florida. Vol. 1.
- Lee, K. J. (1979). Factors effecting cone initiation in pines: A review. *Korean Institute of Forest Genetic Res. Rep.* 15, 45-85.
- Lee, S. J. 1994. Breeding Radiata pine in New Zealand. *Scottish Forestry*, 48: 9-15
- Lee, S.S., A.G. Taylor, M.M. Beresniewicz and D.H. Paine. 1995. Sugar leakage from aged leek, onion and cabbage seeds. *Plant Varieties and Seeds*, 8: 81-86
- Letchamo, W. and Gosselin, A. (1996). Light, temperature and duration of storage govern the germination and emergence of *Taraxacum officinale* seed. *Journal of Horticultural science*. 71:373-377
- Li, X.J., P.J. Burton and C.L. Leadem. 1994. Interactive effects of light and stratification on the germination of some British Columbia conifers. *Canadian Journal of Botany*, 72: 1635-1646
- Longman, K. A. (1961). Factors affecting flower initiation in certain conifers. *Proc. Linn. Soc. Lond.* 172, 124-127.
- Mason, W.L., G. Negussie, and M.K. Hollingsworth. 1995. Seed pretreatments and nursery regimes for raising Macedonian pine (*Pinus peuce* Grisebach). *Forestry*, 68: 255-264
- Matthews, J. D. (1963). Factors affecting the production of seed by forest trees. *Forest Abstracts*. 24, i-xiii.
- McLemore, B.F. and J.P. Barnett. 1966. Loblolly pine seed dormancy influenced by cone and seed handling procedures and parent tree. U.S. Forest Service Research Note, SO-41

- Mendham, N.J., P.A. Shipway and R.K. Scott. 1981. The effects of seed size, autumn nitrogen and plant population density on the response to delayed sowing in winter oil-seed rape (*Brassica napus*). *Journal of Agricultural Science*, 96: 417-428
- Menzies, M.I., J.C. van Dorsser and J.M. Balneaves. 1985. Seedling quality - radiata pine as a case study. In: D.B. South (ed), *Proceedings of the International Symposium on Nursery Management Practices for the Southern Pines*, August 4-9, Montgomery, Alabama, pp 384-415
- Minko, G. and G. Craig. 1976. Radiata pine nursery research in north-eastern Victoria. Forestry Commission of Victoria, Melbourne.
- Mirov, N. T. (1956). Photoperiod and flowering in pines. *Forest Science*. 2, 328-332.
- Mohamed-Yasseen, Y. and Splittstoesser, W. E. (1990). The role of the onion (*Allium cepa* L.) seed coat in aging and ultrastructural changes in root tips of low vigor seedlings. *Proceedings of the Plant Growth Regulator Society of America*. (17th) 10-15.
- Murphy, J.B. and M.F. Hammer. 1993. Temperature effects on gene expression of dormant sugar pine (*Pinus lambertiana* Dougl.) seeds. In: D.G.W. Edwards (ed) *Dormancy and barriers to germination*, pp 87-95. *Proceedings of an International Symposium of IUFRO Project Group P2.04-00*
- Murray, G.A., J.B. Swensen and G. Beaver. 1992. Emergence of spring- and summer-planted onions following osmotic priming. *HortScience*, 27(5): 409-410
- Neal, C.A. and L.A. Ellerbrock. 1986. Optimising time of harvest for seed of *Allium cepa* L. *Journal of Seed Technology*, 10: 37-45
- Noland, T.L. and J.B. Murphy. 1986. Protein synthesis and aminopeptidase activity in dormant sugar pine seeds during stratification and warm incubation. *Journal of Plant Physiology*, 124: 1-10
- Ogawa, T. (1961). Studies on the seed production of onion. II. The relation between the development of seeds and their germinating ability. *Journal of the Japanese Society for Horticultural Science*. 30, 325.
- Outcalt, K.W. 1991. Stratification increases germination of Ocala sand pine seed in dry soil. *Seed Science and Technology*, 19: 511-517
- Owens, J. N. and Molder, M. (1984). The reproductive cycle of lodgepole pine. *British Columbia Min. Forest Inf. Serv. Br., Victoria*. P. 30.
- Owens, J. N. and M.D. Blake. 1985. Forest tree seed production: a review of literature and recommendations for future research. *Can. For. Serv., Info. Rep. PI-X-53*, 61p.
- Peishi, Z., Plummer, J.A., Turner, D.W., Choengsaat, and Bell, D.T. (1999). Low and high temperature effects on viability and germinability of seeds of three Australian Asteraceae. *Australian journal of Botany*. 47:2, 265-275
- Perez, G.F., Come, D., and Corbineau, F. (1993). Germination behaviour of *Onopordum acanthium* L.: effect of the origin of the cypsela. *Proceedings of the fourth international workshop on seeds: basic and applied aspects of seed biology*. Anger France. Vol2, 417-422
- Perry, D.A. 1982. The influence of seed vigour on vegetable seedling establishment. *Scientific Horticulture*, 33: 67-75
- Pharis, R. P. and Ross, S. D. (1985). The hormonal promotion of flowering in the Pinacea. In: Halvey, A. H., ed. *Handbook of Flowering*. CRC Press Inc. Boca Raton, Florida. Vol. 5.
- Phillipson, J. J. (1990). Prospects for enhancing flowering in conifers and broadleaves of potential silvicultural importance in Britain. *The Journal of the Institute of Chartered Foresters*. 63, 223-240.
- Pollock, C.J. and E.J. Lloyd. 1994. The metabolism of fructans during seed development and germination in onion (*Allium cepa* L.). *New Phytologist*, 128: 601-605

- Poulson, K.M. 1995. Application of the IDS-method to *Pinus caribaea* seed. *Seed Science and Technology*, 23: 269-275
- Powell, A. A. and Matthews, S. (1981). Evaluation of controlled deterioration, a new vigour test for crop seeds. *Seed Science and Technology*. 9, 633-640.
- Rabinowitch, H.D. 1985. Onions and other edible alliums. In: CRC Handbook of Flowering. Volume I. A.H. Halvey (ed). CRC Press
- Rabinowitch, H.D. 1990a. Physiology of flowering. In: Onions and Allied Crops. Volume I. H.D. Rabinowitch and J.L. Brewster (eds). CRC Press
- Rabinowitch, H.D. 1990b. Seed development. In: Onions and Allied Crops. Volume I. H.D. Rabinowitch and J.L. Brewster (eds). CRC Press
- Reich, P.B., J. Oleksyn and M.G. Tjoelker. 1994. Seed mass effects on germination and growth of diverse European Scots pine populations. *Canadian Journal of Forest Research*, 24: 306-320
- Richie, G. A. and Hinckley, T. M. (1975). The pressure chamber as an instrument for ecological research. *Advances in Ecological Research*. 9, 165-254.
- Rimbawanto, A., P. Coolbear, A.M. Dourado and A. Firth. 1988. Seed maturation precedes cone ripening in New Zealand *Pinus radiata*. *New Zealand Journal of Forestry Science*, 18: 139-148
- Rimbawanto, A., P. Coolbear and A. Firth. 1989. Morphological and physiological changes associated with the onset of germinability in *Pinus radiata* (D. Don) seed. *Seed Science and Technology*, 17: 399-411
- Roos, E.E. 1980. Physiological, biochemical, and genetic changes in seed quality during storage. *HortScience*, 15: 781-784
- Ross, S.D. 1977. Influences of gibberellins and cultural practices on early flowering of douglas-fir seedlings and grafts. Third World Consultation on Forest Tree Breeding. Session 4: Constraints on progress. Canberra, pp 997-1007
- Rudolf, P.O. 1950. Cold soaking - A short-cut substitute for stratification? *Journal of Forestry*, 48: 31-32
- Sahlen, K. and K. Abbing. 1995. Effects of artificial environmental conditions on anatomical and physiological ripening of *Pinus sylvestris* L. seeds. *New Forests*, 9, 205 - 224.
- Sahlen, K. and K. Wiklund. 1995. Anatomical and physiological effects of osmotic priming on *Pinus sylvestris* seeds of different maturity. *Seed Science and Technology*, 23: 725-737
- Salama, A. M. and Pearce, R. S. (1993). Ageing of cucumber and onion seeds: Phospholipase D, lipoxygenase activity and changes in phospholipid content. *Journal of Experimental Botany*. 44, 1253-1265.
- Sandhu, J. S., Nandpuri, K. S. and Thakur, J. K. (1972). Studies on the germination of freshly harvested mature and immature onion seeds. *Indian Journal of Horticulture*. 29, 339-341.
- Sarvas, R. (1962). Investigations on the flowering and seed crop of *Pinus sylvestris*. *Inst. For. Fenn.* 53, 1-198.
- Schneider, W.L. and D.J. Gifford. 1994. Loblolly pine seed dormancy. I. The relationship between protein synthesis and the loss of dormancy. *Physiologia Plantarum*, 90: 246-252
- Schultz, R. P (1971). Stimulation of flower and seed production in a young slash pine orchard. USDA Forest Service, Research PaperNo. SE 91. 10 p.
- Shende, P.V., Sorte, N.V., Banginwar, A.D., Sunita, B., and Bankar, S. (1998). Evaluation of various methods to improve seed quality in sunflower. *Journal of Soils and Crops*. 8:2, 201-203

- Shim, S.I., Lee, S.G., and Kang, B.H. (1998). Effects of several chemicals and burial of seeds into the soil on dormancy breaking of weed species. *Korean journal of Weed science*. 18:4, 295-303
- Simak, M. 1985. Seed germination after incubation at different moisture stress. "Seed Problems". Proceedings of the international symposium on seed problems under stressfull conditions. International Union of Forest Research Organisations. Project Group: P2.04.00 Ed. J. Nather. Vienna and Gmunden, Austria.
- Simpson, J. D. and Powell, G. R. (1981). Some factors influencing cone production on young black spruce in New Brunswick. *Forest Chronical*. 57, 267-269.
- Skordilis, A. and C.A. Thanos. 1995. Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis*. *Seed Science Research*, 5: 151-160
- Smith, W. H. and Stanley, R. G. (1969). Cone distribution in crowns of slash pine *Pinus elliottii* Englem. in relation to stem, crown, and wood increment. *Silvae Genetica*. 18, 86-91.
- Stanley, R.G. 1958. Gross respiratory and water uptake patterns in germinating sugar pine seed. *Physiologia Plantarum*, 11: 503-515
- Steiner, J.J. and D.C. Akintobi. 1986. Effect of harvest maturity on viability of onion seed. *HortScience*, 21: 1220-1221
- Stoehr, M.U., S.J. L'Hirondelle, W.D. Binder, and J.E. Webber. 1998. Parental environment aftereffects on germination, growth, and adaptive traits in selected white spruce families. *Can. J. For. Res.*, 28, 418 - 426
- Stone, S. and D.J. Gifford. 1997. Structural and biochemical changes in loblolly pine (*Pinus taeda* L.) seeds during germination and early-seedling growth. I. Storage protein reserves. *International Journal of Plant Sciences*, 158: 727-737
- Stumpf, C.L., S.T. Peske and L. Baudet. 1997. Storage potential of onion seeds hermetically packaged at low moisture content. *Seed Science and Technology*, 25: 25-33
- Sweet, G.B. and M.P. Bollmann. 1970. Seasonal growth of the female strobilis in *Pinus radiata*. *New Zealand Journal of Forestry Science*, 1(1): 15-21
- Sweet, G. B. and Hong, S. O. (1978). The role of nitrogen in relation to cone production in *Pinus radiata*. *New Zealand Journal of Forest Science*. 8, 225-238.
- Sweet, G. B. and Krugman, S. L. (1978). Flowering and seed production problems – a new concept of seed orchards. Pages 749-759. In; *Proceedings Third World Consult Forest Tree Breeding*. Vol. 2. CSIRO, Canberra, Australia.
- Sweet, G. B. and Will, G. M. (1965). Precocious male cone production associated with low nutrient status in clones of *Pinus radiata*. *Nature*, 196, 739.
- Tanaka, Y. and D.W.G. Edwards. 1986. An improved and more versatile method for prechilling *Abies procera* Rehd. seeds. *Seed Science and Technology*, 14: 457-464
- Tanner, C.B. and S.M. Goltz. 1972. Excessively high temperatures of seed onion umbels. *Journal of the American Society for Horticultural Science*, 97: 5-9
- Taylor, A.G. 1997. Seed storage, germination and quality. In: *The physiology of vegetable crops*. H.C. Wein (ed). CAB International
- TeKrony, D.M. and D.B. Egli. 1991. Relationship of seed vigour to crop yield: a review. *Crop Science*, 31: 816-822
- Tillman-Sutela, E. and A. Kauppi. 1995a. The morphological background to imbibition in seeds of *Pinus sylvestris* L. of different provenances. *Trees*, 9: 123-133

- Tillman-Sutela, E. and A. Kauppi. 1995b. The significance of structure for imbibition in seeds of the Norway Spruce, *Picea abies* (L.) Karst.. *Trees*, 9: 269-278
- Tompsett, P. B. and Fletcher, A. M. (1977). Increased flowering of the Sitka spruce (*Picea sitchensis* (Bong.) Carr.) in a polythene house. *SilviaeGenetica*. 26, 84-86.
- Valdes, V.M. and K.J. Bradford. 1987. Effects of seed coating and osmotic priming on the germination of Lettuce seeds. *Journal of the American Society for Horticultural Science*, 112: 153-156
- Van de Walle, C. 1987. Germination uniformity of *Fraxinus excelsior* controlled by seed water content during cold treatment. *Physiologia Plantarum*, 69: 645-650
- Vleeshouwers, L.M., H.J. Bouwmeester and C.M. Karssen. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology*, 83: 277-282
- Voss, R. E. (1979). Onion production in California. Division of Agricultural Science, University of California, Davis.
- Wakely, P.C. 1954. Planting the southern pines. U.S. Department of Agriculture, Agriculture Monograph 18, Washington.
- Wang, B.S.P., B. Downie, S. Wetzel, D. Palamarek and R. Hamilton. 1992. Effects of cone scorching on germinability and vigour of lodgepole pine (*Pinus contorta* var. *latifolia*) seeds in Alberta. *Seed Science and Technology*, 20: 409-419
- Ward, F. H. and Powell, A. A. (1983). Evidence for repair processes in onion seeds during storage at high seed moisture contents. *Journal of Experimental Botany*. 34, 277-282.
- Wareing, P. F. (1958). Reproductive development in *Pinus sylvestris*. Pages 643-654 in Thimann, K. V., ed. *The Physiology of Forest Trees*. Ronald Press, New York.
- Wilcox, M.D. and A. Firth. 1980. Artificial ripening of green *Pinus radiata* cones does not reduce seed germination or seedling vigour. *New Zealand Journal of Forestry Science*, 10: 363-366
- Wheeler, N. C., Ying, C. C. and Murphy, J. C. (1982). Effect of accelerating growth on flowering in lodgepole pine seedlings and grafts. *Canadian Journal of Forest Research*. 12, 533-537.
- Wheeler, T.R. and R.H. Ellis. 1994. Effects of seed quality and temperature on pre-emergence root growth of seedlings of onion (*Allium cepa*). *Seed Science and Technology*, 22: 141-148
- Wiebe, H.J. 1994. Effects of temperature and daylength on bolting of leek (*Allium porrum* L.). *Scientia Horticulturae*, 59: 177-185
- Wu, W. -S. (1979). Survey on seed-bourn fungi of vegetable seed. *Plant protection Bulletin, Taiwan*. 21, 206-219.