Cardinal temperatures and vernalisation requirements for a selection of vegetables for seed production

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by

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Abstract of a Thesis submitted in partial fulfilment of the requirement for the Degree of Bachelor of Agricultural Science

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The cardinal temperatures and vernalisation requirements for a selection of vegetables for seed production were studied. Experiment one assessed the rate of germination (1/days) of selected vegetable species over a range of 5-40 °C, in order to calculate the cardinal (base, optimum and maximum) temperatures for germination. The cardinal temperatures for carrot and red beet were 0.1, 30.9 and 40.7 °C, and 4.2, 35.9 and 44.4 °C respectively. Experiment two examined the vernalisation response of imbibed cabbage, carrot and red beet seeds, plus perennial ryegrass as a comparison over duration treatments of 0-12 weeks at 4 °C. Red beet and ryegrass had positive vernalisation responses with anthesis occurring in plants from the 4-12 week durations in red beet, and all durations in ryegrass. The number of days to anthesis and the final number of main stem leaves did not differ significantly (P = 0.143 and P = 0.323 respectively) among vernalisation durations in red beet, but did differ significantly in ryegrass (P <0.001 and P = 0.004 respectively). The number of days to anthesis decreased by 3.6 days for every one week increase in vernalisation duration. The same pattern was observed for main stem leaf number in ryegrass, with a decrease of 0.1 leaves for every one week increase in vernalisation duration. This response is likely to be attributed to perennial ryegrass having a lower base temperature (1.1 °C) than the vernalisation treatment (4 °C), leading to continued development towards anthesis throughout the vernalisation period. Red beet had a base temperature of 4.6 °C, so may not have experienced development towards reproduction, or may have reached vernalisation saturation at ≤ 4 weeks, resulting in a minimum number of leaves produced for all
durations. There is potential for the sowing date of red beet to be changed to spring for successfully vernalised seeds, which would reduce the expenses of weed, pest and disease control and give opportunity of other land uses over the winter period.

**Keywords:** biennial, cabbage (*Brassica oleracea*), cardinal temperatures, carrot (*Daucus carota*), crop rotation, disease, germination, perennial ryegrass, photoperiod, red beet (*Beta vulgaris*).
# TABLE OF CONTENTS

ABSTRACT ......................................................................................................................... i

Table of Contents ............................................................................................................. iii

List of Tables ....................................................................................................................... v

List of Figures ...................................................................................................................... vi

List of Plates ....................................................................................................................... vii

List of Appendices ............................................................................................................. viii

1 INTRODUCTION ........................................................................................................... 1

2 REVIEW OF THE LITERATURE .................................................................................. 3

2.1 Introduction ..................................................................................................................... 3

2.2 Current practices for vegetable seed production ......................................................... 3

2.2.1 Length of season ....................................................................................................... 3

2.2.2 Weeds, pests and diseases ......................................................................................... 4

2.2.3 Crop rotation ............................................................................................................ 9

2.3 Physiology of biennial crops ........................................................................................ 11

2.4 Cardinal temperatures ............................................................................................... 12

2.4.1 What are cardinal temperatures? ............................................................................. 12

2.4.2 Calculating base temperature .................................................................................. 12

2.4.3 Vegetable seed base temperatures .......................................................................... 14

2.5 Vernalisation ............................................................................................................... 17

2.5.1 Explaining vernalisation .......................................................................................... 17

2.5.2 Juvenile stage .......................................................................................................... 20

2.5.3 Optimum vernalisation ............................................................................................ 21

2.5.4 De-vernaliisation ..................................................................................................... 24

2.6 Photoperiod ................................................................................................................. 25

2.6.1 Explaining photoperiod .......................................................................................... 25

2.6.2 Vegetable photoperiod examples ............................................................................. 27

2.7 Conclusions .................................................................................................................. 27

3 MATERIALS AND METHODS .................................................................................... 29

3.1 Experiment 1: Determination of cardinal temperatures ............................................. 29

3.1.1 Experimental design ............................................................................................... 29

3.1.2 Measurements ......................................................................................................... 29

3.1.3 Statistical analysis .................................................................................................. 30
3.2 Experiment 2: Determination of vernalisation requirement ........................................ 31
  3.2.1 Experimental design ............................................................................................... 31
  3.2.2 Temperature and lighting ....................................................................................... 32
  3.2.3 Measurements ......................................................................................................... 32
  3.2.4 Statistical analysis .................................................................................................. 33
4 RESULTS .......................................................................................................................... 34
  4.1 Base temperature experiment .................................................................................... 34
    4.1.1 Germination percentage ......................................................................................... 34
    4.1.2 Germination rate and estimation of cardinal temperatures ................................. 37
  4.2 Vernalisation experiment .......................................................................................... 40
    4.2.1 The number of plants that flowered ....................................................................... 42
    4.2.2 Vernalisation effect on the number of days to anthesis ...................................... 43
    4.2.3 Vernalisation effect on the number of main stem leaves produced .................... 45
5 DISCUSSION .................................................................................................................... 48
  5.1 Cardinal temperature experiment ............................................................................ 48
    5.1.1 Cumulative germination percentage ..................................................................... 48
    5.1.2 Germination rate and cardinal temperatures ..................................................... 49
      5.1.2.1 Methods ........................................................................................................... 49
      5.1.2.2 Moisture .......................................................................................................... 51
      5.1.2.3 Origin ............................................................................................................... 52
  5.2 Vernalisation ............................................................................................................. 52
    5.2.1 Determining vernalisation response ................................................................. 52
    5.2.2 Cabbage ............................................................................................................... 53
    5.2.3 Carrot .................................................................................................................. 54
    5.2.4 Red beet ............................................................................................................ 56
      5.2.4.1 Days to anthesis .............................................................................................. 56
      5.2.4.2 Main stem leaf number .................................................................................. 57
    5.2.5 Perennial ryegrass ............................................................................................... 57
      5.2.5.1 Days to anthesis ............................................................................................. 57
      5.2.5.2 Main stem leaf number .................................................................................. 58
    5.2.6 Application to the vegetable seed industry ......................................................... 59
  5.3 Conclusions ............................................................................................................... 61

Acknowledgements ........................................................................................................... 62
References ........................................................................................................................... 63
Appendices .......................................................................................................................... 68
**LIST OF TABLES**

| Table 2.1 | The main pathogens of carrot crops (from George, 2009) | 5 |
| Table 2.2 | The percentage of leaves infected with *Alternaria radicina* or *A. dauci* in a field spray trial in New Zealand (adapted from Soteros, 1979) | 7 |
| Table 2.3 | The effect of weed competition on yield (fresh weight of roots) and leaf area of carrot plants. Measurements are expressed as a percent reduction from the weed free controls (adapted from Shadbolt and Holm, 1956) | 8 |
| Table 2.4 | The percent reduction from the weed free checks of the yield (fresh weight of roots) of red beet plants. Measurements were taken early in the season at the time that the weeds were removed (adapted from Shadbolt and Holm, 1956) | 9 |
| Table 2.5 | The base temperatures for a selection of vegetable species | 15 |
| Table 2.6 | The minimum, optimum and maximum temperature range for vernalisation resulting in flowering, and the duration of cold exposure required in some important vegetable seed crops (adapted from Wiebe, 1990) | 22 |
| Table 2.7 | Effects of different constant temperatures, and length the plants were exposed to temperature, on subsequent flowering in the carrot cultivar ‘Chantenay Red Cored’ (From Atherton *et al.*, 1990) | 23 |
| Table 3.1 | The vegetable species used in this experiment, sourced from South Pacific Seeds Ltd (Darfield, NZ) | 29 |
| Table 3.2 | The average monthly temperature in Fletcher glasshouse, Lincoln University | 32 |
| Table 4.1 | The base, optimum and maximum temperature (°C) for germination determined by linear regression for the selected vegetable species and perennial ryegrass | 40 |
LIST OF FIGURES

Figure 2.1  The effect of constant temperature on the rate of germination for different germination percentages of the carrot seed population. Extrapolation of the linear regression fitted to data for the 50th percentile to the temperature axis (Tb) is shown (from Finch-Savage et al., 1998). .........................................................13

Figure 2.2  Vernalisation response in days to flowering in Ryegrass (Lolium) species (from Leopold and Kriedmann, 1975). .................................................................18

Figure 2.3  Temperature effectiveness on vernalisation (from Weir et al., 1984). ..............19

Figure 2.4  The percentage of winter rye seeds remaining vernalised vs. duration of vernalisation, after de-vernalising treatment (from Purvis and Gregory, 1952). .................................................................25

Figure 2.5  An explanation of red light and far red light accumulation in plants (adapted from Kendrick and Frankland, 1983). .................................................................26

Figure 4.1 (1 of 2) The average cumulative germination percentage at temperature treatments of 5 °C (●), 10 °C (○), 15 °C (▼), 20 °C (▲), 25 °C (■), 30 °C (□), 35 °C (◆) and 40 °C (◇) for Asian radish (a), cabbage (b), carrot (c), Chinese cabbage (d), mustard (e) and onion (f). Time is the number of days from initial imbibition to germination. Error bars represent the standard error of the mean for the final germination percentages. .........................................................35

Figure 4.2  (1 of 2) Rate of germination (1/days) to 50% germination over a range of temperatures (°C) increasing in 5 °C increments used to find the base, optimum and maximum temperatures for germination for Asian radish (a), cabbage (b), carrot (c), Chinese cabbage (d), mustard (e) and onion (f). ..............38

Figure 4.3  The number of plants that flowered per pot (out of a possible 4) over vernalisation durations of 0-12 weeks, for perennial ryegrass (○) and red beet (●). The effect of vernalisation (LSD 0.537, p <0.001) and species (LSD 0.287, P <0.001) was significant with no interaction. .........................................................43

Figure 4.4  The number of days on average from the end of the vernalisation period until anthesis occurred in perennial ryegrass over a vernalisation range of 0-12 weeks. The vernalisation treatment had a significant effect on the number of days to anthesis (LSD 8.15, P <0.001). .........................................................44

Figure 4.5  The number of days on average from the end of the vernalisation period until anthesis occurred in red beet over a vernalisation range of 0-12 weeks. The vernalisation treatment did not have a significant effect on the number of days to anthesis (P = 0.143). .........................................................45

Figure 4.6  The main stem leaf number for perennial ryegrass over vernalisation durations of 0-12 weeks. The vernalisation treatment had a significant effect on the number of main stem leaves (LSD 0.817, P = 0.004). .........................................................46

Figure 4.7  The main stem leaf number for red beet over vernalisation durations of 0-12 weeks. The vernalisation treatment did not have a significant effect on the number of main stem leaves (P = 0.323). .........................................................47
LIST OF PLATES

Plate 4.1  The visual effect of vernalisation durations of 0-12 weeks on flowering in red beet, with no reproductive changes in 0-2 week plants. .................................41
Plate 4.2  Vegetative carrot plants from 0-12 week vernalisation durations with no visual signs of reproductive change in any duration. .................................................41
Plate 4.3  Vegetative cabbage plants from 0-12 week vernalisation durations with no visual signs of reproductive change in any duration. .................................42
Plate 5.1  Microscopic view of the vegetative stage of the growing point (apex) of a cabbage plant exposed to 12 weeks of vernalisation (at 4 °C). .................................54
LIST OF APPENDICES

Appendix 1 The vernalisation experiment complete randomised block design for the layout of the pots in Fletcher glasshouse

68
1 INTRODUCTION

The seed industry is an important part of New Zealand’s economy and land based industries. Annual seed exports from New Zealand in 2010 and 2011 were valued at approximately $136 million per year, excluding cereal seeds (Hampton et al., 2012). From this figure, carrots (Daucus carota) contribute an average of $11.35 million per year. Carrot plants for seed production have a biennial lifecycle, meaning they take two years (13-14 months) to reach maturity. Carrot seed crops are of particular interest in this study, as they are a long term land commitment and high capital input crop due to disease susceptibility and low competitiveness against weeds. A decrease in the growing period of biennial seed crops such as carrot is likely to reduce disease and weed issues and minimise production expenses.

Biological processes in plants respond to temperature, and the responses can be quantified by base, optimum and maximum temperatures (cardinal temperatures) for development. Base temperature is the lowest temperature at which metabolic processes result in plant development, and therefore a gain in above ground biomass and further stages of development (Yang et al., 1995). Cardinal temperatures are useful knowledge for aligning the timing of sowing, germination and emergence with favourable environmental conditions for seedling growth and development (Monks et al., 2009). Base temperature is important in calculating the thermal time requirements of a crop, and is also an important concept in vernalisation (cold period) research. Base temperature is usually calculated statistically rather than physiologically due to difficulties with varying developmental phases. Literature on the base temperature for germination in vegetable seeds is limited. There are various models available for predicting and calculating the base temperature for plants, but the estimates of base temperatures from experiments are variable, and the methods are often tedious and lack a theoretical foundation in mathematics (Yang et al., 1995).

Many biennial vegetable species require a vernalisation period before they become reproductive and flower (Alessandro and Galmarini, 2007). The stimulus for stem elongation and flowering has long been known to be vernalisation, but the range of effective temperatures, the duration required and receptive stages vary for different
species and cultivars, and have not been precisely determined (Chouard, 1960; Atherton et al., 1990; Alessandro and Galmarini, 2007 etc.). Red beet (*Beta vulgaris*) has been stated to have the ability to receive vernalisation as a seed (Chouard, 1960), which may suggest that other species could also have this ability. This could lead to the potential to plant a seed crop post-winter, resulting in a shortened lifecycle and decreased pest and disease issues. Other species of interest in this study include red beet and cabbage (*Brassica oleracea*), as they are also biennial plants with similar growing seasons and pest and disease issues to carrot.

The aim of this thesis was to calculate cardinal temperatures for germination for a selection of vegetable species and to determine if cabbage, carrot and red beet could be vernalised as imbibed seed and therefore reduce the period to flowering.
2 REVIEW OF THE LITERATURE

2.1 Introduction
The objective of this review is to gain an insight into the physiology of biennial crops, explain base temperature, vernalisation and photoperiod, and discuss some basic principles of vegetable seed production. An understanding of these topics will help to assess the potential of changing the sowing date of some biennial vegetable seed crops to after the winter.

2.2 Current practices for vegetable seed production

2.2.1 Length of season
Due to the species of interest in this review having a biennial lifecycle when grown for seed, the length of season is greater than twelve months. This is a long term land commitment causes major difficulties with weed competition and diseases, and well as some inconvenience with crop rotation with other species. The species of interest in this review (mainly carrot and red beet, and cabbage to a lower extent) all have similar season lengths, generally being planted in late summer/early autumn and harvested early autumn the following year. The length of season for carrot is explained in more detail; for carrot seed production using the seed-to-seed method, sowing generally takes place in January-February in Southern Hemisphere (McDonald and Copeland, 1997; Kelly and George, 1998). After germination, the juvenile seedling produces leaves and a tap root before being vernalised over the winter period, and proceeding to flower in the spring and produce seed for harvest in late February to March (McDonald and Copeland, 1997). There is some variation in the literature on the growing season for carrot seed crops, as Alessandro and Galmarini (2007) state that there are early flowering cultivars available that are able to be sown from January to July (summer to early winter), and achieve flowering in the same year. It was also stated that late flowering cultivars need to be sown early in summer (January) to achieve flowering late in the following spring (November), so it could be assumed that majority of the literature discussed is referring to late flowering carrot cultivars.
There may be potential to shorten the growing season of vegetable seed crops with an obligate vernalisation requirement, by exposing imbibed seeds to a cold period, as opposed to a post-juvenile plant. If this was successful the sowing date of vegetable seed crops such as carrot may be able to be changed to after winter. This would mean the length of season/time in the ground would be much shorter (less than 12 months) which may have a positive effect on crop rotation and pest and disease issues. There is an ‘opportunity cost’ of being able to do something else with the land between autumn and spring if the planting of crops like carrot and red beet was able to be moved to spring.

If the length of time that a carrot seed crop is in the ground is reduced by planting after winter, then a winter cover crop such as oats (*Avena sativa*) could be planted. Winter cover crops are used reduce soil erosion and loss of plant available nutrient s from leaching and runoff (Dabney *et al.*, 2001). Some other factors cover crops may also help with include weed suppression, increasing soil quality, increasing beneficial insect populations and some disease reduction (Dabney *et al.*, 2001). There is opportunity for income from grazing or taking silage cuts off a winter cover crop in place of where carrots or red beet would usually be growing. Winter is the most susceptible time period for these crops to develop disease and weed issues (see Section 1.2). Carrots especially require continuous disease control, usually in the form of fungicide and chemical sprays which may cost up to $1000/ha for the period of February-August (R.Chynoweth, 14/10/2013, Pers.Comm). This means there is also opportunity cost to save money on chemical expenses and make income from the cover crop. The current length of carrot seed season running from January/February until March/April the following year (McDonald and Copeland, 1997) places some restrictions on the use of winter cover crops which would need to be sown in March, or earlier for sufficient dry matter production (see Section 2.2.3)(Dabney *et al.*, 2001).

### 2.2.2 Weeds, pests and diseases

It is well known that carrots, and red beet are susceptible to many diseases, insects, and weeds and that crop rotation is one of the most effective production practices for minimising their occurrence (McDonald and Copeland, 1997), as well as host resistance and chemical use.
George (2009) produced a table of the main pests and seed-borne pathogens effecting carrot crops (Table 2.1).

**Table 2.1 The main pathogens of carrot crops (from George, 2009).**

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Common names</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria dauci</em></td>
<td>Carrot leaf blight</td>
</tr>
<tr>
<td><em>Alternaria radicina</em></td>
<td>Black root rot, seedling blight</td>
</tr>
<tr>
<td><em>Cercospora carotae</em></td>
<td><em>Cercospora</em> blight of carrot, leaf spot</td>
</tr>
<tr>
<td><em>Erysiphe heraclei</em></td>
<td>Powdery mildew</td>
</tr>
<tr>
<td><em>Phoma rostrupii</em></td>
<td><em>Phoma</em> root rot</td>
</tr>
<tr>
<td><em>Xanthomonas campestris</em></td>
<td>Bacterial blight, root scab</td>
</tr>
<tr>
<td><em>Ditylenchus dipsaci</em></td>
<td>Eelworm</td>
</tr>
</tbody>
</table>

Beet crops are also known to have serious pathogen issues, with mildews (such as powdery mildew (*Blumeria graminis*)), *Cercospora* leaf spot, damping off (caused by numerous pathogens), root rot (from water moulds such as *Phytophthora* species) and dry rot canker (*Corticium vagum*) being some of the most common problems affecting crop yields (Errakhi et al., 2007).

The pathogens presented in Table 2.1 have been produced from northern hemisphere literature, but there has been literature published from the southern hemisphere on some of the same pathogens, particularly the *Alternaria* species. *Alternaria radicina* and *Alternaria dauci* were stated in literature by Soteros (1979) to be infectious leaf pathogens of carrot crops in New Zealand, causing considerable damage for both root and seed crops.

*Alternaria radicina* is a fungal pathogen primarily of carrot that can cause black root rot, leaf and umbel blight and pre and post-emergence damping off (condition where seed or seedling dies/weakens), and is both seed and soil borne (Soteros, 1979; Farrar et al., 2004; Hampton et al., 2012). It is believed to have been introduced in New Zealand in the late 1960’s (Soteros, 1979) via imported carrot seed. Infections in carrot seed crops may lead to reduced seed yield due to root rot and foliar damage, and severe infections can cause the entire umbel to become necrotic and produce no seed at all (Farrar et al.,
Another major problem caused by *A. radicina* is reduced carrot seed germination. Reduced germination led to carrot seed lots not meeting contracted standards, and being rejected by European seed house markets (Hampton *et al.*, 2012). Control of *A. radicina* can be difficult as it can spread by seed, soil, wind and crop residue (Farrar *et al.*, 2004), but is necessary to prevent destruction of the photosynthetic surface area of the plants (Soteros, 1979). *Alternaria dauci* causes leaf blight, but can also infect developing seeds by infecting buds and flowers, which may lead to non-viable seed (Soteros, 1979; Farrar *et al.*, 2004). If seed is infected with *A. dauci* fungus then it can spread new strains of the pathogen to existing carrot production areas, or transmitted it to other areas of carrot production.

Control methods for the *Alternaria* carrot pathogens include crop rotation (see Section 2.2.3), cultivation, fungicide application, clean seed, sanitation/seed treatments and cultivar selection (Soteros, 1979; Farrar *et al.*, 2004). For management of the disease under high pressure, no single control measure is sufficient. Literature on carrots produced for the harvestable root product has stated that intensive foliar fungicide application is routine for adequate control of *Alternaria* pathogens (Ben-Noon *et al.*, 2001; Farrar *et al.*, 2004). Fungicide seed treatment can also be used, although it is often expensive and may reduce seed germination (Farrar *et al.*, 2004). It could be assumed that these control methods would be the same for carrot seed crops, as they go through the same lifecycle as plants which are harvested for the root product.

In a trial on the effectiveness of three field spray fungicides on four carrot cultivars, Soteros (1979) found that on an average of 66% of carrot seedlings were infected with either or both of the *Alternaria* pathogens in the untreated control (Table 2.2). The carrot cultivars were not defined. All of the fungicides effectively reduced the number of infected leaves, but triphenyltin hydroxide had the best control with an average of only 9.4% of leaves infected after being sprayed seven times over a three month period. This fungicide however was withdrawn from sale in New Zealand not long after the trial, and focuses were aimed towards a fungicide called propineb which had been shown to have successful control of *Alternaria* pathogens in glasshouse trials (Soteros, 1979).
Table 2.2 The percentage of leaves infected with *Alternaria radicina* or *A. dauci* in a field spray trial in New Zealand (adapted from Soteros, 1979).

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Number of sprays</th>
<th>Cultivar 1</th>
<th>Cultivar 2</th>
<th>Cultivar 3</th>
<th>Cultivar 4</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triphenyltin hydroxide</td>
<td>7</td>
<td>8.5</td>
<td>10.2</td>
<td>10.5</td>
<td>8.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Triphenyltin acetate plus maneb</td>
<td>6</td>
<td>20.2</td>
<td>25.5</td>
<td>27.2</td>
<td>24.7</td>
<td>24.4</td>
</tr>
<tr>
<td>Captafol</td>
<td>6</td>
<td>24.7</td>
<td>30.5</td>
<td>32.2</td>
<td>28.2</td>
<td>28.9</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>50.5</td>
<td>76.2</td>
<td>78.7</td>
<td>58.2</td>
<td>65.9</td>
</tr>
</tbody>
</table>

Iprodione and chlorothalonil were the most popular choices of fungicide in the United States in the early 2000’s, as well as azoxystrobin and pyraclostrobin showing excellent control in field evaluations for control of leaf blight (Farrar *et al.*, 2004). Fungicide sprays are generally initiated before the first appearance of disease, and are followed with subsequent sprays for the rest of the growing season, which has been seen in some literature to be up to 40 sprays (Ben-Noon *et al.*, 2001). This is likely to be labour and cost intensive and a shorter growing season could be beneficial.

Carrots do not compete well with weeds, as weeds tend to establish faster, and can quickly out compete the crop in the early stages of the season (Sasnauskas *et al.*, 2012). Sasnauskas *et al.* (2012) also confirm that under intensive agricultural conditions, chemical application is the most effective method of controlling weeds. Weeds can dominate carrot crops because in the beginning of the carrot growth cycle, the root of the plant is the photosynthetic sink and is growing at the expense of the above ground canopy. This means weeds generally have a much greater ground cover (Sasnauskas *et al.*, 2012). Control of weeds in carrot seed crops is almost totally dependent on chemicals, as any physical alteration to the seedbed may affect the seed placement and result in poor establishment (McDonald and Copeland, 1997).

The ability of weeds to dominate crops was shown in an experiment by Shadbolt and Holm (1956) where the yield of carrots (fresh weight of roots) was significantly decreased by weed stands of 30 and 50% of the crop area competing for more than four and a half weeks (Table 2.3). The spring measurements were taken after weeds were removed early.
in the growing season. At this stage where carrots had been competing with weeds for four and a half and five and a half weeks at 50% of the crop area there was a 92.9 and 90.9% reduction respectively in the fresh weight of carrot roots. The reductions in the autumn measurement were still significantly different from weed free control plots, however the reductions were not as great as in the spring measurement. This means the recovery from weeds was greater and can be related to crop maturity and competition ability. This is in agreement with what Sasnauskas et al. (2012) stated in relation to carrots having poor competition ability in early growth stages.

Table 2.3 The effect of weed competition on yield (fresh weight of roots) and leaf area of carrot plants. Measurements are expressed as a percent reduction from the weed free controls (adapted from Shadbolt and Holm, 1956).

<table>
<thead>
<tr>
<th>Weed Stand (%)</th>
<th>Duration of competition (weeks)</th>
<th>Percent reduction in yield (fresh weight of roots)</th>
<th>Percent reduction in leaf area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spring</td>
<td>Autumn</td>
</tr>
<tr>
<td>15</td>
<td>3.5</td>
<td>11.6</td>
<td>8.4</td>
</tr>
<tr>
<td>30</td>
<td>3.5</td>
<td>4.5</td>
<td>9.1</td>
</tr>
<tr>
<td>50</td>
<td>3.5</td>
<td>11.6</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>4.5</td>
<td>73.6 *</td>
<td>30.1 *</td>
</tr>
<tr>
<td>30</td>
<td>4.5</td>
<td>81.2 *</td>
<td>30.6 *</td>
</tr>
<tr>
<td>50</td>
<td>4.5</td>
<td>92.9 *</td>
<td>44.5 *</td>
</tr>
<tr>
<td>15</td>
<td>5.5</td>
<td>77.7 *</td>
<td>38.7 *</td>
</tr>
<tr>
<td>30</td>
<td>5.5</td>
<td>87.4 *</td>
<td>47.4 *</td>
</tr>
<tr>
<td>50</td>
<td>5.5</td>
<td>90.9 *</td>
<td>61.9 *</td>
</tr>
</tbody>
</table>

Note: * Significantly different from the weed free check at 1% level

Leaf area was also reduced in the spring measurements by as much as 73.8% in the 50% weed cover for five and a half weeks (Shadbolt and Holm, 1956). This would effectively reduce the plants photosynthetic ability, with smaller leaf area not being able to intercept as much light (Taiz and Zeiger, 2010). Weeds in America may differ to New Zealand weeds, but it has already been established that carrots struggle with weed competition, so this data simply quantifies the extent of damage to carrot crops. The root of vegetable species like carrots and red beet is important for seed production, as the plant would die without it. The root is in the ground for a long period of time (>12
months) with crops such as these, so it is important for the root to stay protected from pests and disease.

Shadbolt and Holm (1956) also included red beet in their weed trial, and found similar effects with weeds generally reducing the yield with increasing weed stands and competition duration (Table 2.4). The damage of weeds on red beet plants was not as severe as the carrot plants in this study.

**Table 2.4** The percent reduction from the weed free checks of the yield (fresh weight of roots) of red beet plants. Measurements were taken early in the season at the time that the weeds were removed (adapted from Shadbolt and Holm, 1956).

<table>
<thead>
<tr>
<th>Weed Stand (%)</th>
<th>Duration of competition (weeks)</th>
<th>Reduction in yield (% fresh weight of roots)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>4</td>
<td>16.7</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>27.2</td>
</tr>
<tr>
<td>50</td>
<td>4</td>
<td>58.4</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>56.5</td>
</tr>
<tr>
<td>30</td>
<td>5</td>
<td>59.8</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>63.3</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>16.4</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>53.2</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>61.7</td>
</tr>
</tbody>
</table>

### 2.2.3 Crop rotation
Crop rotation is one of the most effective production practices for minimising the occurrence of weeds, pests and diseases in crops, as well as being important for maintaining soil physical conditions and plant nutrition (McDonald and Copeland, 1997; George, 2009).

Crop rotation is an important part of the control of the *Alternaria* carrot diseases in the soil, but the rotation must be of sufficient duration to allow carrot debris to completely decompose and eliminate fungus (Farrar *et al.*, 2004). Kelly and George (1998) recommend a break of at least three years between carrot seed crops, and an increase in this break if there has been presence of soil-borne pathogens such as *Sclerotinia*.
sclerotiorum (white mold/watery soft rot). Hampton et al. (2012) suggested that a seven to eight year gap between carrot seed production in the same paddock is required to avoid soil-borne issues from A. radicina, after finding the pathogen present in soil six years after carrot seed was harvested, but absent seven or more years after harvested. Farrar et al. (2004) also suggested at least eight years rotation for heavily infested soils. Non-host crops such as faba bean, alfalfa (lucerne), wheat or barley which may have antifungal root exudates, could be included in the crop rotation to reduce levels of A. radicina more quickly (Farrar et al., 2004; Hampton et al., 2012).

The long growing season of carrot and red beet grown for seed can cause problems with the timing of sowing of other species. Carrot needs to be sown in January/February, but many other commonly grown crops in New Zealand such as wheat and white clover are not harvested until February (R.Chynoweth, 14/10/2013, Pers.Comm). This causes species and time limitations. If a carrot or red beet seed crop could be sown later then there would be time for a stale seed bed to be implemented to control some weed issues before sowing. Many farmers plough paddocks in hope to minimize/eliminate weeds and diseases in the soil, however Hampton et al. (2012) stated that in the case of Alternaria diseases ploughing only reduces the inoculum in the top 0-20 cm for one year. Issues with crop rotation also arise at the harvest end of seed crops like carrot and red beet, as harvest does not usually occur until late March/April. Common crops such as wheat and ryegrass preferably need to be sown by April in New Zealand. It is possible to follow carrots with crops such as these, but this is usually only in cases where the season is favourable and harvest is not dull or pushed back by wet spells. Slight delays in harvest are the difference between sowing a crop in autumn, or having to wait until spring, which then causes income losses by lowered yields. A fallow period over winter is undesirable from an environmental point of view also, with mineralisation causing nutrient leaching (particularly nitrogen) as there are no plant roots present to take up the nutrients.
2.3 Physiology of biennial crops

A biennial crop is one which completes its lifecycle in two years (Taiz and Zeiger, 2010). Almost all biennial crops can also be annual crops, or have early flowering, depending on their end use (Alessandro and Galmarini, 2007). Many vegetable species fall into this category, being annual crops if they are grown for food production, or biennial for seed production (Wiebe, 1990). Using carrots as an example, the first year of growth is vegetative and involves a basal rosette of leaves being produced along with a tap root for carbohydrate storage (Alessandro and Galmarini, 2007). If this root is harvested as a food product, the plant is classed as having an annual growth pattern. If the carrot is grown for seed then it remains in the ground and the stem elongates, flowers and produces seed in the following season. Some other examples of biennial vegetables may include cabbages, beets, onion, parsnip, radish and mustard (Wiebe, 1990). Although most vegetables for seed production are biennial, there is occasionally a tendency for a small number of plants to act as an annual and bolt prematurely (Kelly and George, 1998). It is important that these early bolting plants are removed from the seed crop, so that no genes carrying this trait are continued.

Many biennial plants have an obligate vernalisation requirement to initiate flowering, along with specific photoperiod conditions (Wiebe, 1990; Alessandro and Galmarini, 2007). Vernalisation is a cold period which prompts flowering; therefore it is a major determinant in the growth and development of most biennial plants (Yan and Hunt, 1999)(see Section 2.5). The obligation means that without cold exposure they will remain vegetative (Wiebe, 1990). Photoperiod is the period of time each day in which a plant or other organism receives light, or in other terms the day length (Taiz and Zeiger, 2010)(see Section 2.6). Plant size is also an important factor for vernalisation (see Section 2.5.2). Before any of the physiological processes of biennial crops can take place, the seeds need to germinate. Germination has temperature requirements, which are further discussed in Section 2.4.
2.4 Cardinal temperatures

2.4.1 What are cardinal temperatures?
Biological processes in plants respond to temperature, and the responses can be quantified by cardinal temperatures. Cardinal temperatures quantify the range of temperatures that contribute to plant development (Black et al., 2006; Monks et al., 2009), and consists of three points of response; the base temperature, the optimum temperature, and maximum temperature (Cho et al., 2008). Base temperature is the lowest temperature at which metabolic processes result in plant development, and therefore a gain in above ground biomass and further stages of development (Yang et al., 1995). Below the base temperature, plant development ceases. When moisture conditions and oxygen are adequate, seed germination depends primarily on temperature (Bierhuizen and Wagenvoort, 1974; Finch-Savage and Phelps, 1993). The optimum temperature represents the temperature at which the rate of germination is fastest. Cardinal temperatures are useful knowledge for aligning the timing of sowing, germination and emergence with favourable environmental conditions for seedling growth and development (Monks et al., 2009).

2.4.2 Calculating base temperature
Base temperature is an important component in calculating a plants grow degree day (GDD) requirement, which is a measurement of development used to predict plant growth stages such as flowering (Yang et al., 1995; Cho et al., 2008). For this reason, substantial research has been conducted on developing methods to determine base temperature in widely adapted crops such as wheat. Base temperature can be difficult to determine, as each development stage of a plant can have different minimum temperature requirements (Yang et al., 1995). Base temperature is most commonly determined statistically, as opposed to physiologically. Some important methods of determining base temperature which have been reported in the past include; the least standard deviation in GDD, the least standard deviation in days, the coefficient of variation in days and the regression coefficient. Yang et al. (1995) believed there were shortcomings with the four calculation methods used, and provided mathematical formulae to facilitate these calculations which were then tested on a selection of vegetables to prove they could be used to provide an accurate base temperature.
Standard deviation in days, coefficient variation and regression coefficient methods all gave similar results and were superior over to the standard deviation in GDD method (Yang et al., 1995).

Finch-Savage et al. (1998) defined base temperature as the temperature at below which germination does not occur, and calculated this in their experiment on carrot germination with a threshold model. The point where the temperature axis was intercepted was the base temperature ($T_b$), found by extrapolation of linear regression (Figure 2.1).

![Figure 2.1](image-url)

**Figure 2.1** The effect of constant temperature on the rate of germination for different germination percentages of the carrot seed population. Extrapolation of the linear regression fitted to data for the 50th percentile to the temperature axis ($T_b$) is shown (from Finch-Savage et al., 1998).

In this data set, using extrapolation of linear regression, the base temperature of carrot was found to be 2.15 °C. It is relevant to note however that this is under a constant
temperature, which is not realistic in field conditions, so it could be assumed this would differ in diverse environments, and between cultivars.

Yang et al. (1995) stated that this method is simple and logical, but has serious limitations statistically, as it is not acceptable to extrapolate in regression models. However, in the regression method Yang et al. (1995) used, there was no extrapolation involved, as the base temperature was found when the regression coefficient was zero (ie. no development occurred). Zero was found by graphic interpolation. The method was therefore statistically acceptable.

Angus et al. (1981) stated that for data where temperature has been controlled, the simplest model for relating the rate of development (R) to temperature (t) is usually the linear regression model (Equation 1).

**Equation 1** \[ E (R) = b_0 + b_1 t \]

Where \( E (R) \) denotes the expected value of \( R \), and \( b_0 \) and \( b_1 \) are regression estimates of \( y \) intercept and slope respectively related to the base temperature for emergence and the number of degree days above the base temperature between sowing and emergence. To determine base temperature Angus et al., (1980) used the following equation for base temperature where equation 1 is solved for \( t \) when the linear regression intersects the \( x \) axis;

**Equation 2** \[ Tb = \frac{-b_0}{b_1} \]

In 1990 Atherton et al. reported that there appeared to be no other published literature on cardinal temperatures for carrots.

### 2.4.3 Vegetable seed base temperatures

A range of base temperatures (\( T_b \)) have been determined for a selection of vegetables (Table 2.5). Majority of the authors used linear regression methods to determine the base temperature. An exception to this was Cho et al. (2008) who used a quadratic model.
<table>
<thead>
<tr>
<th>Species</th>
<th>Growth stage</th>
<th>$T_b$ (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cotyledons horizontal</td>
<td>1</td>
<td>Bierhuizen and Wagenvoort (1974)</td>
</tr>
<tr>
<td></td>
<td>n/a</td>
<td>6.5</td>
<td>Brewster and Sutherland (1993)</td>
</tr>
<tr>
<td>Carrot (<em>Daucus carota</em> L.)</td>
<td>n/a</td>
<td>1</td>
<td>Brewer and Sutherland (1993)</td>
</tr>
<tr>
<td></td>
<td>Cotyledons horizontal</td>
<td>1.3</td>
<td>Bierhuizen and Wagenvoort (1974)</td>
</tr>
<tr>
<td></td>
<td>n/a</td>
<td>2.1</td>
<td>Whalley <em>et al.</em> (1999)</td>
</tr>
<tr>
<td></td>
<td>n/a</td>
<td>2.15</td>
<td>Finch-Savage <em>et al.</em> (1998)</td>
</tr>
<tr>
<td></td>
<td>n/a</td>
<td>2.1</td>
<td>Whalley <em>et al.</em> (1999)</td>
</tr>
<tr>
<td></td>
<td>Leaves 3-9 appearance</td>
<td>5</td>
<td>Lancaster <em>et al.</em> (1996)</td>
</tr>
<tr>
<td></td>
<td>n/a</td>
<td>5.9</td>
<td>Brewer and Sutherland (1993)</td>
</tr>
<tr>
<td>Pak choi (<em>Brassica rapa</em> L. var. <em>chinensis</em>)</td>
<td>Radicle &gt;1 mm</td>
<td>13.5</td>
<td>Cho <em>et al.</em> (2008)</td>
</tr>
<tr>
<td></td>
<td>n/a</td>
<td>5.6</td>
<td>Brewer and Sutherland (1993)</td>
</tr>
</tbody>
</table>

Note: n/a = information not available

Brewster and Sutherland (1993) found notably higher base temperatures than other authors for cabbage, onion and red beet, with all being greater than 5 °C. It was not clear how they calculated the base temperature of these vegetables or at what growth stage, so comparison is difficult. It was apparent however that the vegetables were germinated in glasshouses which had constant temperatures of 15-20 °C, so this may have had an effect on the base temperature for development. The base temperature given for carrot (1 °C) was consistent with Atherton *et al.* (1990) who stated that the minimum
temperature for growth in carrot is 0 °C, however Krug (1997) found that 5 °C was the minimum temperature for growth. Suojala (2000) reviewed many of the same references as listed above, and concluded that most literature appears to show a base temperature for growth and development in the vegetative stage to be closer to 0 °C.

Pak-choi is an important cool-season leafy Asian vegetable. Cool-season vegetables in general have been shown to have an optimum germination rate between 12.8-18.3 °C in Asia, but the cardinal temperatures have not previously been published (Cho et al., 2008). Cardinal temperatures are commonly defined by extrapolation of germination rates over temperature range, but other models have also been applied to explain the effect of temperature on germination rates (Cho et al., 2008). Cho et al. (2008) used a quadratic model and a parabolic function to estimate base, optimum and maximum temperatures for germination in pak-choi. Cho et al. (2008) reported that other literature stated parabolic models have a fault of assuming a symmetrical response from the optimum temperature, and they do not allow for any dipped curving close to the base temperature, and that this too was the case with their data. Temperate species usually have a base temperature between 0 and 5 °C (Cho et al., 2008), but in this experiment the base temperature for pak-choi was calculated as 13.5 °C, and pak choi was stated to be sensitive to low temperature. Such a high base temperature in comparison to other temperate species may be due to the experimental method used by Cho et al. (2008), as the pak choi seeds were germinated in growth chambers at constant temperature, but the lowest temperature used was 15 °C. The base temperature was estimated by regressing and extrapolating the inverse of time to 50% germination against a temperature gradient, but 15 °C being the lowest temperature meant that the regression line was likely to intercept the temperature axis at a much higher point than it would if lower temperatures were used. Pak-choi and Chinese cabbage are both of the *Brassica rapa* species, so in theory could have similar temperature requirements. The base temperature given in Table 2.5 for Chinese cabbage was 0 °C, so perhaps under the same growing conditions pak-choi would have a lower base temperature than that given in experiments from Asia.
Bierhuizen and Wagenvoort (1974) found that brassica crops and leafy vegetables in general have a low base temperature of around 1 °C. A similar conclusion was made for root crops such as radish (1-2 °C base temperature) which can therefore be sown early in the season due to their low temperature requirement and rapid germination. Root crops such as carrot and onion have the same base temperature in general, but are much slower to germinate and grow.

2.5 Vernalisation

2.5.1 Explaining vernalisation
The initiation and timing of flowering in certain plants such as the biennial vegetables and perennial ryegrass species of interest in this review are determined by changes in photoperiod and temperature, which are the two main environmental cues (Craigon et al., 1995; Porter and Gawith, 1999; Streck et al., 2003 etc.). Temperature affects both development and plant growth (Porter and Gawith, 1999). Vernalisation is a period of low temperature which an imbibed seed or growing plant is exposed to, which gives them the ability to become reproductive (stimulates flowering) (Kane et al., 2006; Taiz and Zeiger, 2010). Vernalisation research in wheat (Triticum aestivum L.) has shown temperature, duration of low temperature, photoperiod and genotype to be factors which influence the response to vernalisation (Wang et al., 1995).

It is commonly believed that dry seeds do not respond to a vernalisation period, as it is an active metabolic process which requires the seed to have imbibed water (Taiz and Zeiger, 2010), however Wiebe (1990) stated that vernalisation may begin while the seed is still on the mother plant in some species such as red beet. If a plant that requires vernalisation does not receive a suitable cold period, then they are likely to remain in the vegetative stage, or have delayed flowering (Wiebe, 1990; Robertson et al., 1996; Taiz and Zeiger, 2010). Appropriate photoperiod conditions (long days for most vegetable species) are sometimes required along with the achieved vernalisation saturation for floral stimulation (Robertson et al., 1996; Kane et al., 2006; Mauseth, 2012).
Whether a plant has a vernalisation requirement or not can be demonstrated in Figure 2.2 with ryegrass (*Lolium* species) (Leopold and Kriedmann, 1975).

![Figure 2.2 Vernalisation response in days to flowering in Ryegrass (*Lolium*) species (from Leopold and Kriedmann, 1975).](image)

*L. temulentum* had no response to vernalisation (number of days to flower stayed the same regardless of vernalisation duration) which indicates this cultivar does not require a cold period to initiate flowering. Both *L. multiflorum* and *L. perenne* hybrid cultivars showed a decrease in the number of days until flowering occurred as duration at 4 °C increased, so had a moderate vernalisation response. *L. perenne* had a substantial response, with the number of days to flowering decreasing rapidly with vernalisation duration until week 5, indicating it has a strong vernalisation requirement for flowering (Leopold and Kriedmann, 1975).

Plants accumulate vernal degree days (VDD) until vernalisation saturation (*V_{sat}*)(fully vernalised) is reached, where beyond this point further contributions do not increase the effect of vernalisation anymore (Weir *et al.*, 1984). This can be described by a function of
vernalisation ($F_v$), with an equation from the ‘Arcwheat’ model in Weir et al. (1984) (Equation 3).

**Equation 3**

$$F_v = \frac{(VDD - V_{base})}{(V_{sat} - V_{base})}$$

Where $V_{base}$ is the number of consecutive cold days required before vernalisation begins, and $V_{sat}$ is when $F_v \geq 1$ (plant is fully vernalised) (Weir et al., 1984). Temperature affects the rate of VDD accumulated (Figure 2.3).

![Figure 2.3](image)

**Figure 2.3** Temperature effectiveness on vernalisation (from Weir et al., 1984).

Vernalisation temperatures in this model are most effective between 3-10 °C, when the V effectiveness equals one. At -4 °C and 17 °C the V effectiveness is zero, so no VDD are accumulated. At about 0 °C V effectiveness is 0.5 which means the contribution to vernalisation is about 50% of the temperature between 3 and 10 °C. Therefore, it would take twice as many days at 0 °C to reach saturation than at 4 °C (Weir et al., 1984).

The main effect of vernalisation is a reduction of the duration of the leaf primordia production (cells forming new leaves) (Robertson et al., 1996), as the number of leaves is how plants delay development. This is due to the initiation of the collar primordium (final leaf) being earlier than usual and therefore reducing the total number of leaves produced.
on the main stem, which results in earlier flowering. The final leaf number is reduced, given the assumption that the production of leaf primordial is dependent only on temperature.

2.5.2 Juvenile stage
Many of the biennial plants which have an obligatory vernalisation requirement have to have reached a certain size/stage in development before they can successfully receive the cold stimulus (Wiebe, 1990). Prior to this stage in development, the plants are called juveniles. The number of leaves is usually the measure for when a plant is past the juvenile stage, and will respond to the cold stimulus.

Atherton et al. (1990) and Alessandro and Galmarini (2007) both state that for carrot plants, once 8-10 leaves have been initiated and the storage root is 4-8mm in diameter, it is no longer in the juvenile stage and becomes responsive to vernalising conditions. Wiebe (1990) also reported eight or more leaves as the changing point for carrot, so this seems to be consistent; however there is some variation on the root diameter. Dias-Tagliacozzo and Valio (1994) reported that other authors consider the juvenile stage as before the storage root reaches 10mm in diameter. A general conclusion could be made that sensitivity to vernalisation increases with carrot seedling age (Dickson et al., 1961). Receptiveness to cold may be harder to predict in Cabbage, as Wiebe (1990) reported the leaf initiation number when the juvenile stage ends to be between 4-15 leaves which are greater than 2 cm long.

Wiebe (1990) found when reviewing other literature that there is no set juvenile stage for all vegetable species, and that some species can become receptive to vernalisation with seed imbibition (Taiz and Zeiger, 2010) or when the radicle emerges at germination. There are a small number of vegetable crops such as red beet, lettuce and peas that may even become receptive during seed development on the mother plant (Chouard, 1960; Wiebe, 1990). Chouard (1960) had a similar statement saying that in some beet species vernalisation can be achieved in very young plants and even in seeds, but the responsiveness increases with plant age. Yan and Hunt (1999) stated that based on their present understanding, vernalisation experiments preferably treat germinated seeds/growing plants as opposed to imbibed seeds yet to geminate. They do not explain
why this is preferable, but it could be assumed that it may be related to specific species
juvenile stages and when they become receptive to vernalisation. In carrot, vernalisation
response at different growth stages changes with cultivars, but in some it is possible as
imbibed seeds (Chouard, 1960). As cited by Chouard (1960), it was found that when some
carrot varieties from the southern region of the former Union of Soviet Socialist Republics
were chilled as wet seeds at 2 °C for 50-80 days, one third of the plants flowered in the
same year.

2.5.3 Optimum vernalisation
The optimum vernalisation temperature range for all obligatory plants has been widely
studied, with a variety of results (e.g. Craigon et al., 1995; Brooking, 1996; Streck et al.,
2003; Taiz and Zeiger, 2010). Weir et al. (1984) proposed that the optimum vernalisation
temperature generally lies between 3 and 10 °C, and the effective range about -4 – 17 °C
(Figure 2.3). McDonald and Copeland (1997) reported that in America, carrot seed
production used to be located primarily in California, however it is now in colder areas
such as Washington as the necessary vernalisation temperature which they state to be
less than 7 °C was not consistently being met in California.

Yan and Hunt (1999) used an equation for optimum vernalisation temperature (Equation
4).

Equation 4

\[ v = V_{\text{max}} \left( \frac{T_{\text{max}} - T}{T_{\text{max}} - T_{\text{opt}}} \right) \left( \frac{T}{T_{\text{opt}}} \right) \frac{T_{\text{opt}}}{T_{\text{max}} - T_{\text{opt}}} \]

Where;

\( v \) is the daily rate of vernalisation progress

\( T \) is temperature

\( T_{\text{opt}} \) is optimum temperature

\( T_{\text{max}} \) is the maximum temperature for vernalisation

\( V_{\text{max}} \) is the maximum rate of vernalisation on a daily basis and occurs at \( T_{\text{opt}} \)

The optimum temperature for vernalisation is generally agreed to be low, but the
effective range of temperatures and specific vernalisation requirements for different
plant species have contrasting opinions (Yan and Hunt, 1999). Wiebe (1990) defined
optimum vernalisation as the temperature which leads to the fastest flower induction. Vernalisation itself has an invisible nature so can only be evaluated by its after-effects, which are mainly related to final leaf number and days to flowering. These effects are highly dependent on photoperiod and temperature conditions (Yan and Hunt, 1999). The minimum, optimum and maximum temperatures for vernalisation that led to flower generation for a range of vegetable species was published from a range of literature gathered by Wiebe (1990), as well as the number of weeks required (Table 2.6).

Table 2.6 The minimum, optimum and maximum temperature range for vernalisation resulting in flowering, and the duration of cold exposure required in some important vegetable seed crops (adapted from Wiebe, 1990).

<table>
<thead>
<tr>
<th>Species</th>
<th>Inductive temp range (°C)</th>
<th>Duration required (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Opt</td>
</tr>
<tr>
<td>Cabbage</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Carrot</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Onion</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Parsnip</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Radish</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Red beet</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

For most of the species in Table 2.6, if vernalisation doesn’t occur at the optimum temperature then the duration of cold exposure needs to be at least doubled. Cabbage is the most extreme example, with only four weeks of cold exposure needed if the temperature is between 4 and 7 °C, but 20 weeks needed if the temperature is at 0 or 12 °C (Wiebe, 1990). The number of days to flowering after vernalisation was not stated, but due to the definition given it could be assumed that the shortest number of days would be associated with plants in the optimum temperature range.

The optimum vernalisation temperature and duration for carrots has been researched by numerous authors with varying results, but all conclude an optimum temperature between 0-10 °C (Atherton et al., 1990; Wiebe, 1990; Yan and Hunt, 1999; Alessandro and Galmarini, 2007 etc). The extent of the response to chilling is cultivar dependent (Atherton et al., 1990).
Yan & Hunt (1999) found the average optimum vernalisation temperature for the carrot cultivar ‘Chantenay Red Cored’ was 6.6 °C, over 9-15 week exposure treatments. Similar results were published by Atherton et al. (1990), where the most effective response to vernalisation in terms of percentage of carrot plants flowering, were plants exposed to temperatures of 5 °C and 7 °C for 12 and 15 week periods (Table 2.7). These were the only treatments where 100% of the plants flowered. It is important however to note that this was also for the carrot cultivar ‘Chantenay Red Cored’, so the results may differ for other carrot cultivars.

Table 2.7 Effects of different constant temperatures, and length the plants were exposed to temperature, on subsequent flowering in the carrot cultivar ‘Chantenay Red Cored’ (From Atherton et al., 1990).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Duration (weeks)</th>
<th>Plants flowering (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>100</td>
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<td>10</td>
<td>9</td>
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<tr>
<td></td>
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<tr>
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<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>12</td>
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<td>16</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>15</td>
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</tbody>
</table>
The results in Table 2.7 are consistent with the information given previously by Wiebe (1990) in terms of optimum temperature range being around 2-6 °C, and definitely below 10 °C. The duration however differs, as Wiebe (1990) reported that at optimum vernalising temperature the duration should be about 5 weeks, whereas in Table 2.7 twelve weeks gave better flowering results than 9 weeks, which is still far greater than 5 weeks. Twelve weeks was given as the duration for minimum and maximum effective vernalisation temperatures, but here it appears to be the optimum duration for flowering. Differences may be due to different carrot cultivars being used.

It is important to note that many vernalisation experiments take place in a controlled environment such as an incubator, where the exposure to cold is constant, which does not mimic realistic field conditions (Sung and Amasino, 2005). This may change the results as the vernal degree days accumulated over a period of time could differ significantly, as temperature fluctuates in uncontrolled conditions.

2.5.4 De-vernalisaton
The effectiveness of the cold period (in terms of seeds remaining vernalised after a de-vernalisating treatment) increases as duration increases (Figure 2.4). High temperatures can reverse vernalisation (de-vernalisating)(Purvis and Gregory, 1952; Wiebe, 1990). The longer a plant or seed is exposed to cold temperature, the more permanent the effect is. Purvis and Gregory (1952) hydrated winter rye (Secale cereale) seeds and exposed them to 5 °C for different lengths of time, and then transferred them immediately to 35 °C for three days. At a vernalisation period of 2 weeks, none of the seeds remained vernalised (100% de-vernalised), whereas at 8 weeks approximately 95% of seeds retained their vernalisation (Purvis and Gregory, 1952).
Figure 2.4  The percentage of winter rye seeds remaining vernalised vs. duration of vernalisation, after de-vernalisng treatment (from Purvis and Gregory, 1952).

2.6 Photoperiod

2.6.1 Explaining photoperiod
Photoperiod is the length of daylight in a 24 hour period (Taiz and Zeiger, 2010). Day length at the equator is equal to night length, and stays this way year round. As one moves further towards the north and south poles from the equator, the day length increases in summer and decreases in winter. Many plants have evolved the ability to distinguish changes in day length in different seasons (Taiz and Zeiger, 2010). Photoperiodism is the response to day length, or night length, and often acts as a seasonal indicator for flowering in plants (Taiz and Zeiger, 2010; Mauseth, 2012). Specific photoperiod responses are strongly related to the latitude of their origin.

Plants can be classed as long day, short day or day neutral plants. Long day plants can have a qualitative response to photoperiod where flowering is only triggered with long days, or a quantitative response where flowering is accelerated by long days (Taiz and Zeiger, 2010). Short day plants are the opposite, where flowering is triggered by, or accelerated with short days. Day neutral plants are ones which have no response to day
length. The essential differentiation between long day plants and short day plants is that promotion of flowering is when a critical day length is exceeded for long day plants, whereas in short day plants it needs to be less than the critical day length (Taiz and Zeiger, 2010).

Technically, it is the length of dark not light which a plant measures and responds to (Kendrick and Frankland, 1983; Taiz and Zeiger, 2010). Plants contain a photoreceptor protein called phytochrome which senses changes in light. During daylight hours plants accumulate the phytochrome form which absorbs red light (Pr), and this converts to the phytochrome far-red light (Pfr) form, which is an active form that triggers flowering (see Figure 2.5)(Hendricks and Borthwick, 1967). During dark hours Pfr converts back to Pr which is inactive. The length of dark therefore determines flowering in plants. In long day plants the night length needs to be short enough that a build-up of Pfr is created because the dark hours are not long enough to convert all of the Pfr back to the inactive Pf form. Short day plants are the opposite, and require a low concentration of Pfr to trigger flowering (Kendrick and Frankland, 1983; Taiz and Zeiger, 2010).

Figure 2.5 An explanation of red light and far red light accumulation in plants (adapted from Kendrick and Frankland, 1983).
2.6.2 Vegetable photoperiod examples
Carrots have been classified as both day-neutral plants (Sakr and Thompson, 1942; Hiller and Kelly, 1979) and long day plants (Atherton et al., 1984). In an experiment by Atherton et al. (1984), plants that had received their vernalisation requirement were returned to warm (at least 16 °C) glasshouses with different photoperiod conditions. The plants which were returned to long photoperiods (16h) flowered, but the plants returned to short photoperiods (8h) remained vegetative. The experiment also showed that the photoperiod conditions during the chilling period have an effect on the flowering response. After the chilling period, when plants were in 16 hour photoperiod and warm growing conditions, a reduction in the number of plants flowering was seen where a photoperiod of greater than 12 hours was used during chilling. The shorter photoperiods of 0h and 8h throughout the chilling resulted in faster flowering responses (28 and 29 days respectively compared to 33 and 47 days at 12h and 16h photoperiods)(Atherton et al., 1984). There are obvious differences in literature, as Sakr & Thompson (1942) and Hiller & Kelly (1979) showed carrots as being day neutral, even after chilling whereas the results discussed above found that plants which were returned to short photoperiods after chilling did not flower and those returned to long photoperiods did flower. Atherton et al. (1984) suggests that differences in published literature may be due to varied plant ages, and that from their results carrots appear to behave as a combination of short and long-day plants with a vernalisation requirement to induce flowering. Red beet is a quantitative long-day biennial with a cold requirement for flower initiation (George, 2009).

2.7 Conclusions

- The seed industry is an important part of New Zealand’s economy and land based industries, with carrots making up an average of $11.35 million per year.

- Seed quality may be reflected by agronomic aspects such as seed maturity and irrigation. Seed quality may also help to improve weed, pest and disease issues.
• Biennial vegetable species for seed have a growing period greater than 12 months, which can enhance issues with weeds, pests and diseases. Carrot crops are particular susceptible, but there could be potential to reduce these by altering their vernalisation receptiveness and shortening their growing period.

• Base temperature is the lowest temperature at which metabolic processes result in plant growth and development, and therefore a gain in above ground biomass. The base temperature of carrot generally lies between 1 and 5 °C. There is a lack of literature on base temperature for a wide variety of vegetable species.

• Photoperiod and temperature are the two main environmental cues for initiating flowering. Most biennial vegetable species for seed production require a vernalisation period to induce flowering, and the optimum vernalisation temperature and duration generally lies between 3 and 10 °C, and the effective range about -4 – 17 °C, depending on species.

• Many of the biennial plants which have an obligatory vernalisation requirement have to have reached a certain size/stage in development before they can successfully receive the cold stimulus. The exact size/stage varies for species and could be further investigated.
3 MATERIALS AND METHODS

3.1 Experiment 1: Determination of cardinal temperatures

3.1.1 Experimental design
The rate of germination at different temperatures was compared for ten vegetable species and one perennial ryegrass cultivar. The vegetable species used were; Asian radish, cabbage, carrot, Chinese cabbage, mustard, onion, pak choi, parsnip, red beet and red radish (see Table 3.1 for variety and germination percent). Three replicates of 50 seeds per species were placed on moist filter paper in Petri dishes in unlit incubators at constant set temperatures from 5.0 to 40.0 °C (± 0.5 °C) in 5 °C increments. Petri dishes were organised randomly in the incubators. Filter paper was kept moist when necessary with reverse osmosis (RO) water to ensure moisture was non-limiting for germination.

Table 3.1 The vegetable species used in this experiment, sourced from South Pacific Seeds Ltd (Darfield, NZ).

<table>
<thead>
<tr>
<th>Species</th>
<th>Variety</th>
<th>Germination test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot</td>
<td>Hybrid Carrot No 31</td>
<td>88%</td>
</tr>
<tr>
<td>Red beet</td>
<td>Hybrid Red beet KR-333</td>
<td>95%</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Hybrid Cabbage No 164</td>
<td>97%</td>
</tr>
<tr>
<td>Mustard</td>
<td>Hybrid Chinese Mustard CMF-18</td>
<td>99%</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>Hybrid Chinese Cabbage CCF-31</td>
<td>98%</td>
</tr>
<tr>
<td>Pak choi</td>
<td>Hybrid Pak Choi No 4</td>
<td>97%</td>
</tr>
<tr>
<td>Red radish</td>
<td>Hybrid Radish No 108 3.75-4.0</td>
<td>97%</td>
</tr>
<tr>
<td>Asian radish</td>
<td>Hybrid RR Radish N34514</td>
<td>99%</td>
</tr>
<tr>
<td>Onion</td>
<td>OP Onion Baron &gt;2.4</td>
<td>96%</td>
</tr>
</tbody>
</table>

Parsnip seed was not available from South Pacific Seeds Ltd, so was purchased from Mitre Ten gardening centre in Christchurch. The ryegrass seed used was sourced from the Field Service Centre, Lincoln University, and was Samsung AR37 perennial ryegrass treated with Gaucho insecticide/fungicide.

3.1.2 Measurements
Germination of the seeds was counted cumulatively and recorded daily, or twice daily for the first 3 days in high temperatures (>25 °C). Once a seed had germinated it was removed from the dish for ease of counting each day. Germination was counted as a seed
with a radicle of ≥ 1 mm. This measure was by eye, as measuring with any device was not practical. Germination was counted until 50% of the seeds had germinated. In some cases 50% germination was not reached, so the experiment was ceased when no seeds had germinated for more than five consecutive days.

3.1.3 Statistical analysis

The rate of germination was calculated for each temperature treatment for each species when 50% of seeds had germinated. To determine the precise timing of 50% germination, lines of regression were drawn. This consisted of a slope and intercept being calculated using data points from each replicate where the value before 25/50 seeds had germinated and the value at or after where 25/50 seeds had germinated (or closest to 25/50 for species which did not reach 50% germination). The precise point (number of days) where 50% germination occurred was then calculated (See Equation 5).

Equation 5

\[ G_{50\%} = 25 - \frac{\text{intercept}}{\text{slope}} \]

The inverse of duration (1/days) represented the development rate. Multiple (minimum of two) linear regression lines were fitted to mean data points (chosen on visual slopes/changes in response) to determine the cardinal temperatures for each species involved. The base temperature for germination was calculated where the regression for the lower temperature treatments crossed the x axis. The optimum temperature was determined where the regressions intersected at the highest rate of germination. The maximum temperature was calculated where the regression for the higher temperatures crossed the x axis.
3.2 Experiment 2: Determination of vernalisation requirement

3.2.1 Experimental design

Three vegetable species (cabbage, carrot and red beet) and perennial ryegrass were selected for this vernalisation experiment. Imbibed seeds were exposed to a range of cold period durations. The seeds used were from the same source as in experiment one. These vegetable species were chosen as they have similar biennial lifecycles when grown for seed, and ryegrass is a well-documented (successfully vernalised as an imbibed seed) comparison.

This experiment took place in an incubator in the Field Service Centre at Lincoln University. The duration treatments used in this experiment were 0, 2, 4, 6, 8, 10 and 12 weeks. The temperature used for vernalisation of all species was constant 4 °C (± 0.5 °C either side). The experiment began with the longest vernalisation duration being set up first. This consisted of 15 seeds of each species being placed in separate petri dishes on wetted filter paper in the base. This was replicated six times, giving a total of 24 dishes per duration treatment (four species x six replicates each = 24). Petri dishes were kept moist with RO water when necessary.

The longest duration (12 weeks) was started on the 22nd March 2013, followed by the declining durations being added into the incubator every two weeks, so that after 12 weeks all treatments had been in the incubator for the appropriate length of time. The zero week vernalisation treatment was germinated at room temperature, so that no vernalisation was accumulated. Three weeks into the vernalisation period some of the species, particularly cabbage, germinated and began to develop leaves while still in the petri dishes in the 4 °C incubator. The seedlings were planted into trays with potting mix (same mix as used in glasshouse) and moved to another incubator still at 4 °C, but with 24 hour lighting. Light readings were taken with a Skye Spectro Sense at the top bottom and middle of the incubator, and the average light reading was 2.8 µM/m².

On completion of the vernalisation periods (Thursday 13th June, 2013), all seeds/seedlings were planted into pots and kept in a glasshouse. Four seeds/seedlings were chosen from the 15 seeds that were germinated in each petri dish for every species in each duration.
treatment. The four seeds/seedlings were planted into a 2.5 L pot filled with a standard potting mix which was a bark and pumice composite containing the following fertilisers that last for 3-4 months (g/500 L potting mix); Osmocote exact (16-3.5-10) 1500 g, Horticultural lime 500 g and Hydroflo 500 g. This was repeated for each replicate meaning that there were 168 pots in total (4 species x 6 replicates x 7 duration treatments). The pots were arranged in a complete randomised block design in the glasshouse with the replicates blocked (see Appendix 1). Plants were watered when required by staff at the glasshouse with a hand held sprinkler hose.

3.2.2 Temperature and lighting
Air temperature in the glasshouse was measured every two hours and computer logged. The average temperature in the Fletcher glasshouse for each month the experiment ran was between 18.1-19.9 °C (Table 3.2).

<table>
<thead>
<tr>
<th>Month</th>
<th>Average temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June (13th – 31st)</td>
<td>18.1</td>
</tr>
<tr>
<td>July</td>
<td>18.6</td>
</tr>
<tr>
<td>Aug</td>
<td>18.7</td>
</tr>
<tr>
<td>Sept</td>
<td>19.3</td>
</tr>
<tr>
<td>Oct (1st and 2nd)</td>
<td>19.9</td>
</tr>
</tbody>
</table>

The photoperiod was 20 hours consisting of natural and artificial lighting during daylight hours, and artificial lighting during night hours. Each table had a large high pressure sodium lamp hung approximately two meters above the centre of the table used for artificial lighting.

3.2.3 Measurements
The number of days from when the vernalisation period finished (13th June when plants were moved to the glasshouse) to anthesis (flowering) was measured. After flowering, the plant was harvested by cutting off at the base, and the final main stem leaf number was counted. In perennial ryegrass this measurement was taken from the main stem in the primary tiller. These measurements were taken for each plant in each pot, and numbered 1-4 in the order of which plant had flowered first.
3.2.4 Statistical analysis
The statistical analysis was only conducted on ryegrass and red beet species in this experiment, as they were the only species to reach anthesis and therefore produce data.
The number of days from when the plants were potted into the glasshouse until anthesis occurred was analysed in Genstat (15th edition, VSN International Ltd, Hemel Hempstead, UK) individually for both species by a one-way ANOVA to assess if there were significant vernalisation treatment effects, independently due to missing data points.

The number of main stem leaves produced was also analysed individually for ryegrass and red beet by a one way ANOVA to assess if there were significant effects. The significant differences between means for both days to anthesis and main stem leaf number were separated using the least significant difference (LSD) at a 5% level. A regression analysis was conducted on both the number of days to anthesis and the main stem leaf number for red beet and ryegrass.
4 RESULTS

4.1 Base temperature experiment

4.1.1 Germination percentage

The rate and maximum cumulative germination percentage differed with temperature (Figure 4.1 part 1 and 2). At 5 °C Asian radish, Chinese cabbage, mustard and pak choi did not reach 50% germination. At 10 °C Asian radish, Chinese cabbage and mustard again did not reach 50% germination. At 15 °C the only species not to reach $\geq 50\%$ germination was mustard. All species reached $\geq 50\%$ germination in the 20 °C, 25 °C and 30 °C experiments. At 30 °C onion was the only species not to reach 50% germination. At 35 °C onion and parsnip did not reach 50% germination. At 40 °C the only species which successfully reached $\geq 50\%$ germination were Asian radish, Chinese cabbage and pak choi. It is notable that these three were all species which did not reach 50% germination at the lowest temperature (5 °C). Asian radish and Chinese cabbage showed similar germination responses at all of the incubator temperatures. Some similarities can also be seen between onion and parsnip with both not reaching high germination percentages when exposed to temperatures $>30$ °C. Parsnip took at least five days before any germination occurred at any temperature, and was the only species to show this response. The 5 °C temperature was the treatment which showed the slowest germination (longest amount of days for germination to begin), which was 23 days in parsnip.
Figure 4.1 (1 of 2) The average cumulative germination percentage at temperature treatments of 5 °C (●), 10 °C (○), 15 °C (▼), 20 °C (△), 25 °C (■), 30 °C (□), 35 °C (◆) and 40 °C (◇) for Asian radish (a), cabbage (b), carrot (c), Chinese cabbage (d), mustard (e) and onion (f). Time is the number of days from initial imbibition to germination. Error bars represent the standard error of the mean for the final germination percentages.
Figure 4.1 (2 of 2) The average cumulative germination percentage at temperature treatments of 5 °C (●), 10 °C (○), 15 °C (▼), 20 °C (△), 25 °C (■), 30 °C (□), 35 °C (◆) and 40 °C (◇) for pak choi (g), parsnip (h), red beet (i), red radish (j) and perennial ryegrass (k). Time is the number of days from initial imbibition to germination. Error bars represent the standard error of the mean for the final germination percentages.
4.1.2 Germination rate and estimation of cardinal temperatures

The rate of germination graphs were used to find the base, optimum and maximum temperatures for germination for all of the species (Figure 4.2). These were tabulated for ease of comparison (Table 4.1). The germination rates of the vegetable species and perennial ryegrass tested increased linearly with temperature increases from the base temperature up to an optimum. Germination rate declined linearly to zero at the maximum temperature for germination.

The base temperatures of the species involved ranged from -0.2 °C to 6.1 °C. The optimum temperatures ranged from 15.4 °C to 35.9 °C, but most of the species were around 28-30 °C. The maximum temperatures ranged from 37.4 °C to 45.5 °C, but most of the species maximum temperature for germination was around 40-42 °C.

Parsnip had the lowest cardinal temperatures, particularly the optimum temperature at 15.4 °C, which was almost half of that of all of the other species except for onion. Parsnip and onion were the only species which had maximum temperatures of less than 40 °C. Mustard had the highest base temperature at 6.1 °C, but had similar optimum and maximum temperatures to other species. Red beet had the highest optimum temperature at 35.9 °C and the second highest maximum temperature (44.4 °C) after pak choi at 45.5 °C.
Figure 4.2 (1 of 2) Rate of germination (1/days) to 50% germination over a range of temperatures (°C) increasing in 5 °C increments used to find the base, optimum and maximum temperatures for germination for Asian radish (a), cabbage (b), carrot (c), Chinese cabbage (d), mustard (e) and onion (f).
Figure 4.2 (2 of 2) Rate of germination (1/days) to 50% germination over a range of temperatures (°C) increasing in 5 °C increments used to find the base, optimum and maximum temperatures for germination for pak choi (g), parsnip (h), red beet (i), red radish (j) and perennial ryegrass (k).
Table 4.1  The base, optimum and maximum temperature (°C) for germination determined by linear regression for the selected vegetable species and perennial ryegrass.

<table>
<thead>
<tr>
<th>Species</th>
<th>Base temperature (°C)</th>
<th>Optimum temperature (°C)</th>
<th>Maximum temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian radish</td>
<td>3.3</td>
<td>29.7</td>
<td>41.3</td>
</tr>
<tr>
<td>Cabbage</td>
<td>1.4</td>
<td>30.7</td>
<td>41.9</td>
</tr>
<tr>
<td>Carrot</td>
<td>0.1</td>
<td>30.9</td>
<td>40.7</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>4.6</td>
<td>35.2</td>
<td>42.9</td>
</tr>
<tr>
<td>Mustard</td>
<td>6.1</td>
<td>31.1</td>
<td>40.7</td>
</tr>
<tr>
<td>Onion</td>
<td>1.2</td>
<td>21.5</td>
<td>39.6</td>
</tr>
<tr>
<td>Pak choi</td>
<td>3.1</td>
<td>29.4</td>
<td>45.5</td>
</tr>
<tr>
<td>Parsnip</td>
<td>-0.2</td>
<td>15.4</td>
<td>37.4</td>
</tr>
<tr>
<td>Red beet</td>
<td>4.2</td>
<td>35.9</td>
<td>44.4</td>
</tr>
<tr>
<td>Red radish</td>
<td>1.5</td>
<td>28.7</td>
<td>41.6</td>
</tr>
<tr>
<td>Ryegrass</td>
<td>1.1</td>
<td>29.3</td>
<td>40.4</td>
</tr>
</tbody>
</table>

4.2  Vernalisation experiment
Perennial ryegrass and red beet plants showed a positive response to the vernalisation treatment as imbibed seeds, where flowering occurred in plant exposed to vernalisation durations of ≥ 4 weeks (Plate 4.1). Three carrot plants flowered, but were from different vernalisation durations (one each from 6 week, 8 week and 10 week treatments). There were no visual differences for reproductive development among vernalisation durations for carrot (Plate 4.2). No flowering occurred in cabbage plants (Plate 4.3).
Plate 4.1 The visual effect of vernalisation durations of 0-12 weeks on flowering in red beet, with no reproductive changes in 0-2 week plants.

Plate 4.2 Vegetative carrot plants from 0-12 week vernalisation durations with no visual signs of reproductive change in any duration.
4.2.1 The number of plants that flowered

The number of plants which flowered on average per pot (out of four) for perennial ryegrass and red beet generally increased as the vernalisation duration increased (P < 0.001) (Figure 4.3). The interaction between red beet and ryegrass was not significant as their response to vernalisation was the same. There was a significant difference in the number of plants per pot that flowered between species (P < 0.001), with ryegrass having a higher number of plants at every vernalisation treatment. At zero and two week vernalisation treatments, no flowering occurred in any plants from any replicate for red beet. At the four week vernalisation treatment red beet plants began to show some flowering, with one plant per pot on average flowering. This remained the same at the six week treatment, but thereafter for the eight, 10 and 12 week vernalisation treatments, flowering occurred in two to three plants per pot on average. Anthesis in the ryegrass occurred in one plant on average in the zero and two week vernalisation treatments, then increased to two plants in the four week treatment and three to four plants in the six, eight and ten week treatments. At the twelve week treatment all four ryegrass plants flowered in every pot.
The number of plants that flowered per pot (out of a possible 4) over vernalisation durations of 0-12 weeks, for perennial ryegrass (○) and red beet (●). The effect of vernalisation (LSD 0.537, p <0.001) and species (LSD 0.287, P <0.001) was significant with no interaction.

**4.2.2 Vernalisation effect on the number of days to anthesis**

The duration of the vernalisation period had a significant effect (P <0.001) on the average number of days from when the vernalisation treatment ended to when anthesis occurred in perennial ryegrass (Figure 4.4). Plants which were exposed to 12 weeks of vernalisation flowered in the least amount of days on average (68 days). The number of days to anthesis increased continually as vernalisation duration decreased. At the two week vernalisation treatment ryegrass plants took 108 days on average to flower, which was 39 days longer than the 12 week treatment. The greatest change in the number of days to anthesis occurred between two and four weeks of vernalisation (17 days difference). The regression for the number of days to anthesis for ryegrass was significant (P <0.001), explaining 78.6% of the data. The number of days to anthesis decreased by 3.6 days for every one week increase in vernalisation duration.
The number of days on average from the end of the vernalisation period until anthesis occurred in perennial ryegrass over a vernalisation range of 0-12 weeks. The vernalisation treatment had a significant effect on the number of days to anthesis (LSD 8.15, P <0.001).

The duration of the vernalisation period did not have a significant effect (P = 0.143) on the average number of days from when the vernalisation treatment ended to when flowering occurred in red beet (Figure 4.5). The analysis excluded zero and two week vernalisation treatments because no flowering occurred. The number of days to flowering did not change between the eight and twelve week vernalisation treatments. There was an increase in days to flowering on average between the eight and four week treatments (90 and 98 days respectively), but this difference was not significant. No flowering occurred in the zero and two week vernalisation treatments in red beet, therefore it was not included in the analysis. The regression for the number of days to anthesis for red beet was significant (P = 0.034), however it only explains 15.2% of the data. The number of days to anthesis decreased by 0.9 days for every one week increase in vernalisation duration.

Figure 4.4 The number of days on average from the end of the vernalisation period until anthesis occurred in perennial ryegrass over a vernalisation range of 0-12 weeks. The vernalisation treatment had a significant effect on the number of days to anthesis (LSD 8.15, P <0.001).
The number of days on average from the end of the vernalisation period until anthesis occurred in red beet over a vernalisation range of 0-12 weeks. The vernalisation treatment did not have a significant effect on the number of days to anthesis ($P = 0.143$).

### 4.2.3 Vernalisation effect on the number of main stem leaves produced

The duration of vernalisation had a significant effect on the average number of main stem leaves produced in perennial ryegrass ($P = 0.004$)(Figure 4.6). There was little increase in the main stem leaf number (<0.5 leaves) between twelve weeks and eight weeks of vernalisation, but there was a 1.5 leaf increase between the six week and the two week vernalisation treatment. The average main stem leaf number decreased from 5.3 at the two week treatment to 4.9 at the zero week treatment. The regression for the number of main stem leaves for ryegrass was significant ($P < 0.001$), explaining 31% of the data. The number of main stem leaves decreased by 0.1 leaves for every one week increase in vernalisation duration.
Figure 4.6 The main stem leaf number for perennial ryegrass over vernalisation durations of 0-12 weeks. The vernalisation treatment had a significant effect on the number of main stem leaves (LSD 0.817, \( P = 0.004 \)).

The duration of vernalisation did not have a significant effect on the average number of main stem leaves produced in red beet (\( P = 0.323 \))(Figure 4.7). Between the 12 and four week vernalisation treatments the average main stem leaf number of the red beet plants remained between 29 and 33. No flowering occurred in the zero and two week vernalisation treatments in red beet, therefore it was not included in the analysis. The regression for main stem leaf number for red beet was not significant (\( P = 0.42 \)).
Figure 4.7 The main stem leaf number for red beet over vernalisation durations of 0-12 weeks. The vernalisation treatment did not have a significant effect on the number of main stem leaves ($P = 0.323$).
5 DISCUSSION

5.1 Cardinal temperature experiment

5.1.1 Cumulative germination percentage
Seedling germination and emergence in the field is dependent on moisture, oxygen and temperature, so it is important to sow when conditions are optimum. Soil temperatures which seeds are sown into, and experience for germination and emergence fluctuate both daily and seasonally (Moot et al., 2000). Knowledge of the base temperature of different species is therefore important for matching the sowing date to when conditions are most favourable for germination and emergence. The most favourable temperatures for cumulative germination percent for all species were 20, 25 and 30 °C, with these being the only temperatures where all species reached ≥ 50% germination. Bierhuizen and Wagenvoort (1974) stated that carrot and onion were generally slower than other vegetables species to germinate in their experiment. This was consistent with the results from the germination percentage graphs in this experiment, where both species took between 3-5 days for 50% germination in middle range temperatures (15-25 °C) and most other species reached ≥ 50% germination in 0.5-2.5 days. Red beet and ryegrass were not included in the vegetables used in Bierhuizen and Wagenvoort’s (1974) experiment, but they had similar germination time frames to carrot and onion for the selected temperature range in this experiment. Parsnip was the slowest vegetable to germinate at every temperature treatment in this experiment, with no germination occurring at any temperature in less than 5 days. Parsnip is a slow germinating species which may give weeds a competitive advantage at establishment (Monks et al., 2009), so they may populate bare space and capture incoming radiation which compromises the establishment of the slower establishing species.

The reasoning for slower germination in certain species is attributed to thermal time requirements, as the combination of base temperature and heat sums (thermal time in degree days) determines the germination period (Bierhuizen and Wagenvoort, 1974). The heat sum for germination was calculated by integrating the difference between the average daily soil temperature and the base temperature over the germination period. This calculation would differ between constant temperature in a controlled environment,
and conditions in the field, therefore slow germination rates of species such as carrot, onion and parsnip may be even slower or potentially faster in the field depending on temperature fluctuations and degree days accumulated.

5.1.2 Germination rate and cardinal temperatures

5.1.2.1 Methods

The germination rate of the vegetable species and perennial ryegrass tested increased linearly with temperature up to an optimum. Germination rate declined as temperature moved away from the optimum, which was consistent with the favourable germination percentages and fell close to 30 °C for many of the species.

The method which was used in this experiment of finding germination rate by defining 1/days to germination of a defined percentile in the sample is a widely adapted system (Marshall and Squire, 1996). A survey by Angus et al. (1981) showed that responses to temperature of 1/time to 50% of the seed sample were predominantly linear, especially in cereals and legumes, but there were some curvilinear responses also. There was no mention of specific vegetable seed results. Linearity allows extrapolation of rates measured at only a few temperatures to a base temperature, and to an optimum and maximum temperature if the range is wide enough, so is an important and widely used method for the estimation of cardinal temperatures (Marshall and Squire, 1996).

Literature shows that variation studies within populations has sometimes revealed non-linearities or inconsistencies (Marshall and Squire, 1996), similarly to what has been found in this base temperature experiment. High germination percentiles often do not occur at temperatures just above the base temperature. This is evident in the experiment at hand where the percent of seeds germinated was less than 50% at low temperatures (5 and 10 °C for some species) and at high temperature (35 and 40 °C). Marshall and Squire (1996) stated that despite non-linearities being present, they are not great enough to cause unacceptable error in linear models used to predict base temperature, and therefore the method is valid and useful for defining and comparing non-dormant seed lots of most species.
The decisions made in the analysis of data in this experiment are in contradiction of what Marshall and Squire (1996) stated, as there was some exclusion of data points from the germination rate analysis on the basis that they were outliers which would produce inaccurate results. The 5 °C data was removed from the mustard analysis because it only reached 12% germination, so the calculated 50% germination rate (0.01) was so low that it was not realistic to include it as a point above base temperature in a linear regression. If this point was included then the straight line linear regression would force the intercept with the temperature axis to be lower than where the realistic base temperature for where germination would begin to occur. Removing the lowest temperature point however, also causes the estimation of base temperature to be higher than the point where germination actually ceases. An appropriate base temperature is required for practical reasons in the field when planting, as the difference between 5 and 10 °C soil temperature is likely to change the sowing date by months and the establishment and competitiveness of the crop against weeds for any species. For this reason, the removal of unrealistic data points in this experiment improved the base temperature in a practical sense. Using mustard as an example, if the 5 °C data point had not been removed then the base temperature calculated would have been less than 5 °C and a farmer who planted the crop at this lower temperature may only observe 12% germination, whereas with the removal of this point the base temperature was lifted to 6.1 °C where a farmer would observe higher germination rates.

No germination at all occurred at 35 or 40 °C for parsnip, so the 40 °C data point was excluded from the analysis for a more accurate estimate of maximum germination temperature. Similarly with onion, the 40 °C point was excluded from the linear regression line, but was still plotted on the graph, as the rate of germination was unusually high. The inclusion of this point would therefore make the intercept with the X axis unusually high. This could be a limitation of using straight line regression analysis, as a curved line could account for more variable germination rates, and give more accurate estimations of cardinal temperatures. Curved line analysis however, is statistically more difficult to perform and explain. Curved line analyses also has faults, for example in a
parabolic model as used by Cho et al. (2008) for pak choi, a symmetrical response about the optimum temperature for germination is assumed which does not allow for concave curvature near the base or maximum temperatures. Despite this pak choi experiment using a parabolic model instead of a linear model, it is a useful representation of how the base temperature estimation is high (13.5 °C) because the temperature range tested only went as low as 15 °C. Therefore, the fitted line intercepts the temperature axis at a much higher point than if germination rate had been calculated for a wider range of low temperatures.

Base temperature may sometimes be calculated to be below 0 °C, which is difficult to explain biologically (Yang et al., 1995). The only species which was calculated in this experiment to have a base temperature below zero was parsnip, at -0.2 °C.

The base temperature calculated in this experiment for carrot was 0.1 °C, which is consistent with the literature where Suojala (2000) concluded most authors to have found the base temperature for germination in carrots to be close to 0 °C. The calculated base temperature however is lower than any of the base temperatures given in Table 2.5 for carrot. Differences could be related to the methods used by the author, although most have also used regression analysis, or to genetic differences in carrot cultivars used.

5.1.2.2 Moisture

The rate of germination at high temperatures such as 35 and 40 °C may have been negatively affected by moisture levels in the Petri dish. Imbibition in seeds is generally triphasic, where the first phase includes an initial rapid increase in water content, the second phase is a period of little change in water content, and the third phase has another increase in water content when the radicle emerges and grows (Finch-Savage and Phelps, 1993). At high temperatures metabolic processes may have been rapid, and all of the available moisture is likely to have been used in the first phase of imbibition, and/or evaporated. Moisture may then have not been adequate for radicle emergence. Finch-Savage and Phelps (1993) found that in onion, the transition from phase two to radicle emergence was extremely moisture sensitive and was a rate limiting step in germination, as progress ceased when moisture was limiting. This may be the reasoning
for onion in this experiment not reaching levels higher than 30% germination for 30, 35 and 40 °C treatments. When moisture and oxygen are not limiting, temperature is the main dependent of germination, however in this case poor germination rates were likely to have been due to a combination of moisture constraints and heat damage. Germination rates may have been higher if moisture levels were checked more than twice daily, but it is likely to be a combination of these factors, not purely temperature or moisture causing low germination rates.

5.1.2.3 Origin

It is possible that species origin may have an effect on their germination responses over a range of temperatures. Asian radish, Chinese cabbage and pak choi are all likely to have a more tropical origin in Asia than most of the other temperate species used, which could be a factor contributing to their low germination rates at 5 and 10 °C, and superior germination rates compared to all other species at 40 °C. Angus et al. (1981) defined the base temperature differences among a range of temperate and tropical crops. It was found that temperate species had a base temperature of less than 4 °C, where tropical crops were all between 10 and 14 °C.

5.2 Vernalisation

5.2.1 Determining vernalisation response

Various methods of expressing changes in development induced by vernalisation have been used in literature, and this experiment has used the number of days from the end of the vernalisation period to anthesis, and the final number of leaves on the main stem. The final number of leaves on the main stem has been stated as the most appropriate approach because it directly reflects differences in the timing of the transition from the vegetative phase to the reproductive phase in the apex (Wang et al., 1995; Robertson et al., 1996). The final number of leaves on the main stem of a plant is determined by the number of primordia that are initiated up until floral transition (Robertson et al., 1996), therefore the final leaf number subsequently affects the rate of development to anthesis. The number of days to anthesis is an after effect of vernalisation, so does not consider
the effects of continued development during vernalisation; however this can be reflected in the final leaf number.

The effect of vernalisation on the number of leaves which are initiated on the shoot apex at the time of vernalisation saturation can be illustrated by a function of the time taken to reach saturation and the number of leaf primordia initiated during that time, plus the number of leaf primordia present in a seed embryo at germination (Robertson et al., 1996).

5.2.2 Cabbage
No flowering occurred in any cabbage plants from any vernalisation duration. This could be assumed to be due to the imbibed seeds being insensitive to vernalisation (juvenile stage not able to be overcome). This is supported by literature from Wiebe (1990) who stated cabbage was only sensitive to vernalisation when a plant had 4-15 leaves initiated. Other possibilities could include photoperiod effects or the vernalisation temperature used (4 °C) not being cold enough. In a vernalisation experiment on carrots by Atherton et al. (1984), it was stated that shorter photoperiods of 0 hours and 8 hours throughout the chilling resulted in faster flowering responses than 12 hour and 16 hour photoperiods. It is possible that this could be a reason why flowering in cabbage had not been observed, as most of the cabbage plants were exposed to 24 hour photoperiods while in the incubator. Robertson et al. (1996) stated that observation of leaf development on the shoot apex of a plant can give an idea of its vegetative development stage, and if there is going to be a transition to reproductive development. This was performed in this experiment, with the growing point of cabbage plants from all vernalisation treatments being assessed under a high powered microscope for signs of the growing point changing from vegetative to reproductive. There was also no visual evidence of cabbage plants from any vernalisation duration producing reproductive organs (the growing point was dome like and continually producing new leaves)(Plate 5.1), which makes the previous theory related to photoperiod unlikely.
Plate 5.1 Microscopic view of the vegetative stage of the growing point (apex) of a cabbage plant exposed to 12 weeks of vernalisation (at 4 °C).

The effectiveness of vernalisation changes over temperature ranges, which was illustrated by Weir et al. (1984) in the review of the literature. This was a general illustration, and it could be a possibility that 4 °C was not cold enough for cabbage to be vernalised. This suggestion however is not supported by literature from Wiebe (1990), where cabbage is said to have an optimum vernalisation temperature of 4-7 °C, and at this temperature vernalisation saturation should be reached in just four weeks. It could therefore be concluded that the most explainable cause for no flowering in cabbage is the inability to receive vernalisation as an imbibed seed.

5.2.3 Carrot
Three carrot plants out of the entire vernalisation experiment flowered, but there was no consistency in results, as they were not from the same vernalisation treatment. It is therefore hard to draw conclusions as to why these plants flowered. It is however notable that the three plants that did flower were all from vernalisation durations of ≥ 6 weeks, so perhaps there was some vernalisation effect. Kelly and George (1998) stated that
although most vegetables for seed production are biennial, there is occasionally a
tendency for a small number of plants to act as an annual and bolt (stem elongate)
prematurely. This could be a possible reason for the three carrot plants flowering. It may
also be due to genetic variation in the carrot seed used. Although information was very
limited, it was stated that some carrot cultivars in the past have been known to be able to
be vernalised as imbibed seeds (Chouard, 1960), which is contrasting to almost all other
literature, but suggests there may be potential for plant breeding to develop this trait.
Wang et al. (1995) stated that genotype had been shown to be a factor affecting the
vernalisation response in wheat, but techniques for the characterisation of genetic
variation were not well developed.

The reasoning for majority of the plants not flowering is likely to be the same as cabbage,
where imbibed seeds were not sensitive to accumulating vernal degree days. This was
supported again by Wiebe (1990) who stated that carrot required at least 8 leaves
initiated to be receptive to vernalisation. An experiment by Atherton et al. (1990) also
suggested that carrots have a strong juvenile period after plants which were 2 and 4
weeks old with less than three initiated leaves did not show any flowering after a 12
week vernalisation period at 5 °C. Plants older than six weeks with ≥ 7.5 leaves initiated
flowered and showed increases in the number of plants flowering as their age and
number of leaves increased. It is interesting to note that Wiebe (1990) reported that
carrots need short day photoperiods during the vernalisation period, and long day
photoperiod after vernalisation for flowering to occur, but this is a quantitative factor.
This means that vernalisation is the dominating influence and specific photoperiod will
decrease the time to flowering, but flowering would still occur eventually if photoperiod
was not optimum. This could have had an effect on the carrots used in this experiment
because some of the plants (particularly the long vernalisation durations) were shifted
into a 24 hour photoperiod when they began to grow and needed light while still under
the vernalisation treatment.

The apical meristem (growing point) of carrots from all vernalisation treatments were
also observed under a high powered microscope to assess if there were reproductive
organs present. As with cabbage, there was no sign of any of the treatments moving in to
the reproductive phase of development (the growing point was still producing leaf primordia).

5.2.4 Red beet

5.2.4.1 Days to anthesis

The number of days to anthesis was not significantly affected by vernalisation duration in red beet \( (P = 0.143) \), but it is important to note that this was over the 4-12 week durations because no flowering occurred at all for the 0-2 week durations. This suggests that imbibed red beet seeds cannot accumulate enough vernal degree days (VDD) in two weeks at 4 °C to trigger flowering. The fact that there was no significant difference in the number of days to anthesis between 4-12 week vernalisation durations also suggests that 4 weeks at 4 °C was enough time to accumulate the amount of VDD for vernalisation saturation to be achieved. Beyond this point further contributions did not increase the effect of vernalisation anymore, as described by Weir et al. (1984) in the literature for wheat. Wiebe (1990) stated that when red beet is vernalised at its optimum temperature of 5-9 °C it should only need to be exposed to the low temperature for three weeks to initiate flowering. When vernalised at temperatures higher or lower than the optimum range, exposure time for flowering is still low at only five weeks. The results gained in this dissertation experiment were consistent with Wiebe’s (1990) information, as the vernalisation temperature used (4 °C) was slightly lower than the optimum range given for red beet, but flowering only occurred in plants from durations of four weeks and greater. This confirmed that two weeks of cold exposure was not enough for red beet to be fully vernalised and for flowering to occur. Red beet has an obligate vernalisation requirement, so will not flower if it has not received a sufficient cold period.

Large statistical variance resulted in no significant difference in the number of days to anthesis between vernalisation durations. The four plants in each pot of a vernalisation duration, and replicate did not respond in time with each other, creating a large variance in the number of days to flowering.
5.2.4.2 **Main stem leaf number**

As previously mentioned, the number of days to anthesis is affected by the final main stem leaf number. The main stem leaf number did not change significantly over vernalisation duration treatments in red beet plants, which is why there was also no significant differences in the number of days to anthesis. This may be due to red beet having a calculated base temperature of 4.2 °C, which was higher than the vernalisation temperature the imbibed seeds were exposed to, therefore no development would have begun while in the vernalisation treatment. Another possible reason for no significant differences in the main stem leaf number could be due to a minimum number of leaves possible being formed in all vernalisation treatments. The minimum number of leaves which can be produced has been well document for wheat as seven main stem leaves, but no literature was found for red beet.

Vernalisation studies on wheat have found photoperiod to have an effect on the final main stem leaf number between when a plant is fully vernalised to when flowering occurs (Wang *et al.*, 1995). A decrease from a photoperiod of 17 hours to 9 hours increased the final leaf number in wheat from 7 to 13.7. The photoperiod in the glasshouse in the vernalisation experiment in this dissertation was a constant 20 hours, so photoperiod would not have had any effect on the number of main stem leaves produced in red beet or ryegrass.

5.2.5 **Perennial ryegrass**

5.2.5.1 **Days to anthesis**

The number of days to anthesis was significantly affected by vernalisation duration in perennial ryegrass (*P* <0.001). There was less variance in the flowering date among replicates and plants in each pot in the ryegrass compared to the red beet, which made the response more linear and significantly different among the vernalisation durations. The timing of anthesis depends on the number, and the rate of appearance of leaves (Robertson *et al.*, 1996).

The number of days to anthesis for ryegrass species was illustrated in the review of the literature by Leopold and Kriedmann (1975), and perennial ryegrass showed a strong
vernalisation response with no flowering occurring in plants exposed to no vernalisation, and plants exposed to two weeks vernalisation taking almost 160 days to flower. The results gained from the vernalisation experiment in this dissertation (Figure 4.4) differ in that there was some flowering in the zero week treatment, and flowering occurred after about 105 days in the two week vernalisation treatment. Other than these differences, the general response pattern was similar, with a reduction in days to anthesis with an increase in vernalisation duration. The differences may be due to the vernalisation, and glasshouse conditions, as well as differences in perennial ryegrass cultivars and if they have an obligative or facultative vernalisation requirement, which is often unknown. It is assumed that Leopold and Kriedmann (1975) would have used growth chambers with constant temperature, whereas the glasshouse conditions used in this experiment were not constant. For the zero week plants to flower, the glasshouse temperature must have been cold enough for sufficient VDD to be accumulated for flowering to occur. A general model for vernalisation effectiveness over a range of temperatures was given in the review of the literature (Figure 2.3), which showed that some accumulation of VDD was still possible up to 17 °C, however this was for wheat. The glasshouse average temperature for the month of June when the plants were first entered in the glasshouse was 18.1 °C, meaning that there were temperatures lower than this recorded. The maximum temperature where VDD can still be accumulated varies for different species, so this may be higher than 17 °C for perennial ryegrass. It is important to consider the effect that the number of plants that flowered per pot (Figure 4.3) would have had on the results. If more than one of the zero week ryegrass plants on average had flowered then the results may have been more of a curved line response with zero weeks of vernalisation taking the longest to reach anthesis, as opposed to the results gained where zero weeks on average reached anthesis in fewer days than two week plants. The data was restricted by time constraints of the experiment, and future flowering dates could not be statistically estimated.

5.2.5.2 Main stem leaf number
Perennial ryegrass main stem leaf number increased as vernalisation duration decreased, which is likely to be due to continued development and primordium production while in the incubator. This caused a confounding effect between vernalisation effectiveness
lowering the final leaf number, and response of vegetative development to temperature (Robertson et al., 1996). This also affected the number of days to anthesis. Continued development is possible in the ryegrass in this case because the vernalisation temperature used was 4 °C, and the base temperature for germination and development in perennial ryegrass in the experiment was less than this, at 1.1 °C.

Development throughout vernalisation treatments has been reported occasionally in the literature (eg. Robertson et al., 1996), but in young plants as opposed to imbibed seeds. Craigon et al. (1995) considered the possibility of development in young wheat plants during vernalisation treatment, and suggested that plants would advance towards reproductive development at an increasing rate as vernalisation accumulated towards saturation. It may be possible that this could also occur in imbibed seeds, with the maximum number of primordia already developed on the apex by the end of the vernalisation period (the seed is fully vernalised).

5.2.6 Application to the vegetable seed industry
Arable farmers which grow biennial vegetable seed crops such as cabbage, carrot and red beet are often faced with crop rotation, weed, pest and disease issues related to the length of time the crop is in the ground, and this period including winter. The positive vernalisation response of imbibed red beet seeds suggests that there is potential for the sowing date to be after winter, as opposed to late summer/early autumn for a seed crop. This would shorten the lifecycle from greater than 12 months, to 7-8 months. Vernalisation as imbibed seeds does not appear to be a solution for cabbage or carrot seed crops. The ability of red beet seeds to be successfully vernalised means that this process could take place in a controlled environment, and the seeds could be transplanted into the field in optimum growing conditions in spring. The removal of the winter period from the red beet lifecycle would potentially decrease the overall costs of weed, pests and disease control, as this period is where many issues arise. The number of species which could be included in the crop rotation may also be improved by changing the sowing date of red beet seed crops to spring, as well as allowing for the opportunity for other land uses over the winter period. Chouard (1960) stated that there must be a reduction in the total number of leaves produced before flowering as the vernalisation
duration increases for a vernalisation response to be proven for a particular temperature, which would suggest that a vernalisation response was not proven for red beet, but data was not obtained from 0-2 week durations. The objective of this experiment however, was to assess if imbibed seeds could be successfully vernalised, which proved to be positive for red beet. Further research is required for logistics on the optimum vernalisation temperature and duration for red beet seeds.
5.3 Conclusions

- Cardinal temperatures were determined for a range of vegetable species. The base temperature, optimum temperature and maximum temperature for germination in carrot were 0.1, 30.9 and 40.7 °C respectively. Red beet cardinal temperatures were 4.2, 35.9 and 44.4 °C.
- Cabbage and carrot were not successfully vernalised as imbibed seeds, which was most likely due to insensitivity to vernalisation as a juvenile seed or plant. Red beet showed a positive vernalisation response as an imbibed seed, resulting in anthesis in 4-12 week vernalisation durations.
- Ryegrass also responded positively to vernalisation, as expected, with anthesis occurring in all vernalisation durations, confirming the vernalisation technique.
- The number of days from the end of the vernalisation period to anthesis differed significantly in ryegrass, with a general decrease in days as vernalisation duration increased. This is related to continued development and faster reproductive changes with minimised leaf primordial production. Red beet did not have significant differences in the number of days to anthesis between 4-12 week vernalisation durations.
- The final main stem leaf number links with the number of days to anthesis, so showed similar patterns with significant differences in the number of leaves produced between durations in ryegrass, but no significant differences in red beet.
- There is potential for the sowing date of red beet seed crops to be moved to spring, resulting in decreased weed, pest and disease expenses, and the possibility of opportunity cost of a short term winter crop.
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REFERENCES


Suojala, T. 2000. Pre-and postharvest development of carrot yield and quality University of Helsinki, Finland


APPENDICES

Appendix 1 The vernalisation experiment complete randomised block design for the layout of the pots in Fletcher glasshouse.

<table>
<thead>
<tr>
<th>Block 1 (Rep 1)</th>
<th>Block 2 (Rep 2)</th>
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<td>10 wk cabbages</td>
</tr>
<tr>
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<td>6 wk cabbage</td>
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<tr>
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<td>4 wk RG</td>
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