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Quantification of vernalisation for six forage brassica crops

A dissertation

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

Quantification of vernalisation for six forage brassica crops

by

C. B. Jamie

The vernalisation response of six forage brassica crops was studied. The experiment examined phyllochron, days and thermal time to bud, and the thermal time between bud and flower over 0, 3, 6, 9 and 12 weeks of duration of vernalisation treatments of 4°C, 8.1°C, 12°C and 18°C. There was a range of phyllochron values for each crop, where lower vernalisation temperatures and longer durations of exposure resulted in a shorter phyllochron. For 'Mainstar' rape, the range was 35.2 to 88.4°Cd/leaf. For 'Hawkestone' swede, the range was 55.8 to 137°Cd/leaf. For 'Firefly' kale, the range was 75.2 to 178°Cd/leaf. For 'Endurance' radish, the range was 48.3 to 133°Cd/leaf. For 'Hunter' leafy turnip, the range was 22.0 to 80.8°Cd/leaf. For 'CC Pallaton' raphanobrassica, the range was 116 to 149°Cd/leaf. Rape, swede and kale had an obligate vernalisation requirement. A minimum of six weeks at 4°C was required for all plants to develop buds in rape and swede, while kale required the same treatment for any amount of plants to develop buds. Radish, leafy turnip and raphanobrassica indicated a facultative vernalisation response. Buds formed in all radish plants, and in some leafy turnip and raphanobrassica plants, without vernalisation. Three weeks at 4°C were required for all leafy turnip plants to develop buds, while no vernalisation treatment resulted in all raphanobrassica plants with buds. The thermal time to bud decreased with longer durations of colder vernalisation treatments. However, the effect diminished as thermal time approached a crop-specific minimum threshold for bud development. The 18°C temperature treatment did not reduce the thermal time to bud, so it could be beyond the range of inductive temperatures for vernalisation. The thermal time between bud and flower was consistent among durations within the same temperature treatment in kale (303°Cd), leafy turnip (163°Cd), and

raphanobrassica (275°Cd). Variation was observed in rape, swede and radish and was likely attributable to the location of temperature measurements and the frequency of data collection. There is an indication that if vernalisation and photoperiod requirements are saturated, raphanobrassica may not have an overlap in flowering time with rape or leafy turnip and could be planted without isolation.

Keywords: brassica, vernalisation, phyllochron, bud, flower, thermal time

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1 INTRODUCTION

The seed industry is an important component of the New Zealand land based economy. In 2023, $281 million of New Zealand seed were exported to 60 countries. Over 80% of this production occurred in the Canterbury region due to its favourable soil, climate, industry infrastructure and locality of specialized expertise (NZGSTA 2024). New Zealand has an international reputation supported by a high quality seed certification system that export markets trust and value (McKinnon 2018).

Forage brassicas are sown in New Zealand grazing systems for their dry matter yield and nutritional value, and for counter-season seed production for northern hemisphere growers. They are commonly used as supplementary feed during periods of low pasture quality or shortage through summer, autumn and winter; as break crops in farm rotations; to finish stock; and in pasture renewal programs (Charlton & Stewart 2000; Thomson et al. 2016; PGG Wrightson Seeds 2024). The production of genetically homogenous seed is essential for the success of these agricultural practices.

The genomes of agricultural brassica crops diverged from a common ancestor and separately evolved through spontaneous hybridization events and selection for agronomic traits (Wei et al. 2023). This shared genetic lineage increases the risk of hybridisation in the field, which can compromise the genetic purity of seed lots (Sohn et al. 2022). Contamination of seed lots can lead to rejected contracts or legal liability for farmers, which impacts both individual producers and the wider industry. Despite its significance, literature on spontaneous hybridisation among *Brassica* is limited as it occurs at very low frequencies in the field. Hybridisation requires the transfer of viable pollen between plants (cross pollination), which depends on an overlap of flowering periods.

Plants optimise timing of reproductive development through environmental cues (Springthorpe & Penfield 2015). Development between phenological stages (eg. emergence to bud visible; bud visible to flowering) can be described by the accumulation of thermal time until a target is met. In *Brassica*, exposure to a prolonged period of cold temperature (vernalisation) is either required, an obligate response, or can reduce the thermal time requirement for floral initiation, a facultative response. However, the range

of effective temperatures, the required duration, and the consistency of response among species is unclear (Whish et al. 2020).

The aim of this dissertation was to quantify vernalisation requirements for a selection of forage brassica crops to identify those that may pose risk of hybridisation due to overlapping flowering periods. This will help improve seed production management and ensure genetic purity in brassica seed crops.

2 REVIEW OF THE LITERATURE

2.1 Introduction

The objective of this review is to understand brassica seed production in New Zealand, the processes that enable hybridisation and how to quantify the requirements for phenological development. This helps to assess the risk of genetic contamination posed by closely sown brassica varieties.

2.2 Current practices of brassica seed production

2.2.1 Forage brassica use in New Zealand

The Brassicaceae family is morphologically diverse. Annual, vernalisation independent, and biennial, vernalisation dependent, forms exist within many species (Schiessl et al. 2017). Some forage brassicas produce bulbs, such as swedes (*Brassica napus* spp. *napobrassica*), or long stems, such as kale (*B. oleracea* spp. *acephala*). Others can be leafy, such as leafy turnips (*B. rapa*), rape (*B. napus* spp. *biennis*), radish (*Raphanus sativus*) and raphanobrassica (*B. oleracea* spp. *acephala* x *R. sativus*). Other important *Brassica* crops include oilseed rape, cabbage and broccoli (Charlton & Stewart 2000; Dumbleton et al. 2022).

Brassicas used for forage in New Zealand are biennials as they grow vegetatively in the first season and produce seed in the second season. Brassicas are tolerant of frost, which allows them to grow throughout autumn and winter when cool season perennial ryegrasses and legumes have limited growth (Adams et al. 2005). Brassica crops produce dry matter yields of 5 to 20 t DM/ha, which can be stored *in situ* and grazed with little decline in feed value (White & Hodgson 1999). They are widely sown in New Zealand both as supplementary feed and as an alternative for pasture renewal in animal production systems. There is a range of available forage brassica types that are selected according to livestock type and required time of grazing (Fletcher et al. 2012). This review, however, will focus on the production and management of brassica seed crops.

2.2.2 Seed production

Vegetable seed crops are typically grown at latitude 43-44°. In the southern hemisphere, this aligns with the South Island and the southern North Island of New Zealand, as well as southern Chile, where conditions are often too wet for seed production. Northern hemisphere breeding programs leverage the long cool springs, effective quarantine controls, lack of wild vegetable species and stable government in New Zealand to multiply new varieties. The country as a seed multiplication market is internationally respected and trusted with intellectual property in the form of patented hybrid plant material (McKinnon 2018).

The Canterbury region produces 80% of all seed in New Zealand over more than 40,000 hectares of certified crop (NZGSTA 2024). The regions proximity to the Southern Alps results in a reduction in air temperature overnight. This is thought to slow the seed maturation process and optimise seed quality, vigor and viability. Additionally, air masses from the Tasman Sea are dehydrated and warmed as they descend the eastern side of the Southern Alps, which creates a warm and humidity-reducing wind. This is ideal for reducing seed moisture from >50% at physiological maturity to 10-14% where it is safe for harvest and storage. This combination of environmental conditions has supported the emergence of the seed export industry, which is worth in excess of $281 million (McKinnon 2018; Chin 2024).

Brassica seed crops often follow a pasture phase when low levels of disease and contamination can be achieved. In New Zealand, the sowing date is optimised to ensure adequate moisture at sowing, but also sufficient crop cover before winter. Sowing too early results in drought risk while sowing too late results in risk of frost damage. The ideal sowing date in Canterbury is early March to early April for biennial brassicas so that they undergo vernalisation through the winter, flower in the spring and produce seed in the summer (Fasi et al. 2012). The late harvest of these crops is well suited ahead of autumn sowing of wheat (Dynes et al. 2010). The growing cycle is typically 12-14 months from sowing to harvest. The optimum sowing depth for brassica seed is 1 to 1.5 cm (De Ruiter et al. 2009). The main insect pests of brassicas are springtails, diamondback moth, white butterfly and aphids (cabbage grey aphid and green peach aphid). The main fungal diseases are club root

and dry rot. The main viruses are turnip mosaic virus and turnip yellows virus (De Ruiter et al. 2009).

2.2.3 Practices to limit contamination

Crops of different *Brassica* subspecies require isolation distances to prevent gene flow between fields. In New Zealand, this is largely managed through the Seed Crop Isolation Distances (SCID) scheme. This is a digital tool developed by the Foundation of Arable Research (FAR) in 2005 and currently managed by AsureQuality to minimise the risk of crop contamination through cross pollination, and to better utilise the available areas for seed crop production. However, it is a voluntary mapping scheme and therefore growers should not rely exclusively on SCID to identify potential issues (NZGSTA 2020).

Brassica seed can stay viable in soil for more than 5 years after harvest, which can contaminate new sowings (Simard et al. 2002). There should be a minimum of five years before brassicas are resown into the same paddock (PGG Wrightson Seeds 2024). Brassica seed crops are sown with buffer, or 'guard row', plants. These limit gene flow as pollen contamination from outside a crop decreases exponentially from the edges to the center of a crop (Section 2.3.2.2) (Stewart 2002). The width of the border area can in some cases have more influence on contamination rate than further isolation (Pedersen et al. 1969). For crops that require a high level of purity, a larger border distance is recommended, especially when there is also a market for the lower purity seed from the border area. For example, seed used for further seed production or hybrid vegetable brassica used for human consumption generally requires a higher level of purity than forage brassica seed used for grazing. Interspecific and interploidy hybrids often produce seeds that are small and shriveled. This allows a degree of separation during seed processing. Larger seed identified from size grading will have a lower contamination rate than field harvested seed. Potential contamination can also occur from the pollen carried by bees when hives are shifted. The viability of pollen declines over one week and therefore bee hives should not be shifted from one brassica crop to another within 7 days before the crop flowers (Stewart 2002).

2.3 Hybridisation

In Brassicaceae, successful fertilisation requires the acceptance of compatible pollen by the receptive pistil. Pollen lands on the stigma and is followed by pollen grain adhesion, pollen tube foot formation, pollen hydration and germination. Then the pollen tube emerges, penetrates the stigmatic surface and grows through the stigma, style and septum toward the ovule and enters the micropyle. There, two sperm cells are delivered for double fertilisation (Figure 2.1) (Chapman & Goring 2010; Robinson et al. 2021).

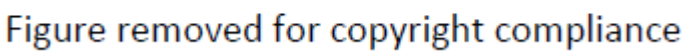

Figure 2.1 Diagram of a pollinated *Brassica* pistil (from Chapman and Goring (2010))

Cross pollination is the delivery of pollen from the stamen of one plant to the stigma of another plant, either within the same species (intraspecific) or between different species (interspecific). Defining hybridisation independent of species circumvents the problem of species definition (Goulet et al. 2017). Therefore, hybridisation is the fertilisation between plants from populations that are different from each other on the basis of one or more phenotypic characters (Arnold 1992).

2.3.1 Genetic compatibility

2.3.1.1 Evolution of Brassica genomes

The relationships between six important agricultural species in the *Brassica* genus is explained by the 'Triangle of U' (U 1935). *B. juncea, B. napus,* and *B. carinata* are the derived allotetraploids from natural pairwise hybridisation of the diploids *B. rapa, B. nigra* and *B. oleracea* (Figure 2.2, Table 2.1) (Kim et al. 2018). Allopolyploids have sub-genomes derived from different species (Kagale et al. 2014; Yang et al. 2021). The *Brassica* and *Arabidopsis* genomes diverged from a common ancestor approximately 43.2 million years ago (Yang et al. 2006). The B genome first diverged from the *Brassica* lineage approximately 9 million years ago, followed by the R, A and C genomes approximately 4.5 million years ago (Mun et al. 2009; Koenig & Weigel 2015; Kim et al. 2018). *B. napus* is an allotetraploid derived from natural hybridization and chromosome doubling of *B. rapa* and *B. oleracea* approximately 7500 (Chalhoub et al. 2014) to 51,000 years ago (Yang et al. 2016). Raphanobrassica is an artificial allotetraploid of *B. oleracea* and *R. sativus* created through colchicine-mediated chromosome doubling and embryo rescue (Table 2.1) (Zhang et al. 2021; Dumbleton et al. 2022).

Table 2.1 Common name, species and genetic composition of six brassica crops

Common name	Species	Genetic composition	
Raphanobrassica	*Brassica oleracea* spp. *acephala x Raphanus sativus*	CCRR	2n = 4x = 36
Swede	*Brassica napus* spp. *napobrassica*	AACC	2n = 4x = 38
Radish	*Raphanus sativus*	RR	2n = 2x = 18
Forage Rape	*Brassica napus* spp. *biennis*	AACC	2n = 4x = 38
Leafy Turnip	*Brassica rapa*	AA	2n = 2x = 20
Kale	*Brassica oleracea* spp. *acephala*	CC	2n = 2x = 18

Adapted from Agrawal et al. (2020); Zhang et al. (2021)

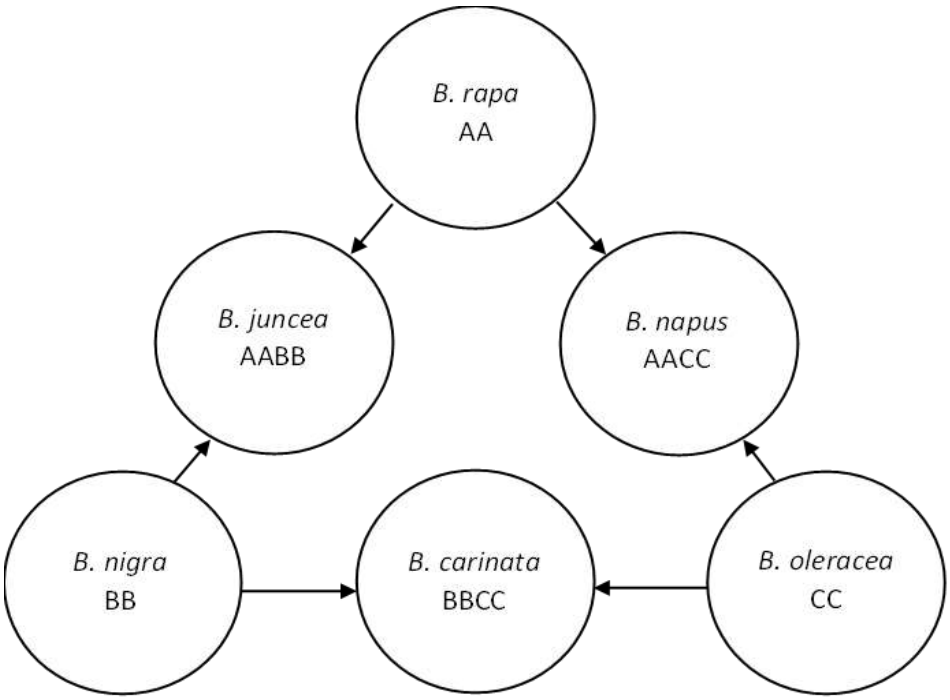

Figure 2.2 The 'Triangle of U' describes the genetic relationships between *Brassica* allotetraploids and their derived diploid species (Adapted from U (1935))

2.3.1.2 Hybridisation in Brassica

It is difficult to simply summarise genetic compatibility and incompatibility among *Brassica* (Scheffler & Dale 1994; Katche et al. 2019). Many species have partial reproductive barriers and different genotypes within a species may differ in their propensity to cross (FitzJohn et al. 2007). The risk of increases as the phylogenetic distance between the constituent genotypes decreases (Katche et al. 2019).

Reports of spontaneous hybridisation (cross pollination under natural conditions) are limited as very low natural hybridisation rates make it difficult to detect (Arnold 1997). Experimental hybridisation (manual hand pollination) allows for measurement of reproductive compatibility and identification of incompatible species combinations (FitzJohn et al. 2007). However, the risk of natural hybridisation between adjacent plants is as much as 20 times less than the seed set reported by hand crossing, and as much as 10,000 times less outside of adjacent fields due to pre-pollination and other ecological barriers (Section 2.3.2) (Stewart 2002). For example, *B. napus* and *B. rapa* have 100% experimental hybridisation by hand pollination in a greenhouse, while spontaneous

hybridisation in the field ranges from 0.02 to 2.78% (Metz et al. 1997; Sohn et al. 2022). Data from controlled experiments therefore significantly overestimate risk of hybridisation in field conditions.

Several studies have produced synthetic *Brassica* hybrids but all lines were meiotically unstable (Abel et al. 2005; Girke et al. 2012; Jesske et al. 2013; Karim et al. 2014). In a hybrid with different chromosome numbers, one or more chromosomes lack a homologous partner and fail to pair correctly during meiotic prophase I (Zamariola et al. 2014). This results in multivalent (three or more chromosomes associate instead of two) or univalent (single, unpaired) chromosomes. Chromosomes must be highly similar in nucleotide sequence and structure for the synaptonemal complex to form correctly. This complex facilitates synapsis and exchange of genetic material through recombination (Zamariola et al. 2014). Failure to correctly align and segregate due to mismatched or incompatible chromosome sets can lead to formation of imbalanced (aneuploid) gametes (Zamariola et al. 2014; Boideau et al. 2021). Aneuploid gametes have missing or extra chromosomes, and often result in inviable zygotes or embryos, or abnormal offspring with reduced vigour and fertility (De Storme & Mason 2014; Mercier et al. 2015).

Non homologous chromosome pairing has been associated with abnormal plant morphology, low pollen viability, low seed set and low fertility in *Brassica* (Katche et al. 2023). *Brassica* hybrids are often unable to produce F_2 or backcross progeny (Scheffler & Dale 1994; Heenan et al. 2007; Sohn et al. 2022). FitzJohn et al. (2007) compiled reported reproductive compatibility within *Brassica* and allied genera (Figure 2.3). Successful experimental crosses in this study resulted in very low rates of hybrid production (median 0.007 hybrids per pollination).

Figure 2.3 Reported successes and failures of hybrid production between *Brassica* crops and relatives. Crops are divided into cases where the crop was the female parent (F) or male parent (M). Numbers in cells indicate how many successful and unsuccessful trials have been reported (successes:failures). Cells are shaded to indicate the proportion of trials that were successful (from FitzJohn et al. (2007)).

2.3.2 Physical compatibility

2.3.2.1 Reproductive barriers in Brassica

Brassicaceae either require cross pollination or can be facultatively cross pollinated. Most wild species in the *Brassica* genus have a sporophytic self-incompatibility (SI) system controlled by the *S*-locus. Recognition of 'self' pollen by the epidermal cell on the stigma surface prevents pollen to germinate and produce pollen tubes that can elongate into the epidermal cell wall (Kachroo et al. 2002; Rea & Nasrallah 2008). The haplotypes and dominance relationships between *S*-alleles inherited from the parent plants drive phenotypic expression of the pollen and stigma (Thompson & Taylor 1966). Diploids *B. rapa*, *B. oleracea* and *R. sativus* exhibit predominant SI (Stewart 2002). Self-compatibility (SC) is common in tetraploid *Brassica* because allopolyploidisation likely occurred in only a few individual plants, and SC acts as an evolutionary advantage for survival of small populations. *B. napus* (Tochigi et al. 2011) and 'Raphanobrassica' (Lewis & Crowe 1958) exhibit predominant SC with 20-33% outcrossing. Some individual tetraploid plants may exhibit SI (Cuthbert & McVetty 2001).

Pollen from the same species is more effective at fertilising ovules compared with pollen from other species (Stewart 2002). Jinling (1990) reported that in *B. napus*, less than 10% of *B. rapa* and *B. oleracea* pollen tubes were able to enter the style. The ability of pollen to adhere to the stigma is independent of SC or SI mechanisms, it is determined by the genetics of the pollen donor (Luu et al. 1997).

After fertilisation, endosperm developmental failure can lead to embryo arrest and seed abortion. This occurs more often in one direction, when the genotype is used as the maternal parent but not when it is used as the paternal parent, or vice versa (Figure 2.3) (Haig & Westoby 1991). This is a widely observed reproductive barrier in interspecific and interploidy crosses (He et al. 2020).

2.3.2.2 Pollen transfer

Hybridisation can only occur when the flowering periods of two compatible genotypes overlap, which enables the transfer of pollen between plants. The closer the flowering time between crops, the greater the percentage of hybrid seedlings (Verdial et al. 2001).

Brassica pollen can be transported by both wind and insect vectors. The relative contribution of each vector is unclear and depends on environmental and topographical conditions as well as research methodology (Eastham & Sweet 2002; Stewart 2002).

Pollen is released in a diurnal pattern which peaks in the middle of the day. Wind transported pollen is believed to travel short distances (Stewart 2002). Tetraploid *Brassica* species have larger and heavier pollen (Sun 1946), which suggests diploid pollen poses higher risk of wind contamination. Hymenoptera (honey-bees) are the most common and important pollinators in mass flowering New Zealand *Brassica* crops, followed by Diptera, Coleoptera and Lepidoptera (Eastham & Sweet 2002; Mesa et al. 2013). Seed set is positively correlated with quantity (Korpela 1988) and activity (Steffan-Dewenter & Tscharntke 1999) of pollinating insects. Foraging distance is related to proximity and quality of flowers. Strong winds and temperatures below 13°C reduce insect pollinator activity. Hedges and other topographical barriers reduce transfer of wind and insect borne pollen, however is difficult to quantify (Bateman 1947; Stewart 2002).

Cross pollination risk decreases at an exponential rate with the distance between compatible plants. This is likely the sum of two factors, one for wind pollination and one for insect pollination that each decrease at a different rate (Stewart 2002). Bilsborrow et al. (1998) reported pollen density of *B. napus* decreased by 50% at 2 m from the crop edge. However, at 360 m, it remained up to 10% of the edge density. Low frequency (0.07%) cross pollination can still occur up to 1.5 km (Timmons et al. 1995) to 3 km (Rieger et al. 2002) from the source field. A report by FAR (2007) found that 98% of bees stayed within 1km of a radish crop, and the furthest distance travelled was 2km. However, insect pollination up to 10 km has been reported where there is a lack of suitable flowers (Ramírez & Davenport 2013).

2.4 Physiological mechanism of phenological development to flowering

During the transition from vegetative growth to reproductive growth, the shoot apical meristem is reprogrammed to produce an inflorescence meristem. The floral transition is regulated by a network of genetically defined but interacting pathways that perceive and respond to a variety of endogenous and environmental stimuli (Akter et al. 2021). These

pathways converge on the floral integrator genes FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) which activate the floral meristem identity genes that promote the irreversible conversion from a vegetative to a floral shoot apical meristem (Tudor 2018). Despite the identification and characterisation of many genes involved, there is a lot that is not understood (Srikanth & Schmid 2011). Current research indicates that the main regulatory pathways governing flowering are conserved between the model plant *Arabidopsis* and *Brassica* species (Itabashi et al. 2019).

2.4.1 Vernalisation

Vernalisation is "the acquisition or acceleration of the ability to flower by a chilling treatment" (Chouard 1960). In natural ecosystems, vernalisation serves to synchronise a plant's reproductive phase with favourable environmental conditions after winter to promote successful reproduction (Henderson et al. 2003). 'Biennial' plants have an obligatory vernalisation requirement, in which a chilling period is required for the plant to flower. 'Winter annual' plants show a facultative response, in which flowering is accelerated but not required (Wiebe 1990).

The vernalisation pathway involves transcriptional and epigenetic regulation mechanisms (Song et al. 2013; Schiessl et al. 2019). Genetic studies on *Arabidopsis* have shown the vernalisation requirement is mainly controlled by two dominant genes, FRIGIDA (FRI) and FLOWERING LOCUS C (FLC) (Strange et al. 2011).

When FLC expression is high, FT expression is suppressed and it overrides activating effects from the other input signals (Srikanth & Schmid 2011). Prolonged exposure to cold temperatures epigenetically represses FLC expression through histone modifications and chromatin remodeling. This repression is maintained throughout subsequent cell divisions and persists even after plants return to warm growth temperatures. This allows the floral transition to occur when a photoperiodic requirement is met in the spring (Kim et al. 2009; Kim & Sung 2014). FRI upregulates transcription of FLC. When active FRI and FLC alleles are present, a vernalisation treatment promotes competence to flower by releasing the FLC-mediated block on flowering (Tallis 2011). Therefore, the response to vernalisation is correlated with the level of FLC expression (Kim & Sung 2014; Tudor 2018; Kim 2020).

Natural accessions of *Arabidopsis* have shown variation in response to vernalisation (Shindo et al. 2006; Tallis 2011). This has been attributed to allelic variants at FLC and FRI (Michaels et al. 2003; Méndez-Vigo et al. 2011). Allelic variation at FRI explains a large proportion of the vernalisation requirement (ie. obligate requirement or not), whereas allelic variation at FLC explains a large proportion of the variation in vernalisation response (ie. how much cold is required to satisfy the vernalisation requirement) (Hou et al. 2012; Li et al. 2014; Irwin et al. 2016).

Unlike *Arabidopsis*, however, a whole genome triplication event during the evolution of *Brassica* has led to duplicate genes that were free to functionally diverge (Wang et al. 2011b). The presence of multiple paralogs, each with sequence variation and different expression patterns, complicates the genetic regulation of flowering in *Brassica*. Multiple paralogues of FLC and FRI have been identified in *Brassica* in comparison to the single gene in *Arabidopsis*. Four copies of *FLC* have been identified in *B. rapa* and *B. oleracea* (Akter et al. 2023), while *B. napus* has nine (Akter et al. 2021).

Allelic variation in paralogs of FLC have been associated to the vernalisation requirement in *B. rapa* (Wu et al. 2012), *B. oleracea* (Irwin et al. 2016) and *B. napus* (Hou et al. 2012; Itabashi et al. 2019). This variation occurs both at the expression level of FLC before vernalisation (which may be affected by single nucleotide polymorphisms in promoter regions), and in the rate of repression of FLC during vernalisation (which may be affected by transposable elements) (Itabashi et al. 2019; Yin et al. 2020). The additional complexity to vernalisation when multiple FLC paralogs are present is not well understood (Itabashi et al. 2019).

2.4.2 The thermosensory pathway

The ambient temperature during vegetative development can affect flowering. This was demonstrated by Srikanth and Schmid (2011) where natural accessions of *Arabidopsis* were grown at elevated ambient temperatures and accelerated flowering was observed. However, Del Olmo et al. (2019) reported that warm temperatures led to delayed flowering in *B. rapa* through the accumulation of histone modifications at the FT locus. This contrasting response suggests that even closely related species may have different

temperature requirements for flowering as an adaptation to their local environment (Li et al. 2024).

2.4.3 Plant age

Many biennial species with an obligate vernalisation requirement must reach a certain stage of development before they become susceptible to cold stimulus (Wiebe 1990). This 'juvenile phase' serves to ensure that the plant has sufficient resources to sustain flower and seed production (Jackson 2009). During this period, the shoot apical meristem it is not competent to flower even if exposed to appropriate environmental cues (Lawson & Poethig 1995; Tallis 2011). The termination of juvenility is commonly defined by the number of initiated leaves (Hand & Atherton 1987; Wurr et al. 1993; Axeisson et al. 2001). By contrast, many annual plants have no clear juvenile phase and stimulus reception can begin in metabolically active seeds (Matar et al. 2021), with emergence of the radicle, or even during seed development on the mother plant (Wiebe 1990).

Wiebe (1990) reported the end of the juvenile phase for most *Brassica* species was 4 leaves greater than 2 cm in length. However, the length of the juvenile period varies can vary between *Brassica* species (Wiebe 1990; Wurr et al. 1994; Hadley & Pearson 1999). It can also vary among different genotypes of the same species. Wiebe (1990) reported that kale, cauliflower and brussel sprouts, which are subspecies of *B. oleracea*, had minimum requirements of 4, 12 and 15 true leaves respectively. Grevsen (1998) reported that the juvenile stage ended at 3.7 true leaves on average in three cultivars of broccoli, while Tallis (2011) reported that the end of the juvenile stage varied from 10.2 to 15 true leaves among four different cultivars of brocolli (*B. oleracea italica*).

2.4.4 Photoperiod

Photoperiod is the length of the light period over 24 hours (Roeber et al. 2022). In plants, it is perceived through the detection of light via photoreceptors and the measurement of time via the circadian clock. These factors influence the CONSTANS (CO) protein to regulate FT expression (Golembeski & Imaizumi 2015; Freytes et al. 2021). Both the duration and quality of light affect CO transcription (Tudor 2018).

Expression of the transcription factor CYCLING DOF FACTOR (CDF) is regulated by a plants internal circadian clock. In the morning, phytochromes perceive light which activates CDF, and CO transcription is repressed. In the afternoon and evening, CDF is repressed. This results in a daily oscillation of CO mRNA levels, with a minimum in the morning and a maximum at night under long days (Freytes et al. 2021). Photoreceptors measure light quality, which regulates CO protein accumulation. In darkness, CO is degraded by the COP1-SPA complex. In the morning, CO is degraded by the absorption of red light. In the afternoon, CO is stabilised by the absorption of far-red and blue light. Under long days, high CO mRNA coincide with stable CO protein accumulation to promote FT activation and floral transition (Freytes et al. 2021; Li et al. 2024).

The phytohormone gibberellic acid (GA) promotes flowering through activation of FT expression in leaves under long day conditions (Song et al. 2013; Li et al. 2024). This process is mediated by DELLA proteins which suppress the transcriptional activity of FT mediators such as CO and PHYTOCHROME INTERACTING FACTOR 4 (PIF4). Under long days, GA production promotes DELLA degradation, which results in increased FT expression. Disruption of GA biosynthesis genes or GA-signaling components results in delayed flowering in *Brassica* (Poza-Viejo et al. 2022).

2.4.5 The autonomous pathway

The autonomous pathway is independent of the vernalisation pathway, but also functions to suppress FLC expression (Kim & Sung 2014). The autonomous pathway includes genes that encode proteins that regulate RNA metabolism. Loss of function of any of these genes led to delayed flowering in *Arabidopsis* from increased FLC expression, but the plants still responded to photoperiod and vernalisation signals (Kim & Sung 2014; Whittaker & Dean 2017; Tudor 2018). Some proteins in the autonomous pathway are involved in the modification of the chromatin structure at the FLC locus. Others are directly involved in binding and processing of RNA transcripts related to FLC. For example, cold-induced long non-coding RNAs (COOLAIRs) recruit chromatin-modifying complexes to add repressive histone marks at the FLC locus which result in histone modification that repress FLC transcription (Kim & Sung 2014). Despite low sequence conservation, transcripts with

secondary structures similar to COOLAIRs have been found in *Brassica* species, which indicates that they have functional similarity (Hawkes et al. 2016).

2.5 Quantifying phenological development

Tracking phenological development of plants at a molecular level is impractical over a large spatial and temporal scale. Therefore, quantifiable metrics that proxy molecular mechanisms have been developed and used in simulation models to predict plant development (Watt et al. 2022).

2.5.1 Cardinal temperatures

Temperature influences the development rate in plants through its impact on enzymatic activity. At low temperatures, enzymes cannot undergo the necessary conformational changes for reactions to take place. At high temperatures, enzymes coagulate and lose their catalytic ability (Bonhomme 2000; Andreucci 2013). These temperature boundaries for development are quantified by cardinal temperatures, which are the base, optimum and maximum temperatures. The base (T_b) and maximum temperatures (T_{max}) are the lower and upper threshold temperatures, respectively, for development. The optimum temperature (T_{opt}) is when rate of development is maximum (Bonhomme 2000). The cardinal temperatures can be estimated by the extrapolation of the inverse of germination rate (Rickman & Klepper 1995; Andreucci et al. 2012). Estimation of T_b is particularly important for biennial brassicas as they grow throughout the winter, accumulating thermal time at suboptimal temperatures (De Ruiter et al. 2009).

Cardinal temperatures are generally conserved within species. Andreucci et al. (2016) reported consistency in cardinal temperatures among species within the *Brassica* genus. This is consistent with reports for other temperate and tropical forage crops (Angus et al. 1980; Moot et al. 2000). A summary of reported cardinal temperatures for crops tested in this experiment is shown on Table 2.2.

Table 2.2 Cardinal temperatures (°C) for development of six brassica crops

Common name	Species	T_b	T_{opt}	T_{max}
Raphanobrassica	*Brassica oleracea* spp. *acephala x Raphanus sativus*	0.40	28.3	35.0
Swede	*Brassica napus* spp. *napobrassica*	1.20	29.0	31.0
Radish	*Raphanus sativus*	1.50	28.7	41.6
Rape	*Brassica napus* spp. *biennis*	3.30	32.9	38.0
Leafy Turnip	*Brassica rapa*	5.10	30.5	48.1
Kale	*Brassica oleracea* spp. *acephala*	-0.10	26.8	35.2

Adapted from Andreucci et al. (2012); McCormick et al. (2013)

2.5.2 Thermal time

Once cardinal temperatures have been defined, thermal time (Tt) accumulation models can be created to quantify Tt requirements and predict upcoming developmental stages (Pessotto et al. 2023). Tt uses cardinal temperatures to normalise plant response to temperature. The accumulated Tt is the sum of daily Tt ($\triangle$Tt) and modified by vernalisation (F_v, Section 2.5.2.1) and photoperiod (F_p, Section 2.5.2.2) (Equation 1).

Equation 1 $$\mathbf{Tt} = \sum(\triangle \mathbf{Tt} \times \mathbf{F_v} \times \mathbf{F_p})$$

In its simplest form, $\triangle$Tt can be calculated by subtracting the base temperature from the daily mean temperature (T_{avg}) (Equation 2).

Equation 2 $$\triangle \mathbf{Tt} = \mathbf{T_{avg}} - \mathbf{T_b}$$

However, this fails to reflect the reduced effectiveness of temperature beyond T_{opt}. the simplest and most widely adopted modification is to include a linear decrease in $\triangle$Tt above T_{opt} down to T_{max} (Equation 3 and Equation 4)(Andreucci et al. 2012).

Equation 3 $$\triangle \mathbf{Tt} = \left(\mathbf{T_{avg}} - \mathbf{T_b}\right) \text{ when } T \leq T_{opt}$$

Equation 4 $$\triangle \mathbf{Tt} = \left(\mathbf{T_{max}} - \mathbf{T_{avg}}\right) \text{ when } T \geq T_{opt}$$

2.5.2.1 Vernal effect on thermal time

As a biological process, vernalisation can be characterised by a unique set of cardinal temperatures which determine the effectiveness of temperature in vernal accumulation (V_{eff}). The effectiveness is zero or negligible below T_{min} and increases to maximum effectiveness at T_{opt}. The effectiveness then declines from T_{opt}, until it reaches zero at and above T_{max}. The fastest floral induction occurs at T_{otp}. In the upper and lower ranges, the temperature must occur longer to be inductive (Lumsden 1980). The vernalisation response is therefore a function of both the temperature (which dictates the effectiveness), and the time spent at that temperature (Wiebe 1990; Tallis 2011). Plants delay development by forming new leaves. The main effect of vernalisation is a reduction of the duration of leaf primoridia production by bringing forward the time of initiation of the collar primordium (Robertson et al. 1996).

Vernal degree days (VDD) are the unit used to quantify a vernalisation requirement that accounts for the effects of both the temperature and the time spent at that temperature. Although rarely used when calculating vernal time, estimates of VDD are also more accurate when daily temperatures are segmented into smaller intervals (Whish et al. 2020). This accounts for accumulation of VDD in days with low minimum and high maximum temperatures, whereas none is accumulated when the average daily temperature approach is used in the same environment.

Plants accumulate VDD until a saturation point (V_{sat}) is reached, beyond which there is no further response (Tallis 2011). At $F_v \geq 1$, the vernalisation requirement is saturated. In the ARCWHEAT 1 model developed by Weir et al. (1984) for winter wheat, this process can be described by the function of vernalisation (Equation 5).

Equation 5 $$\mathbf{F_v = (VDD - V_{base}) / (V_{sat} - V_{base})}$$

Where V_{base} is a minimum amount of VDD before a vernalisation response is observed.

For example, Lumsden (1980) used a three stage linear function for winter wheat where effectiveness equaled zero at -4°C (T_{min}) and 17°C (T_{max}), and one between 3°C (T_{opt1}) and 10°C (T_{opt2}). It would take twice as many days to reach saturation at 0°C (where $V_{eff} \approx 0.5$)

than it would at 6°C. When V_{sat} is set at 33 and V_{base} at 8, F_v = 1 when VDD = 33 and the vernalisation requirement is saturated (Weir et al. 1984). This model can be applied to other plants with vernalisation requirements (Whish et al. 2020).

Wiebe (1990) reviewed literature and found that the temperature that resulted in the fastest floral induction (T_{opt}) in vegetables occurred between 5°C and 8°C, and the maximum inductive temperature (T_{max}) between 10°C and 14°C, with few exceptions. The author also reported that the minimum time to flower was achieved after 3-5 weeks at 6°C for *B. oleracea*, 2-4 weeks at 7°C for *B. napus* and 1-4 weeks at 5-8°C for *Raphanus sativus*. Robertson et al. (2002) reported that the minimum time to flower across 17 cultivars of *B. napus* was achieved after 25 days at 3°C. In the APSIM model for *B. napus*, Tt from emergence to the end of the juvenile stage is reduced by accumulation of VDD (Robertson & Lilley 2016).

2.5.2.2 Photoperiodic effect on thermal time

Photoperiodism is the developmental response of plants to the length of the dark period (Hamner & Bonner 1938). The number of photoperiod effective hours (P_H) is calculated daily based on the site latitude and Julian day number to describe the relative movement of the earth and the sun.

In the ARCWHEAT 1 model developed by Weir et al. (1984) for winter wheat, this process can be described by the function of photoperiod (Equation 6).

Equation 6 $$\mathbf{F_P = (P_H - P_{base}) / (P_{opt} - P_{base})}$$

Where P_{base} is the minimum photoperiod for development to occur and P_{opt} is the photoperiod where development is accelerated the most. The F_v function is normalised between 0 and 1 so it can reduce, but not increase, the accumulation of Tt (Weir et al. 1984).

In most annual crop species, there is a pre-inductive phase from sowing in which development is insensitive to photoperiod. This is tested by transferring plants from long- to short-day regimes and vice versa various times between sowing and flowering

(Robertson & Lilley 2016). However, Robertson et al. (2002) found that vernalized seedlings of *B. napus* respond immediately to the length of the photoperiod.

Robertson et al. (2002) observed that 17 genotypes of *B. napus* responded to photoperiods between 10.8 and 16.3 hours, with a rate of phenological shortening between 61 and 156°Cd/hour. Nanda et al. (1996) observed a maximum photoperiod response between 12 and 14 hours, with a diminished response up to 16 hours. The rate of response was 108°Cd/hour, which lies in this range. King and Kondra (1986) observed a photoperiod response up to 18 hours in some genotypes of *B napus.* and *B campestris*.

Miglietta (1989) reported that vernalisation and photoperiod influence time of flowering in *Triticum* spp. by their effect on the final leaf number on the meristem. Field studies of *B. napus* have shown that vernalisation and long days reduce the time to flowering, primarily by shortening the period from emergence to the initiation of floral primoridia (Robertson & Lilley 2016). When vernalisation is obligate, the photoperiod generally only has facultative effects (Wiebe 1990). *Brassica* are therefore defined as facultative long-day plants where the duration from sowing to flowering is shortened under long days (Wiebe 1990; Axeisson et al. 2001; Robertson et al. 2002; Tallis 2011). However, there is a lack of literature that describes the interactions been photoperiod and vernalisation. As a result, these processes are usually treated independently in crop models which may give rise to errors (Robertson & Lilley 2016; Whish et al. 2020).

2.5.2.3 Phyllochron

The phyllochron is the interval between visual appearance of leaves of successive phytomers (Wilhelm & McMaster 1995). The most frequently used hypothesis testing model is the linear model, which is based on the fact that the production rate of new phytomers is often found to be nearly constant when expressed in Tt (Rickman & Klepper 1995). In this model, the phyllochron is the inverse of the slope of the linear function and is the amount of degree days needed for a leaf to appear (°Cd/leaf). The assumption of this model is the constant rate of leaf production and that it is not affected by the environment. However, some authors have shown that phyllochron can be affected by other factors such as temperature and photoperiod (Cao & Moss 1989; Tamaki et al. 2002).

Slafer and Rawson (1995) reported a linear increase in phyllochron between 10°C and 22°C, followed by a sharp decline in rate above 22°C in wheat (*Triticum aestivum*). Cao and Moss (1989) reported an exponential increase in the phyllochron of wheat and barley (*Hordeum vulgare*) as temperature increased, as a result of lower thermal efficiency. These are both in contrast with findings from Jamieson et al. (2008), who reported a constant phyllochron but only when the temperature was measured near the apical meristem. The discrepancy was suggested to be attributed to the location of temperature data collection, where leaf appearance rate responded linearly to shoot apex temperature. The results of Jamieson et al. (2008) supported the conclusion of Jamieson et al. (1995), that the apex and leaf expansion zone respond directly to the temperature they perceive. This highlights potential systematic error in phyllochron estimation due to lack of accuracy of temperature measurements. The shoot apex in cereals remains below or near the soil surface until stem elongation, therefore requiring soil temperature data. This may be less relevant for *Brassica*, as the growing point, after emergence, is above ground (Andreucci et al. 2012).

Adams et al. (2005) reported a constant phyllochron (T_b = 4°C) for 'Gruner' kale of 65°Cd/leaf, 'Kestrel' kale of 68°Cd/leaf, 'Goliath' rape of 61°Cd/leaf, and 'Green Globe' turnip of 52°Cd. Collie and McKenzie (1998) reported a phyllochron for four cultivars of turnip of 40°Cd/leaf. Chakwizira (2008) reported a phyllochron for 'Pasja' leafy turnip of 109°Cd/leaf and for 'Regal' kale of 60°Cd/leaf. Dam (2006) reported a phyllochron for two cultivars of fodder radish of 69 and 79°Cd/leaf.

Fletcher et al. (2012) reported a phyllochron for kale of 76°Cd/leaf, rape of 60°Cd/leaf, swede of 51°Cd/leaf and turnip of 47°Cd/leaf. However, the authors also reported that phyllochron may be higher earlier in the growth cycle, and the break in relationship coincided with the approximate time of canopy closure. This was also suggested by Andreucci (2013), that larger leaves eventually shade leaf primordia that are therefore not activated which decreases the rate of leaf appearance after canopy closure.

Andreucci (2013) reported an increase in phyllochron with an increase in mean air temperature for turnip cultivars of 3.6°Cd/leaf/°C between 7 and 21°C. The author also reported that phyllochron ranged from 52 to 106°Cd/leaf for 'Aparima Gold' swede, from

20 to 67°Cd/leaf for turnip cultivars and from 42 to 92°Cd/leaf for 'Goliath' rape. Morrison et al. (1992) also reported a decrease in rate of leaf appearance between 10 and 25°C at a rate of 0.0009 leaves/°Cd/°C. Many authors have reported differences in phyllochron between different sowing dates (Nanda et al. 1995; Collie & McKenzie 1998; Andreucci 2013). Andreucci (2013) reported a decrease in phyllochron with a decrease in photoperiod at 12.7°Cd/leaf/hour for swedes and 13.3°Cd/leaf/hour for forage rape.

2.6 Conclusions

A combination of low overnight temperatures, humidity reducing winds and trust in New Zealand regulatory systems have facilitated the emergence of a brassica seed multiplication export industry in Canterbury. Despite the morphological diversity in the *Brassica* genus, agricultural brassica crops have a high degree of genetic homogeneity which make them susceptible to hybridisation. This is limited in natural systems through pollen adherence barriers, endosperm developmental failure and reduced fertility of hybrids, and further limited in agricultural systems through isolation distances, guard rows, and flowering asynchrony. Vernalisation and photoperiod are the two main environmental cues for floral initiation. *Brassica* are quantitative long day plants with photoperiodic responses up to 18 hours, and can have an obligate or facultative responses to vernalisation with inductive vernal temperatures between 0 and 18°C. The response can vary between genotypes. This study will evaluate the vernalisation requirement of six forage brassica crops to assess the possibility of flowering at the same time. If two crops do not have an overlap of flowering, there is no risk of genetic contamination.

3 MATERIALS AND METHODS

3.1 Experimental Design

Vernalisation requirements of six brassica crops (Table 3.1) were quantified from March to September of 2024 at Lincoln University, New Zealand. All cultivars are developed and commercialised by PGG Wrightson Seeds.

Table 3.1 Common name, species and cultivar of six brassica crops tested from March to September 2024 at Lincoln University, New Zealand.

Common name	Species	Cultivar
Raphanobrassica	*Brassica oleracea* spp*. acephala x Raphanus sativus*	'CC Pallaton'
Swede	*Brassica napus* spp*. napobrassica*	'Hawkestone'
Radish	*Raphanus sativus*	'Endurance'
Rape	*Brassica napus* spp*. biennis*	'Mainstar'
Leafy Turnip	*Brassica rapa*	'Hunter'
Kale	*Brassica oleracea* spp*. acephala*	'Firefly'

3.1.1 Establishment phase

The first stage of the experiment aimed to establish plants before the vernalisation treatment was imposed on them. It was done at the Aluminex glasshouse located at the nursery at Lincoln University, New Zealand (43°38'42.0"S 172°27'42.5"E). Three seeds of each species were planted at 10-15 mm depth into 2.5 L pots. Each pot was filled with a standard potting mix of bark, pumice composite and nutrients. It contained 2500 g of osmocote exact (15-3.9-9.1), 500 g of horticultural lime and 500 g of hydroflo, for each 500 L of potting mix. A total of 480 pots were used for 6 species, 4 temperature treatments, 5 periods of temperature treatment and 4 replicates. Each pot was considered a replicate. Once plants emerged, a 1 cm thick layer of vermiculite was added on top of the potting mix. This layer was added to buffer changes in temperature that could happen due to the artificial lights in the growth chambers, where the temperature treatments were imposed. Pots were placed on plastic trays where water was added. This guaranteed that moisture stress was not a factor in the experiment. Each species remained in the glasshouse until the average number of true leaves per plant reached four. This took 28.5 ± 5.5 days from

sowing. After that, pots were transferred to the growth chambers located at Lincoln University. The experiment was a factorial of temperature by species by duration of the vernalisation period.

3.1.1.1 Glasshouse conditions

Air temperature (2 m from ground; canopy height) was logged hourly by a HOBO MX2302 Ext Temperature/RH Data logger (Table 3.2). After plants reached the 4 leaf stage in the 0 week treatment and after the vernalisation treatment in the growth chambers, photoperiod at the glasshouse was set at 20 hours to saturate any photoperiod requirement. This consisted of natural and artificial light during daylight hours and artificial light during night hours. Artificial light was provided by large high pressure sodium lamps hung approximately 2 m above each table (Figure 3.1). Light quality emitted by lamps at canopy height was 6.19 µmol/m^2/s red (r) to 2.51 µmol/m^2/s far red (fr), or a 2.46 r:fr ratio. Trays were rotated 90 degrees clockwise twice weekly to reduce confounding effects (ie. distance from light, edge effects).

Table 3.2 Monthly average air temperature (°C) in the Aluminex glasshouse from March to September 2024 at Lincoln University, New Zealand.

Month	Average Air Temperature (°C)
March (4^{th}-31^{st})	19.6
April	19.4
May	17.0
June	16.7
July	16.5
August	17.8
September (1^{st}-28^{th})	19.6

Figure 3.1 Supplementary light provided by sodium lamps for artificial 20 hour photoperiod in glasshouse at Lincoln University, New Zealand.

3.1.2 Vernalisation phase

The second stage included each species placed into growth chambers at a constant temperature. Pots were then removed in groups according to the duration treatment. The 0 week treatment remained in the glasshouse and was placed under artificial lights to be exposed to a 20 hour photoperiod. When each duration treatment (Figure 3.2, Table 3.3) was completed, the pots were returned to the glasshouse to be exposed to the 20 hour photoperiod. Two growth chambers were available at a time. The first round of experiments had growth chambers at 4°C (± 0.34°C) and 18°C (± 0.21°C) and the second round of experiments had growth chambers at 8.1°C (± 0.12°C) and 12°C (± 0.11°C).

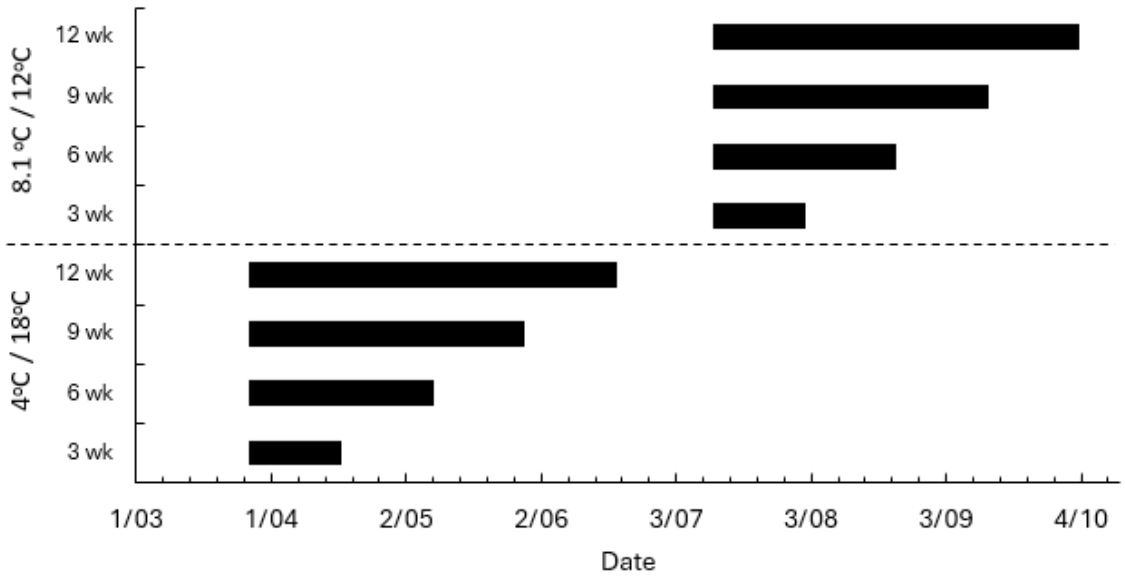

Figure 3.2 Temperature treatments and duration of the vernalisation periods at Lincoln University, New Zealand from March to September 2024.

Table 3.3 Sowing dates and removal dates of six brassica crops from growth chamber vernalisation treatments across five durations at Lincoln University, New Zealand from March to September 2024.

Treatment	Species	Sowing date	4-leaf/0wk	3wk	6wk	9wk	12wk
18°C	Raphanobrassica	04/3	28/3	18/4	09/5	30/5	20/6
4°C	Swede	04/3	28/3	18/4	09/5	30/5	20/6
	Radish	04/3	26/3	16/4	07/5	28/5	18/6
	Rape	04/3	26/3	16/4	07/5	28/5	18/6
	Turnip	04/3	26/3	16/4	07/5	28/5	18/6
	Kale	04/3	28/3	18/4	09/5	30/5	20/6
12°C	Raphanobrassica	07/6	15/7	05/8	26/8	16/9	07/10
8.1°C	Swede	07/6	15/7	05/8	26/8	16/9	07/10
	Radish	07/6	08/7	29/7	19/8	16/9	30/9
	Rape	07/6	08/7	29/7	19/8	09/9	30/9
	Turnip	07/6	08/7	29/7	19/8	09/9	30/9
	Kale	07/6	15/7	05/8	26/8	16/9	07/10

3.1.2.1 Growth chamber conditions

The vernalisation treatments were conducted in Conviron BDW40 Plant Growth Chambers with internal dimensions of 2.36 m (length) x 1.57 m (width) x 2.11 m (height). Fresh air exchange was controlled by motorised dampers and an upward airflow distribution system used sufficient make up air to provide ambient CO_2 levels inside the chamber. Humidity was uncontrolled but measured to ensure there was no water limitation due to low humidity (76.9 ± 11.1% SD). Programmable high intensity metal halide lamps (Phillips Green Power CDM-TP) provided 500 µmol/m^2/s at the top of plant canopy for a photoperiod of 8 hours. Below 8°C, a defrost cycle occurred at approximately 3 hour intervals, during which the main chamber lighting was turned off for approximately 20 minutes. To maintain daylight conditions during the defrost cycles for the 4°C treatment, supplementary LED lighting providing 120 µmol/m^2/s was set up approximately 50 cm above canopy height and programmed to turn on when main lights dimmed below 500 µmol/m^2/s (Figure 3.3). Light intensity was monitored by an Apogee SP110 Pyranometer at 0.5 second intervals and recorded with a Campbell Scientific CS1000 data logger. The 8.1°C treatment was set up to avoid this defrost threshold. To minimize confounding effects (ie. air circulation causing disparate temperature in center and edge), pots were individually rotated within their tray, twice weekly.

Figure 3.3 Supplementary LED lights maintain daylight conditions during defrost in 4°C growth chamber at Lincoln University, New Zealand.

3.2 Pest and disease control

Grey cabbage aphid (*Brevicoryne brassicae*), green peach aphid (*Myzus persicae*) and powdery mildew were documented in the glasshouse and growth chambers. Aphids were mainly present during experiment 1, and are a vector of turnip mosaic virus (Charlton & Stewart 2000; Caciagli 2008) and radish mosaic virus (Dikova 1995). Six turnip plants and eight radish plants presented visual signs of virus, characterised by stunted growth, mottled and crinkled leaves (Charlton & Stewart 2000) (Figure 3.4). Once identified, no further measurements were recorded for these plants due to their abnormal growth.

(a) (b)

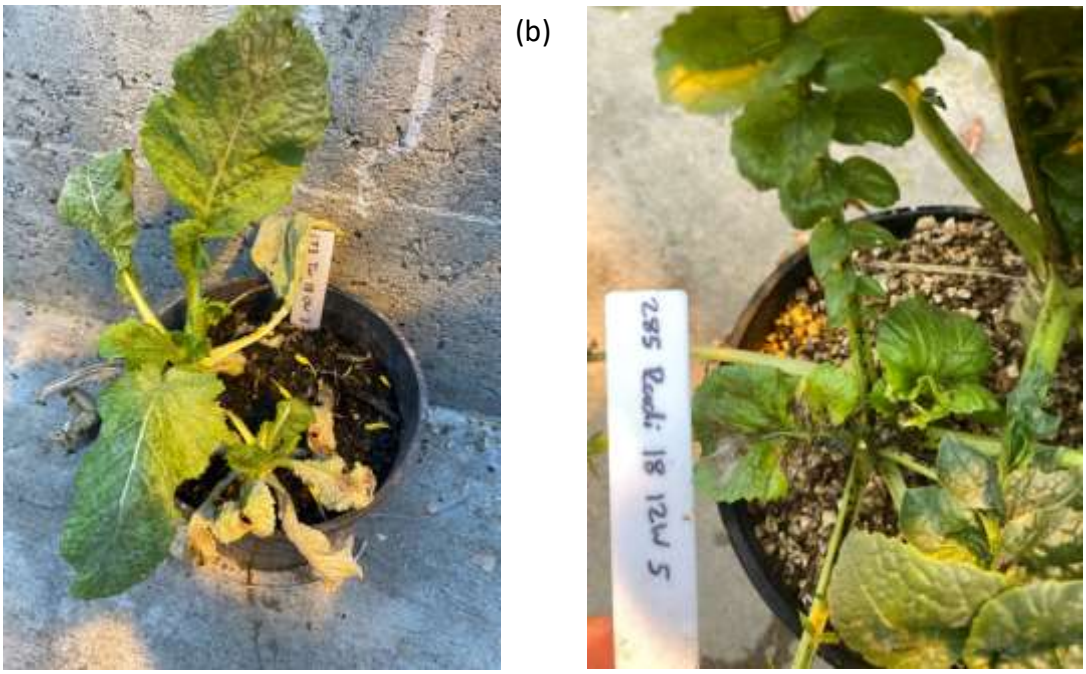

Figure 3.4 Visual identification of turnip mosaic virus (a) and radish mosaic virus (b)

Powdery mildew is caused by the fungus *Erysiphe polygoni* (Hückelhoven & Panstruga 2011). Fungicide application did not completely remove mildew from affected plants, it only stopped the progression of the fungus (Figure 3.5). From visual assessments, leafy turnips were the most affected plants. These were followed by swedes, rape, radish, kale, and raphanobrassica had the least incidence of powdery mildew.

Figure 3.5 Visual identification of powdery mildew

The timeline when agrichemical was applied to control pests or disease is provided in Table 3.4. All applications were made using a knapsack sprayer, each spray was supplemented with Citowett spreader-wetter (1000 g/L exothylated octyl phenol) at a concentration of 0.1 ml/L.

Table 3.4 Insecticide and fungicide inputs applied to six brassica crops at Lincoln University, New Zealand from March to September 2024.

Date	Type	Trade Name	Active ingredient	Rate (mL/L)
25/03	Insecticide	Karate Zeon®	Lambda-cyhalothrin (250g/L)	0.04
24/04	Insecticide	Transform®	Sulfoxafor (240g/L)	2.00
31/04	Insecticide	Transform®	Sulfoxaor (240g/L)	2.00
30/05	Fungicide	Ridomil® Gold	Metalaxyl-M+ (40g/L)	2.50
			Mancozeb (640g/L)	
31/05	Fungicide	Ridomil® Gold	Metalaxyl-M+ (40g/L)	2.50
			Mancozeb (640g/L)	
17/07	Fungicide	Ridomil® Gold	Metalaxyl-M+ (40g/L)	2.50
			Mancozeb (640g/L)	

3.3 Measurements

Emergence recorded daily for each individual plant until no further emergence occurred. Leaf appearance was recorded twice weekly for the duration of the experiment. Using the BBCH-scale by Meier (2001), a leaf was considered 'true' when its petiole was visible or the tip of the next leaf was visible, and it was not a cotyledon (Figure 3.6). Each new true leaf was marked with a permanent marker and recorded.

Figure 3.6 Characterisation of leaf appearance where the marked leaf is considered present, and the unmarked emerging leaf is not yet considered present.

The presence or absence of a bud was recorded twice a week at a minimum. This began as soon as the first reproductive bud was identified. The bud was considered present as soon as it was visible without manually peeling away leaves (Figure 3.7).

(a)  (b) 

Figure 3.7 Characterisation of bud appearance showing a bud that has not yet emerged (a) and an emerged bud (b).

The presence or absence of a flower was observed twice a week at a minimum. Once a single bud had visible petals (Figure 3.8), the date was recorded. Once all plants in a pot showed visible flowers, the date was recorded and the pot was removed with no further data collected.

Figure 3.8 Characterisation of flower appearance was the presence of visible petals from a minimum of one bud.

3.4 Statistical analysis

The data was analysed in Genstat (15th edition, VSN International Ltd, Hemel Hempstead, UK). One-way ANOVAs were conducted within each temperature treatment because data was incomplete. Linear regressions were fitted to the relationship between Tt and leaf number. The phyllochron was calculated from the linear portion of the regression but not forced through the origin because the time to the first leaf represented the emergence period. Days and Tt to bud are reported from the start of the vernalisation period.

Missing values were replaced with the mean when at least three of four replicates were available. Individual plants that developed buds were included in the analysis of the number of plants with buds but excluded from the vernalisation analysis if fewer than three or four replicates were available. Where there was insufficient data, the data was excluded from the analysis but presented for completeness. Any treatments without any results were excluded from all analyses. All plants that did not emerge or died were removed from all analyses.

Where data was not analysed but means are provided for completeness, a standard deviation is reported.

4 RESULTS

4.1 Phyllochron

4.1.1 Rape

The phyllochron of 'Mainstar' rape decreased ($p<0.001$) as the duration of the 4°C vernalisation treatment increased (Table 4.1). It decreased from 88.4°Cd/leaf at 0 weeks to 38.4°Cd/leaf (± 2.35°Cd/leaf) at 9 and 12 weeks. The same trend was observed for the temperatures of 8.1 ($p=0.002$) and 12°C ($p<0.001$). The phyllochron was consistent ($p=0.893$) among durations at 18°C and an average of 84.1°Cd/leaf (± 3.08°Cd/leaf) was observed.

Table 4.1 Phyllochron (°Cd/leaf) of 'Mainstar' rape (T_b = 3.30°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	88.4_a	87.5_a	86.9_a	85.7
3	71.2_b	77.7_b	79.7_b	82.8
6	53.2_c	77.0_b	81.8_{ab}	82.8
9	41.5_d	73.7_{bc}	72.0_c	85.9
12	35.2_d	66.2_c	68.8_c	83.1
Mean	58.0	76.4	77.9	84.1
SEM	2.35	2.76	2.12	3.08
Significance	<0.001	0.002	<0.001	0.893
LSD	7.20	8.50	6.54	ns

Note: LSD refers to the comparison among durations under the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$

4.1.2 Swede

The phyllochron of 'Hawkestone' swede decreased ($p<0.001$) as the duration of the 4°C vernalisation treatment increased (Table 4.2). A decrease in phyllochron was also observed from 0 to 6 weeks at the 12°C treatment ($p<0.001$). For the 6, 9 and 12 weeks durations, the average phyllochron was 91.5°Cd/leaf (± 3.00°Cd/leaf). The phyllochron was consistent ($p=0.107$) among durations at 8.1°C and an average 109°Cd/leaf (± 6.43°Cd/leaf) was observed. In the 18°C treatment, the phyllochron was longer ($p=0.037$) at 12 weeks. The shortest phyllochron was observed at 0 weeks, with intermediate values at 3, 6 and 9 weeks.

Table 4.2 Phyllochron (°Cd/leaf) of 'Hawkestone' swede (T_b = 1.20°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	124_{a}	121	120_{a}	114_{c}
3	111_{b}	115	108_{b}	132_{ab}
6	76.4_{c}	110	93.8_{c}	134_{ab}
9	55.8_{d}	105	94.6_{c}	120_{bc}
12	56.3_{d}	95.0	86.2_{c}	137_{a}
Mean	84.8	109	101	128
SEM	2.97	6.43	3.00	5.26
Significance	<0.001	0.107	<0.001	0.037
LSD	9.14	ns	9.24	16.2

Note: LSD refers to the comparison among durations under the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$

4.1.3 Kale

The phyllochron of 'Firefly' kale decreased ($p<0.001$) from the 0 week duration to the 9 and 12 weeks durations at 4°C (Table 4.3). The same response was observed for the treatments of 8.1 ($p=0.003$) and 12°C ($p=0.015$), with similar phyllochron values for 6, 9 and 12 weeks. The phyllochron was consistent ($p=0.126$) among durations of 18°C and a mean of 165°Cd/leaf (± 6.67°Cd/leaf) was observed.

Table 4.3 Phyllochron (°Cd/leaf) of 'Firefly' kale (T_b = -0.10°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	166_{a}	124_{a}	122_{a}	178
3	146_{a}	113_{ab}	109_{ab}	163
6	118_{b}	99.4_{bc}	106_{abc}	158
9	76.5_{c}	92.0_{c}	92.2_{c}	153
12	75.2_{c}	97.5_{c}	96.3_{bc}	172
Mean	116	105	105	165
SEM	6.78	4.88	5.28	6.67
Significance	<0.001	0.003	0.015	0.126
LSD	20.9	15.0	16.3	ns

Note: LSD refers to the comparison among durations under the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$

4.1.4 Radish

'Endurance' radish decreased its phyllochron from 0 to 6 weeks at 4°C ($p<0.001$), from 0 to 3 weeks at 8.1°C ($p=0.004$) and from 0 to 12 weeks at 18°C ($p=0.037$) (Table 4.4). There were no differences among the durations of 6, 9 and 12 weeks at 4, 8.1 and 18°C. The phyllochron was consistent ($p=0.062$) among durations of 12°C and a mean of 85.3°Cd/leaf (± 4.24°Cd/leaf) was observed.

Table 4.4 Phyllochron (°Cd/leaf) of 'Endurance' radish (T_b = 1.50°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	117_a	104_a	95.4	131_a
3	89.9_b	81.1_b	76.1	133_a
6	58.9_c	76.3_b	80.6	118_{ab}
9	50.2_c	75.0_b	87.2	119_{ab}
12	48.3_c	74.6_b	87.3	105_b
Mean	72.8	82.1	85.3	121
SEM	4.72	4.71	4.24	5.90
Significance	<0.001	0.004	0.062	0.037
LSD	14.6	14.5	ns	18.2

Note: LSD refers to the comparison among durations under the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$

4.1.5 Leafy Turnip

'Hunter' leafy turnip decreased its phyllochron from 0 to 12 weeks at 4°C ($p<0.001$), and from 0 to 9 weeks at 8.1 ($p<0.001$) and 12°C ($p<0.001$) (Table 4.5). There was no difference between the 9 and 12 weeks durations at 8.1 and 12°C. The phyllochron was consistent ($p=0.244$) among durations of 18°C and a mean of 76.9°Cd/leaf (± 5.66°Cd/leaf) was observed.

Table 4.5 Phyllochron (°Cd/leaf) of 'Hunter' leafy turnip (T_b = 5.10°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	80.4_a	70.8_a	71.0_a	77.6
3	54.3_b	54.7_b	59.0_b	85.9
6	33.7_c	41.9_c	55.5_b	72.8
9	38.8_c	35.3_d	42.7_c	80.8
12	22.0_d	34.8_d	38.9_c	67.6
Mean	45.8	47.5	53.4	76.9
SEM	3.80	2.07	3.36	5.66
Significance	<0.001	<0.001	<0.001	0.244
LSD	11.7	6.37	10.4	ns

Note: LSD refers to the comparison among durations under the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$

4.1.6 Raphanobrassica

The phyllochron of 'CC Pallaton' raphanobrassica was consistent among durations at 4°C (p=0.070), 8.1°C (p=0.109), and 18°C (p=0.937) (Table 4.6). The mean phyllochron values were 124°Cd/leaf (± 6.16°Cd/leaf), 148°Cd/leaf (± 5.10°Cd/leaf) and 135°Cd/leaf (± 3.38°Cd/leaf), respectively. In the 12°C treatment, the phyllochron was highest (p=0.023) at the 0 week duration. It then decreased among the 3, 9 and 12 weeks durations with an intermediate value at 6 weeks.

Table 4.6 Phyllochron (°Cd/leaf) of 'CC Pallaton' raphanobrassica (T_b = 0.4°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	140	142	145_a	135
3	130	132	123_b	138
6	120	135	137_{ab}	136
9	116	149	124_b	135
12	116	130	130_b	133
Mean	124	138	132	135
SEM	6.16	5.10	4.41	3.38
Significance	0.070	0.109	0.023	0.937
LSD	ns	ns	13.6	ns

Note: LSD refers to the comparison among durations under the same temperature treatment; means followed by the same letter are similar at α = 0.05

4.2 Vernalisation

4.2.1 Number of plants with buds

4.2.1.1 Rape

Vernalisation treatments at 8.1, 12, and 18°C did not result in any buds until the end of measurements on the 26th of September of 2024 (Table 4.7). At 4°C, the percentage of plants that developed buds increased ($p<0.001$) from 0% at the 0 week duration to 20.8% at 3 weeks and to 100% from 6 weeks onwards.

Table 4.7 Percentage (%) of 'Mainstar' rape plants with buds across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	0_a	-	-	-
3	20.8_b	-	-	-
6	100_c	-	-	-
9	100_c	-	-	-
12	100_c	-	-	-
Mean	64.2	-	-	-
SEM	5.59	-	-	-
Significance	<0.001	-	-	-
LSD	17.2	-	-	-

Note: LSD refers to the comparison among durations at the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$. Empty cells indicate no buds were present until 26/09.

4.2.1.2 Swede

Vernalisation treatments at 8.1, 12, and 18°C did not result in any buds until the end of measurements on the 26th of September of 2024 (Table 4.8). At 4°C, the percentage of plants that developed buds increased ($p<0.001$) from 0 and 8.3% at 0 and 3 weeks durations respectively, to 100% from 6 weeks onwards.

Table 4.8 Percentage (%) of 'Hawkestone' swede plants with buds across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	0_a	-	-	-
3	8.33_a	-	-	-
6	100_b	-	-	-
9	100_b	-	-	-
12	100_b	-	-	-
Mean	61.7	-	-	-
SEM	3.73	-	-	-
Significance	<0.001	-	-	-
LSD	11.5	-	-	-

Note: LSD refers to the comparison among durations at the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$. Empty cells indicate no buds were present until 26/09.

4.2.1.3 Kale

Vernalisation at 8.1, 12, and 18°C did not result in any buds until the end of measurements on the 26th of September of 2024 (Table 4.9). At 4°C, the percentage of plants that developed buds increased (p=0.018) from 0% at 0 and 3 weeks to 62.5% (± 15.5%) at 9 and 12 weeks. The 6 weeks duration was similar to all other durations.

Table 4.9 Percentage (%) of 'Firefly' kale plants with buds across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	0_a	-	-	-
3	0_a	-	-	-
6	45.8_{ab}	-	-	-
9	75.0_b	-	-	-
12	50.0_b	-	-	-
Mean	34.2	-	-	-
SEM	15.5	-	-	-
Significance	0.018	-	-	-
LSD	47.8	-	-	-

Note: LSD refers to the comparison among durations at the same temperature treatment; means followed by the same letter are similar at α = 0.05. Empty cells indicate no buds were present until 26/09.

4.2.1.4 Radish

At 4°C, all durations resulted in 100% of plants with buds (Table 4.10). At 8.1°C, the percentage of plants that developed buds decreased ($p<0.001$) from an average of 80.6% (± 12.7%) at 0, 3 and 6 weeks durations to 12.5% and 0% at 9 and 12 weeks durations, respectively. At 12°C, the percentage of plants with buds went ($p<0.001$) from 83.3% at 3 weeks to 8.33 and 0% at 9 and 12 weeks durations. At 18°C, the percentage of plants with buds ranged ($p=0.042$) from 100% at 0 weeks to 58.3% at 9 weeks. Only the 9 weeks duration was different from the 0, 3 and 6 weeks durations, which averaged 93.1% (± 8.74%). The 12 weeks duration had 75% of plants with buds and was not different from the other durations.

Table 4.10 Percentage (%) of 'Endurance' radish plants with buds across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	100	66.7_{a}	45.8_{b}	100_{a}
3	100	87.5_{a}	83.3_{a}	87.5_{a}
6	100	87.5_{a}	75.0_{ab}	91.7_{a}
9	100	12.5_{b}	8.33_{c}	58.3_{b}
12	100	0_{b}	0_{c}	75.0_{ab}
Mean	100	50.8	42.5	82.5
SEM	0	12.7	12.0	8.74
Significance	<0.001	<0.001	<0.001	0.042
LSD	ns	39.0	37.1	26.9

Note: LSD refers to the comparison among durations at the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$.

4.2.1.5 Leafy turnip

At 4°C, the percentage of plants that developed buds increased (p=0.004) from 58.3% at 0 weeks to 100% at 3, 6, 9 and 12 weeks durations (Table 4.11). At 8°C, the percentage of plants with buds decreased from 70.8% at 6 weeks to 0% at 0, 9 and 12 weeks durations. The 3 weeks duration had 16.7% of plants with buds, and was not different from the 0, 9 and 12 weeks durations. Vernalisation at 12°C did not result in any buds until the end of measurements on the 26th of September of 2024. At 18°C, the percentage of plants with buds decreased from 70.8% at the 0 week duration to 0% at 12 weeks. The 3, 6 and 9 weeks durations had 18.0% (± 11.5%) of plants with buds, and was not different from the 12 weeks duration.

Table 4.11 Percentage (%) of 'Hunter' leafy turnip plants with buds across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	58.3^{b}	0_{b}	-	70.8_{a}
3	100^{a}	16.7_{b}	-	20.8_{b}
6	100^{a}	70.8_{a}	-	25.0_{b}
9	100^{a}	0_{b}	-	8.30_{b}
12	100^{a}	0_{b}	-	0_{b}
Mean	91.7	17.5	-	25.0
SEM	10.1	7.83	-	11.5
Significance	0.004	<0.001	-	0.008
LSD	22.0	24.1	-	35.5

Note: LSD refers to the comparison among durations at the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$. Empty cells indicate no buds were present until 26/09.

4.2.1.6 Raphanobrassica

The vernalisation temperature of 4°C resulted in an average (p=0.489) of 50% (± 17.5%) of plants with buds, independent of duration (Table 4.12). Vernalisation at 8.1°C or 12°C did not result in any buds until the end of measurements on the 26th of September of 2024. The percentage of plants with buds in the 18°C treatment that were not exposed to a vernalisation period was not different to the percentage observed at the 6 weeks duration. These percentages were lower at 3 weeks and were further reduced at 9 and 12 weeks.

Table 4.12 Percentage (%) of 'CC Pallaton' raphanobrassica plants with buds across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	33.3	-	-	66.7_a
3	41.7	-	-	33.3_b
6	58.3	-	-	66.7_a
9	75.0	-	-	0_c
12	41.7	-	-	8.30_c
Mean	50.0	-	-	35.0
SEM	17.5	-	-	7.14
Significance	0.489	-	-	<0.001
LSD	ns	-	-	22.0

Note: LSD refers to the comparison among durations at the same temperature treatment; means followed by the same letter are similar at α = 0.05. Empty cells indicate no buds were present until 26/09.

4.2.2 Days and thermal time to bud from start of vernalisation treatment

4.2.2.1 Rape

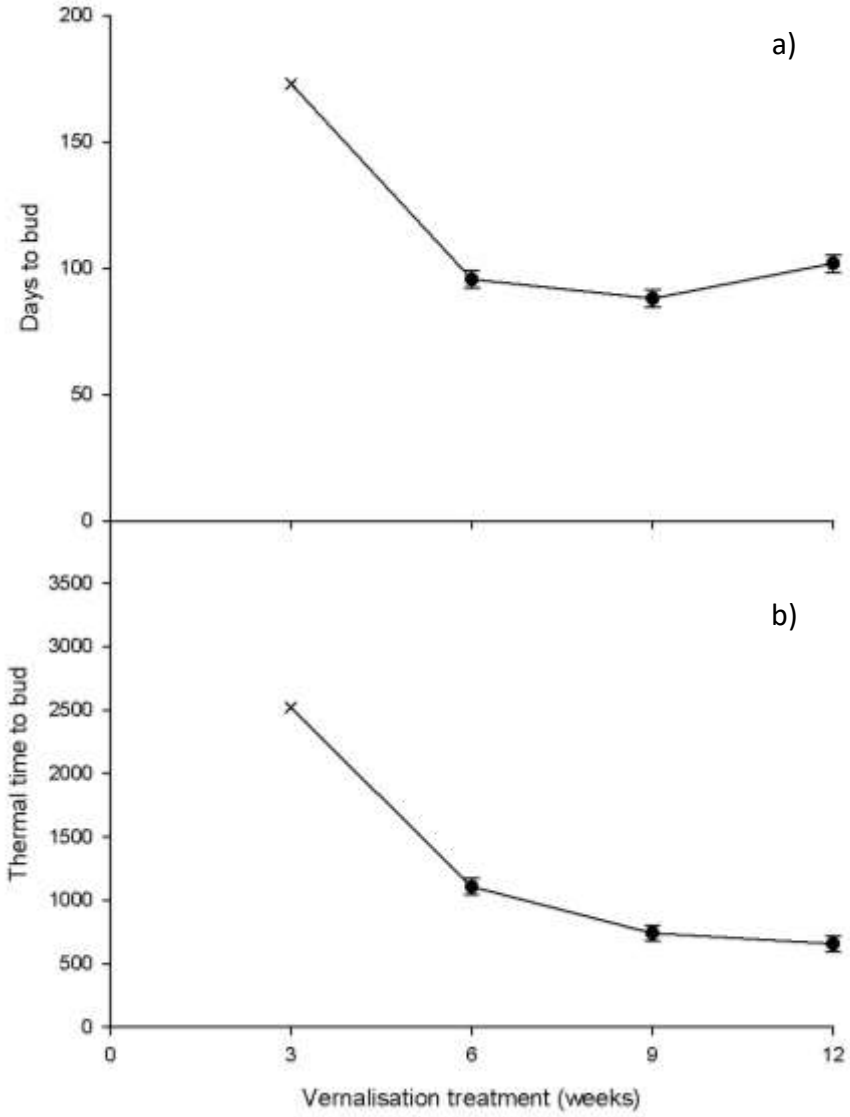

Figure 4.1 Average number of days (a) and thermal time (°Cd) (b) for 'Mainstar' forage rape until bud in relation to the duration of the 4°C (●) vernalisation treatment, from 0 to 12 weeks, at Lincoln University, New Zealand from March to September 2024. The duration of 4°C was not significant for days to bud ($p=0.072$) but was significant for thermal time to bud (LSD 156, $p<0.001$). Error bars represent the standard error of the mean. × indicates insufficient data so was excluded from the analysis but included for completeness.

The number of days to bud was consistent ($p=0.072$) among durations of 4°C and an average of 95.3 days (± 3.40 days) was observed (Figure 4.1). The Tt to bud decreased ($p<0.001$) as the duration of the 4°C vernalisation treatment increased. It decreased from 1110°Cd at 6 weeks, to 698°Cd (± 44.9°Cd) at 9 and 12 weeks.

4.2.2.2 Swede

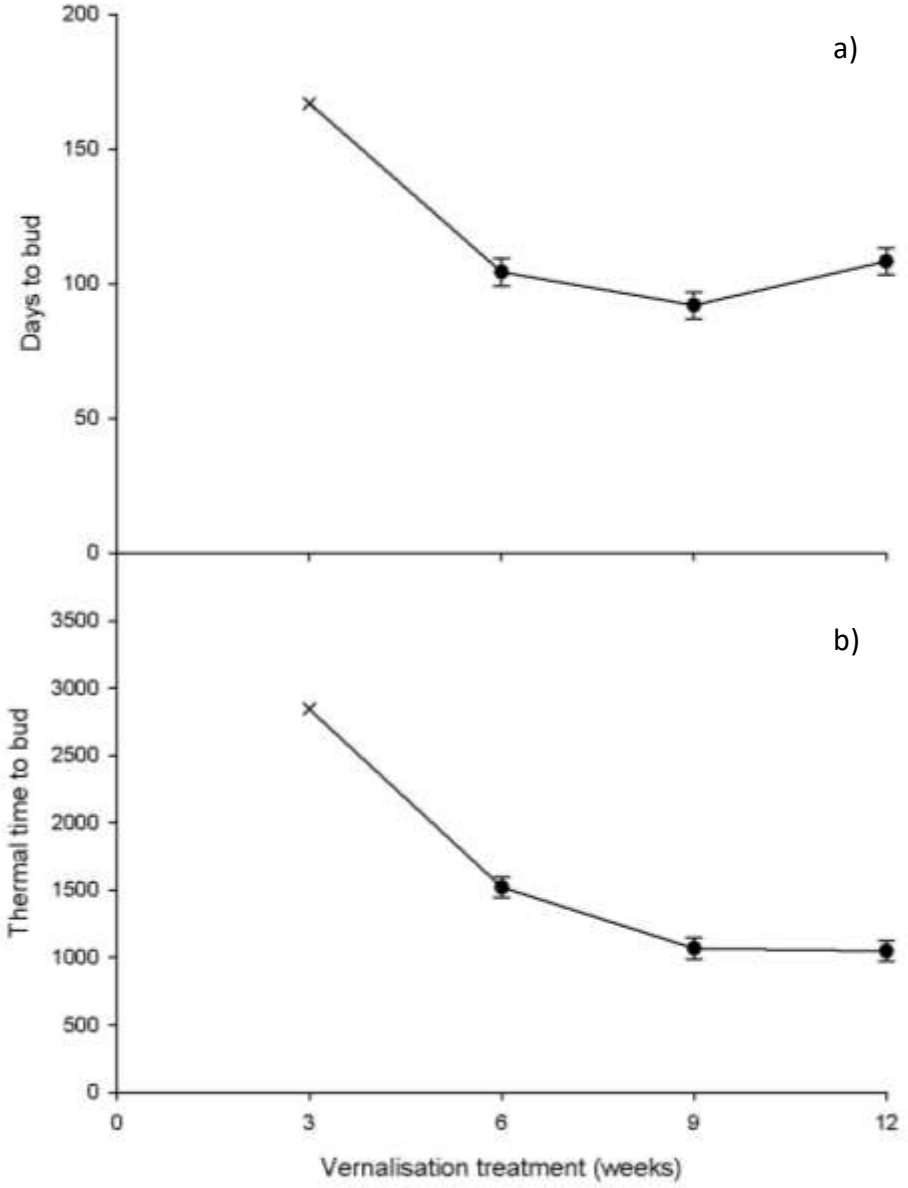

Figure 4.2 Average number of days (a) and thermal time (°Cd) (b) for 'Hawkestone' swede plants until bud in relation to the duration of the 4°C (●) vernalisation treatment, from 0 to 12 weeks, at Lincoln University, New Zealand from March to September 2024. The duration of 4°C was not significant for days to bud (p=0.132) but was significant for thermal time to bud (LSD 272, p=0.009). Error bars represent the standard error of the mean. × indicates insufficient data so was excluded from the analysis but included for completeness.

The number of days to bud was consistent (p=0.132) among durations of 4°C, and an average of 102 days (± 5.01 days) was observed (Figure 4.2). The Tt to bud decreased (p=0.009) as the duration of the 4°C vernalisation treatment increased. It decreased from 1520°Cd at 6 weeks to 1060°Cd (± 78.6°Cd) at 9 and 12 weeks.

4.2.2.3 Kale

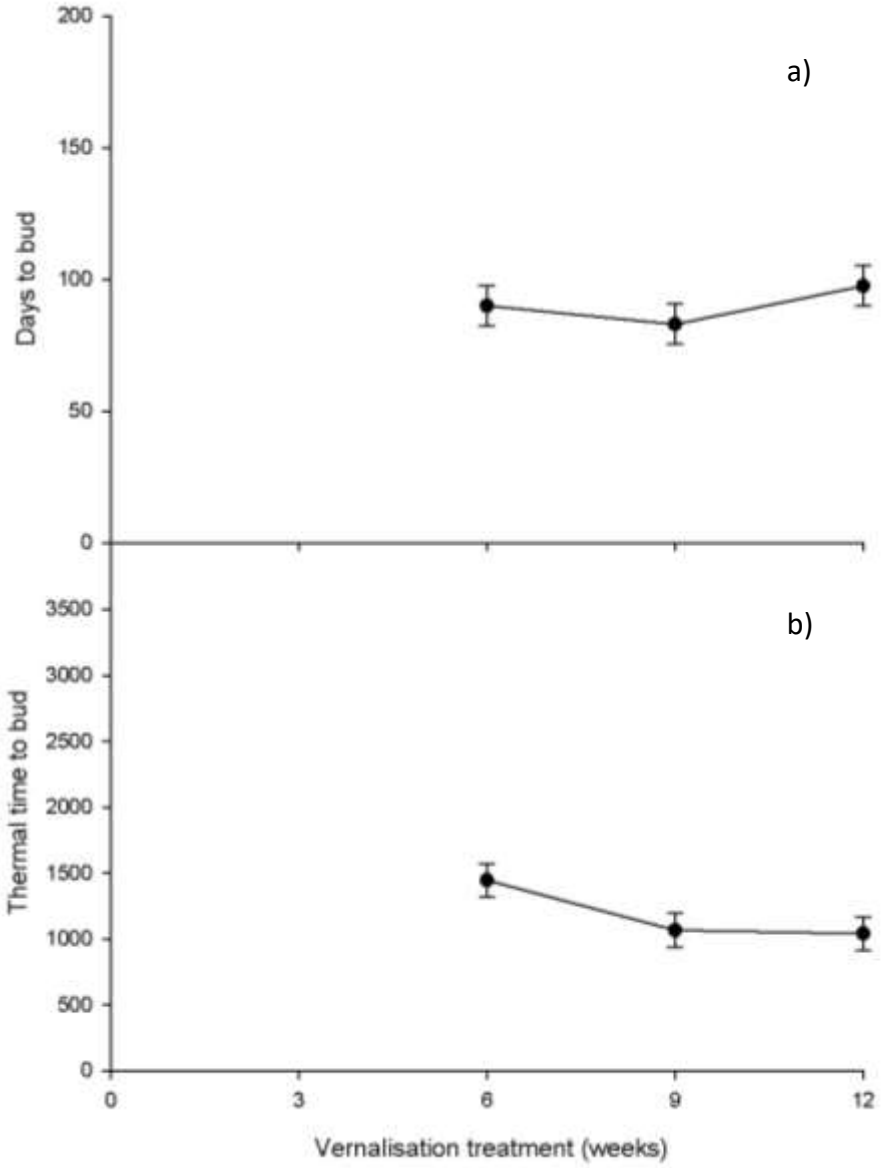

Figure 4.3 Average number of days (a) and thermal time (°Cd) (b) for 'Firefly' kale until bud in relation to the duration of the 4°C (●) vernalisation treatment, from 0 to 12 weeks, at Lincoln University, New Zealand from March to September 2024. The duration of 4°C was not significant for days to bud (p=0.457) or thermal time to bud (p=0.116). Error bars represent the standard error of the mean.

The number of days (p=0.457) and Tt (p=0.116) to bud were consistent among durations of 4°C and an average of 90.2 days (± 7.67 days) or 1190°Cd (± 127°Cd) was observed (Figure 4.3).

4.2.2.4 Radish

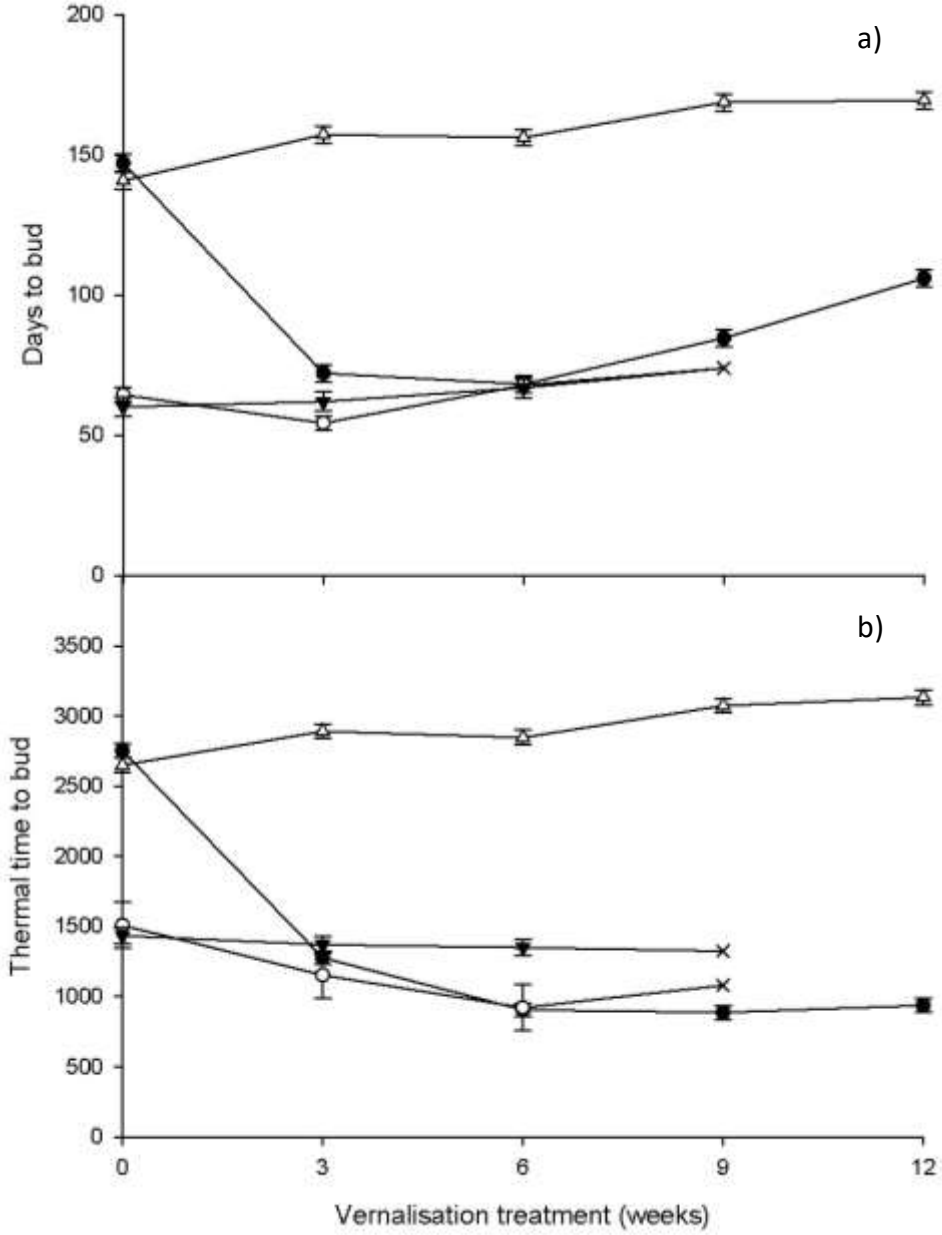

Figure 4.4 Average number of days (a) and thermal time (°Cd) (b) for 'Endurance' radish until bud in relation to the duration of the 4°C (●), 8.1°C (○), 12°C (▼) and 18°C (△) vernalisation treatments, from 0 to 12 weeks, at Lincoln University, New Zealand from March to September 2024. For days to bud, the duration of 4°C (LSD 9.52, $p<0.001$), 8°C (LSD 8.57, $p=0.020$) and 18°C (LSD 9.39, $p<0.001$) was significant, while the duration of 12°C was not ($p=0.410$). For thermal time to bud, the duration of 4°C (LSD 146, $p<0.001$) and 18°C (LSD 162, $p<0.001$) was significant, while the duration of 8°C ($p=0.109$) and 12°C ($p=0.604$) was not. Error bars represent the standard error of the mean. × indicates insufficient data so was excluded from the analysis but included for completeness.

In the 4°C vernalisation treatment, the days to bud decreased ($p<0.001$) from 147 days at the 0 week duration to an average of 70.2 days (± 4.37 days) at the 3 and 6 weeks durations (Figure 4.4). The days to bud then increased to 84.7 days at 9 weeks and to 106 days at 12 weeks. In the 8.1°C treatment, the days to bud decreased ($p=0.020$) from 64.5 days at the 0 week duration to 54.4 days at 3 weeks and it then increased to 67.9 days at 6 weeks. An average of 63.1 days (± 3.48 days) was observed at 12°C, independent of the duration of the treatment. In the 18°C vernalisation treatment, the days to bud increased ($p<0.001$) from 141 days at a 0 week duration to an average 157 days (± 3.05 days) at 3 and 6 weeks. This was followed by a further increase to an average 169 days (± 3.05 days) at 9 and 12 weeks.

In the 4°C vernalisation treatment, the Tt to bud declined ($p<0.001$) from 2750°Cd at the 0 week duration, to 1280°Cd at 3 weeks, to an average of 910°Cd (± 47.4°Cd) at 6, 9, and 12 weeks durations. An average of 1190°Cd (± 164°Cd) and 1390°Cd (± 59.9°Cd) was observed at 8 and 12°C, independent of the duration of the treatment. In the 18°C treatment, the Tt to bud increased ($p<0.001$) from 2650°Cd at the 0 week duration, to 2870°Cd (± 52.7°Cd) at the 3 and 6 weeks durations. This was followed by a further increase to 3100°Cd (± 52.7°Cd) at the 9 and 12 weeks durations.

4.2.2.5 Leafy Turnip

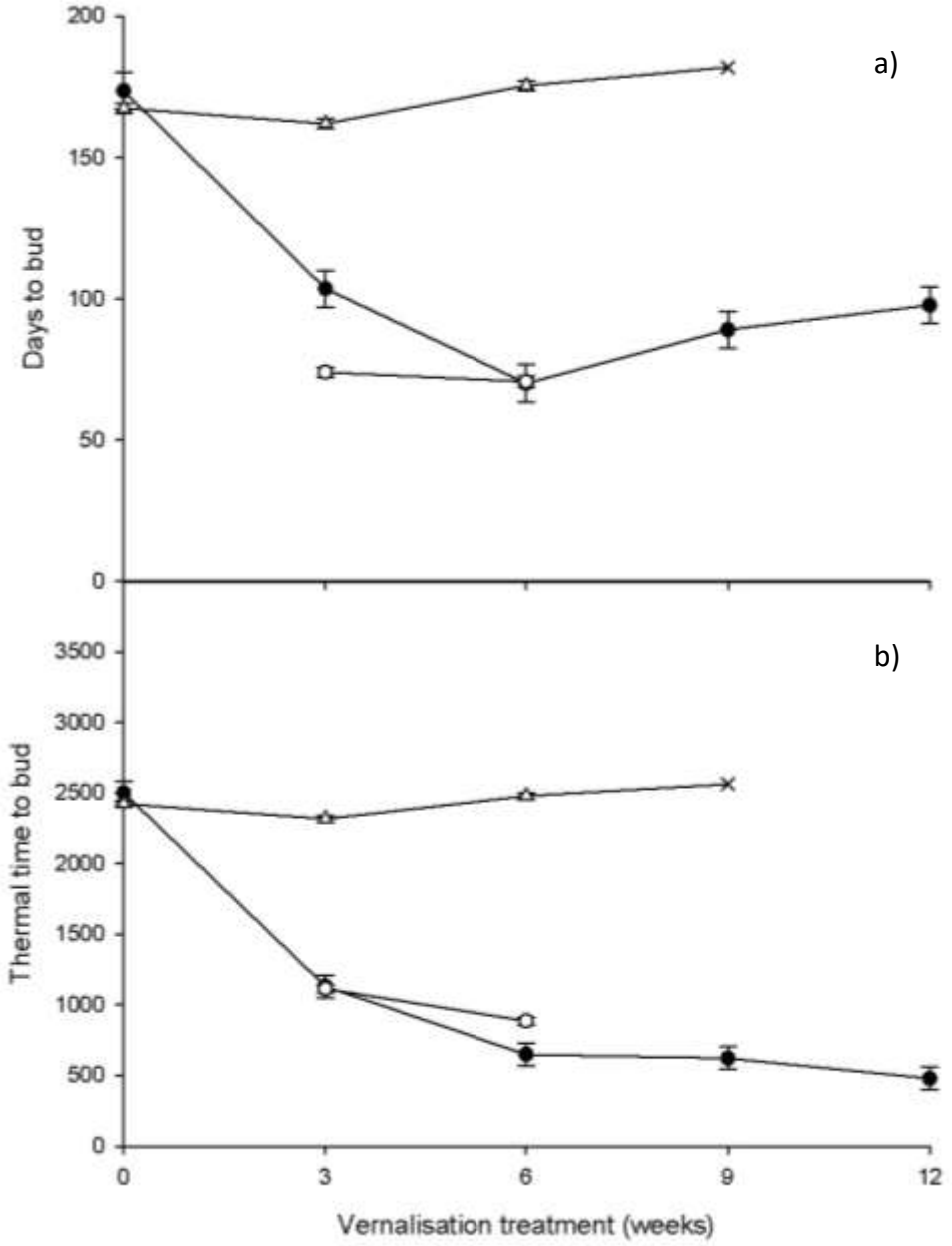

Figure 4.5 Average number of days (a) and thermal time (°Cd) (b) for 'Hunter' leafy turnip until bud in relation to the duration of the 4°C (●), 8.1°C (○) and 18°C (△) vernalisation treatments, from 0 to 12 weeks, at Lincoln University, New Zealand from March to September 2024. For days to bud, the duration of 4°C (LSD 20.2, $p<0.001$) and 18°C (LSD 5.74, $p=0.003$) was significant, while the duration of 8.1°C was not ($p=0.292$). For thermal time to bud, the duration of 4°C (LSD 245, $p<0.001$), 8.1°C (LSD 110, $p=0.007$) and 18°C (LSD 78.0, $p=0.006$) was significant. Error bars represent the standard error of the mean. × indicates insufficient data so was excluded from the analysis but included for completeness.

In the 4°C vernalisation treatment, the days to bud decreased ($p<0.001$) from 174 days at the 0 week duration to 70.1 days at 6 weeks (Figure 4.5). There was no difference in days to bud among the 3, 9 and 12 week durations, nor between the 6 and 9 week durations. The days to bud was consistent ($p=0.292$) among the 3 and 6 weeks durations of 8.1°C, and an average of 72.3 days (± 1.85 days) was observed. In the 18°C treatment, the days to bud decreased ($p=0.003$) from 168 days at the 0 week duration to 162 days at 3 weeks. It then increased to 176 days at 6 weeks.

In the 4°C vernalisation treatment, the Tt to bud decreased ($p<0.001$) from 2500°Cd at the 0 week duration to 1330°Cd at 3 weeks. This was followed by a further decrease to an average of 583°Cd (± 80.0°Cd) at 6, 9, and 12 weeks. In the 8.1°C treatment, the Tt to bud decreased ($p=0.007$) from 1110°Cd at 3 weeks to 886°Cd at 6 weeks. In the 18°C treatment, the Tt to bud averaged 2450°Cd (± 22.5°Cd) at 0 and 6 weeks while at 3 weeks a lower ($p=0.006$) value of 2320°Cd was observed.

4.2.2.6 Raphanobrassica

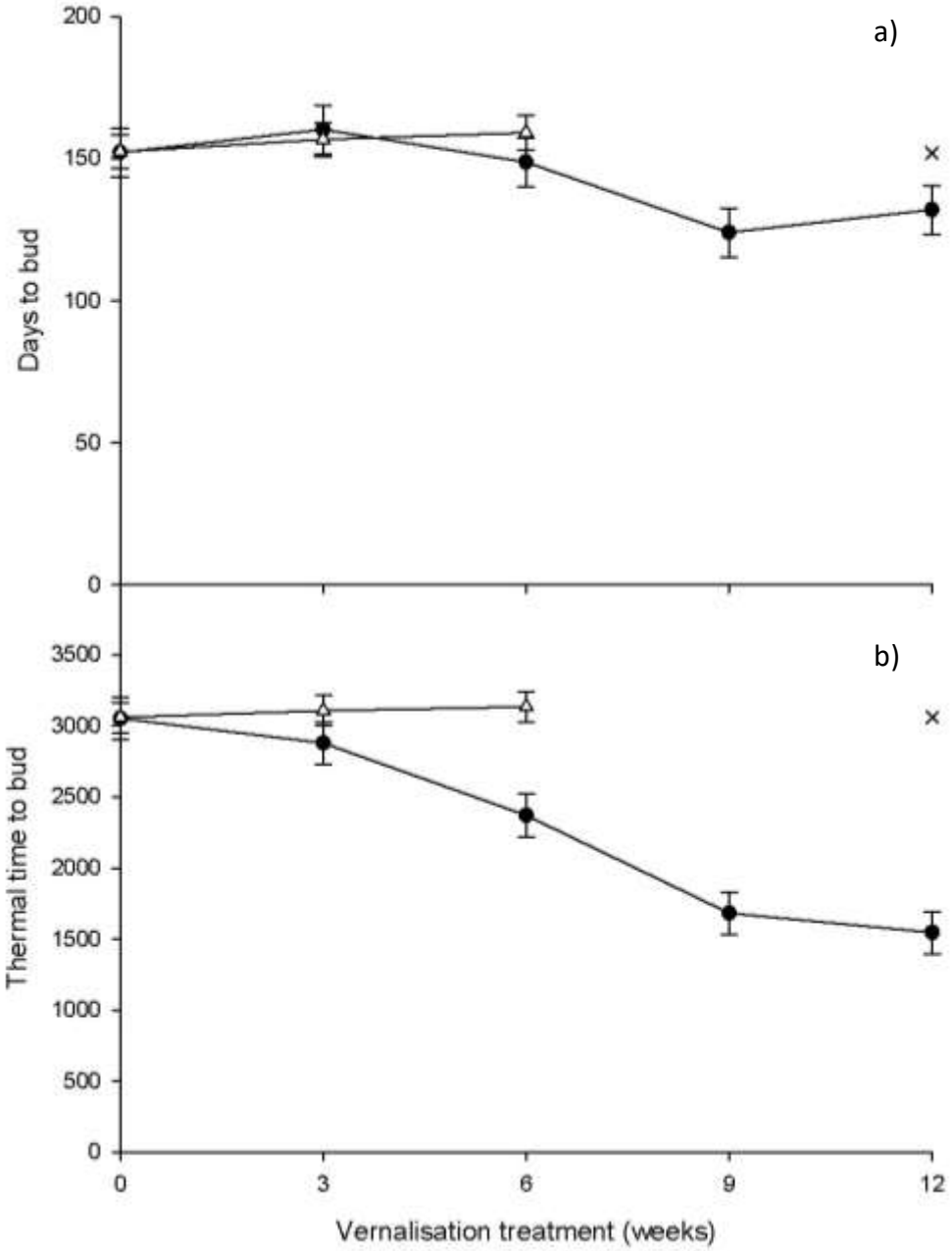

Figure 4.6 Average number of days (a) and thermal time (°Cd) (b) for 'CC Pallaton' raphanobrassica until bud in relation to the duration of the 4°C (●) and 18°C (△) vernalisation treatments, from 0 to 12 weeks, at Lincoln University, New Zealand from March to September 2024. For days to bud, the duration of 4°C ($p=0.085$) and 18°C ($p=0.732$) were not significant. For thermal time to bud, the duration of 4°C was significant (LSD 486, $p<0.001$), but the duration of 18°C was not ($p=0.882$). Error bars represent the standard error of the mean. × indicates insufficient data so was excluded from the analysis but included for completeness.

An average of 144 days (± 8.58 days) was observed for plants to produce a bud at 4°C, independent ($p=0.085$) of duration (Figure 4.6). Plants at the 18°C vernalisation treatment took an average of 156 days (± 5.96 days) to produce a bud, independent ($p=0.732$) of duration.

In the 4°C treatment, the Tt to bud decreased ($p<0.001$) from an average of 2970°Cd (± 149°Cd) at 0 and 3 weeks to 2370°Cd at 6 weeks. Then, a further decrease was observed to an average of 1610°Cd (± 149°Cd) at 9 and 12 weeks. An average ($p=0.882$) of 3100°Cd (± 107°Cd) to bud was observed at the 18°C treatment, independent of duration.

4.2.3 Thermal time between bud and flower

4.2.3.1 Rape

In the 4°C treatment, the Tt between bud and flower decreased (p=0.011) from an average 204°Cd (± 11.8°Cd) at 6 and 9 weeks to 158°Cd at 12 weeks (Table 4.13).

Table 4.13 Thermal time (°Cd) between bud and flower for 'Mainstar' rape (T_b = 3.30°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	-	-	-	-
3	274_x	-	-	-
6	205_a	-	-	-
9	203_a	-	-	-
12	158_b	-	-	-
Mean	188	-	-	-
SEM	11.8	-	-	-
Significance	0.011	-	-	-
LSD	28.8	-	-	-

Note: LSD refers to the comparison among durations at the same temperature treatment; means followed by the same letter are similar at α = 0.05. Empty cells indicate no flowers were present until 26/09. X indicates insufficient data so was not included in the analysis but was included for completeness.

4.2.3.2 Swede

In the 4°C treatment, the Tt between bud and flower decreased (p=0.023) from 301°Cd at 6 weeks to an average of 192°Cd (± 23.3°Cd) at 9 and 12 weeks (Table 4.14).

Table 4.14 Thermal time (°Cd) between bud and flower for 'Hawkestone' swede (T_b = 1.20°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	-	-	-	-
3	222_x	-	-	-
6	301_a	-	-	-
9	204_b	-	-	-
12	180_b	-	-	-
Mean	228	-	-	-
SEM	23.3	-	-	-
Significance	0.023	-	-	-
LSD	80.7	-	-	-

Note: LSD refers to the comparison among durations at the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$. Empty cells indicate no flowers were present until 26/09. X indicates insufficient data so was not included in the analysis but was included for completeness.

4.2.3.3 Kale

An average (p=0.176) Tt between bud and flower of 303°Cd (± 38.8°Cd) was observed for all plants in the 4°C vernalisation treatment, independent of duration (Table 4.15).

Table 4.15 Thermal time (°Cd) between bud and flower for 'Firefly' kale (T_b = -0.10°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	-	-	-	-
3	-	-	-	-
6	260	-	-	-
9	371	-	-	-
12	279	-	-	-
Mean	303	-	-	-
SEM	38.8	-	-	-
Significance	0.176	-	-	-
LSD	ns	-	-	-

Note: Empty cells indicate no flowers were present until 26/09.

4.2.3.4 Radish

In the 4°C treatment, the Tt from bud to flower was higher ($p<0.001$) at the 3 weeks duration (Table 4.16). The durations of 0, 6 and 9 weeks resulted in a lower average of 294°Cd (± 21.8°Cd). The 12 weeks duration had the lowest Tt of 209°Cd. An average ($p=0.669$) of 255°Cd (± 10.1°Cd) was observed for the 8°C treatment, independent of duration. In the 18°C treatment, the Tt from bud to flower decreased ($p=0.003$) from an average of 264°Cd (± 22.9°Cd) for the 0, 3, 6 and 9 week durations to 153°Cd at 12 weeks.

Table 4.16 Thermal time (°Cd) between bud and flower for 'Endurance' radish (T_b = 1.50°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	294_b	229	-	321_a
3	398_a	222	-	271_a
6	295_b	186_x	-	188_a
9	293_b	-	-	275_a
12	209_c	-	-	153_b
Mean	298	255	-	262
SEM	21.8	10.1	-	22.9
Significance	<0.001	0.669	-	0.003
LSD	67.1	ns	-	70.6

Note: LSD refers to the comparison among durations at the same temperature treatment; means followed by the same letter are similar at $\alpha = 0.05$. Empty cells indicate no flowers were present until 26/09. X indicates insufficient data so was not included in the analysis but was included for completeness.

4.2.3.5 Leafy Turnip

No differences were observed among durations at the 4°C treatment (p=0.415) where plants took an average of 163°Cd (± 12.9°Cd) from bud to flower (Table 4.17).

Table 4.17 Thermal time (°Cd) between bud and flower for 'Hunter' leafy turnip (T_b = 5.10°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	160	-	-	148_x
3	157	-	-	220_x
6	169	-	-	148_x
9	183	-	-	-
12	148	-	-	-
Mean	163	-	-	-
SEM	12.9	-	-	-
Significance	0.415	-	-	-
LSD	ns	-	-	-

Note: Empty cells indicate no flowers were present until 26/09. X indicates insufficient data so was not included in the analysis but was included for completeness.

4.2.3.6 Raphanobrassica

The Tt from bud to flower took 275°Cd (± 51.4°Cd) at 4°C, and 331°Cd (± 41.4°Cd) at 18°C, independent of the duration of the treatment (Table 4.18).

Table 4.18 Thermal time (°Cd) between bud and flower for 'CC Pallaton' raphanobrassica (T_b = 0.40°C) across five durations (0, 3, 6, 9 and 12 weeks) at four vernalisation treatments (4, 8.1, 12 and 18°C) at Lincoln University, New Zealand from March to September 2024.

Duration (weeks)	4°C	8.1°C	12°C	18°C
0	319	-	-	298
3	245	-	-	363
6	290	-	-	332
9	293	-	-	-
12	228	-	-	182_x
Mean	275	-	-	331
SEM	51.4	-	-	41.4
Significance	0.722	-	-	0.571
LSD	ns	-	-	ns

Note: Empty cells indicate no flowers were present until 26/09. X indicates insufficient data so was not included in the analysis but was included for completeness.

4.2.4 Thermal time to bud for plants that did not receive vernalisation

All 0 week treatments from the same experiment were used as replicates to determine the Tt to bud for plants that did not receive vernalisation. All raphanobrassica and leafy turnip plants that developed buds without vernalisation were from experiment 1. Leafy turnip required an average of 2460°Cd (± 109°Cd) with a sample size of 8 plants. Raphanobrassica required an average of 3060 (± 142°Cd) with a sample size of 7 plants. For radish, 21 plants from experiment 1, and 12 plants from experiment 2 developed buds. Radish plants from experiment 1 required an average of 2700°Cd (± 117°Cd), while those from experiment 2 required 1470°Cd (± 104°Cd).

5 DISCUSSION

5.1 Phyllochron

The phyllochron of 'Mainstar' rape ranged from 35.2 to 88.4°Cd/leaf (Table 4.1). This is similar but extends the lower range of 42 to 95°Cd/leaf reported by Andreucci (2013), and includes the phyllochron of 61°Cd/leaf reported by Adams et al. (2005), all for 'Goliath' rape. Fletcher et al. (2012) obtained a phyllochron of 60°Cd/leaf for 'Titan' rape and Tian et al. (2017) presented a value of 75°Cd/leaf for oilseed rape in China. This shows that the range presented in Table 4.1 is consistent with the literature on forage and oilseed rape.

The phyllochron of 'Hawkestone' swede went from 55.8 to 137°Cd/leaf (Table 4.2). This extends the upper range of the values of 52 to 106°Cd/leaf reported by Andreucci (2013) for 'Aparima Gold' swede. Fletcher et al. (2012) reported a phyllochron of 51°Cd/leaf for 'Keystone' swede.

The phyllochron of 'Firefly' kale went from 75.2 to 178°Cd/leaf (Table 4.3). This range includes the phyllochron of 76°Cd/leaf reported by Fletcher et al. (2012) for 'Kestrel' kale, and of 109°Cd/leaf presented by Chakwizira (2008) for 'Regal' kale. Adams et al. (2005) reported the phyllochron of 65 and 68°Cd/leaf for 'Gruner' and 'Kestrel' kale, respectively.

The phyllochron of 'Endurance' radish went from 48.3 to 133°Cd/leaf (Table 4.4). This range includes the phyllochron of 69 and 79°Cd/leaf reported by Dam (2006) for fodder radish in the Netherlands.

The phyllochron of 'Hunter' leafy turnip went from 22 to 80.8°Cd/leaf (Table 4.5). This encompasses the values of 60°Cd/leaf reported by Chakwizira (2008) for 'Pasja' leafy turnip, and of 47°Cd/leaf reported by Fletcher et al. (2012) for 'Barkant' bulb turnip. It extends the upper boundary of 20 to 67°Cd/leaf presented by Andreucci (2013) for 'Green Globe' and 'Barkant' bulb turnips.

The phyllochron of 'CC Pallaton' raphanobrassica went from 116 to 149°Cd/leaf. Raphanobrassica showed a narrower range of phyllochron across treatments than other crops. Only the 12°C treatment showed differences (Table 4.6).

Nanda et al. (1996) reported that the rate of leaf appearance differed by up to 35% amongst *B. campestris, B. juncea, B. napus* and *B. carinta*. Additionally, Kasa and Kondra (1986) reported that late maturing genotypes of *B. napus* and *B. campestris* had lower phyllochron values than early maturing genotypes. The phyllochron can vary between and within species which makes studies conducted within New Zealand with crops of close phylogenetic distance particularly relevant for comparison.

Section 4.1 showed that phyllochron was reduced at longer durations at lower vernalisation temperatures (Table 4.1 to Table 4.6). For all crops, the lowest phyllochron values were observed after the period of 12 weeks at 4°C (Table 4.1 to Table 4.6). Andreucci (2013) also reported that lower temperatures resulted in lower phyllochron values for swede, kale and rape. This contrasts with much of the literature that reports phyllochron as constant when expressed in terms of Tt (Jamieson et al. 1995; Wilhelm & McMaster 1995; Bonhomme 2000; Adams et al. 2005). This could be attributed to the effect of temperature on leaf size. Andreucci (2013) reported leaf sizes of turnip, swede and rape increased with mean air temperature. Similarly, Terry (1968) reported leaf area of sugar beet crops increased with temperature from 10 to 24°C. Rodriguez et al. (2015) reported that leaf length and leaf width of *B. oleracea* were at least 50% larger after 14 days at 20°C compared with the same duration at 12°C. Rawson and Hindmarsh (1982) reported a decline in final leaf size with increasing temperature in five cultivars of sunflower. However, the temperature regimes (day/night) were 32/22, 27/17 and 22/12°C which do not reflect the cold treatments tested in this study. While leaf sizes were not measured in this study, it was observed that leaves grown in 18°C were larger than leaves grown in 4°C (Figure 5.1).

(a)  (b) 

Figure 5.1 Leaf sizes of plants 44 days after sowing in the 4°C growth chamber (a) and 18°C growth chamber (b).

As presented in Section 3.3, leaf appearance was recorded only when a leaf was considered expanded, which meant that the top of the leaf was unfolded (red circled area in Figure 5.2). Therefore, the phyllochron was dependent on a growth component, which can make the measurement subjective. In other species, such as grasses, the expansion of the leaf is considered when the ligule is visible (Haun 1973). However, in a brassica it is more difficult to consider a leaf fully expanded. Brassica leaves are complex and large leaves produce sub-leaflets from the petiole (Figure 5.2). Therefore, it could be that leaves that were counted at lower temperatures may have not been fully formed, because the part accounted for as a leaf was the top of the leaf. This could have influenced the interval used to calculate phyllochron, resulting in a faster leaf appearance at lower temperatures.

Figure 5.2 Example of brassica leaf morphology (raphanobrassica)

Where the phyllochron is the Tt requirement for the production of a leaf, the plastochron is the Tt requirement for the initiation of leaf primordia (Strable & Nelissen 2021). However, the true plastochron is a labourious and destructive measurement that depends on dissections of the apical meristem (Davidson et al. 2015). Those were impractical in this experiment. Luo et al. (2018) sampled *B. napus* plants and counted leaf primordia using a microscope and obtained a linear phyllochron across multiple sowing dates. The authors reported no morphological distinction in the leaf primordia of long or short-petiole leaves, or sessile leaves. This method therefore avoids a change in phyllochron between earlier and later leaves attributed to a change in leaf morphology from petiolar to sessile shape, as has been reported by Nanda et al. (1995). For this reason, a destructive method should be used instead. However, this poses other practical limitations as destructive measurements require a large number of plants. This is often not practical in controlled environment experiments because of a lack of space. In maize, a stable phyllochron is found in leaf tip emergence, defined as when the tip exceeds the highest ligule (Zhu et al. 2013). It is suggested that a similar methodology is developed for future brassica studies

where laboratory analysis of leaf primordia is not practical. The data suggests that when this method of tracking leaf appearance is used, phyllochron is not a stable indicator of plant development.

Raphanobrassica was the only crop in this study that did not have a reduction in phyllochron at long durations of low temperatures (Figure 4.6). This could indicate that raphanobrassica had a greater cold tolerance than each of its constituent parent species, which led to a more stable phyllochron. While plant polyploidy has been shown to improve growth vigor as a result of heterosis (Chen 2007), the 'CC Pallaton' raphanobrassica seed used had been through 9 cycles of self-pollination (Dumbleton, pers. comm., 2024). Hence, the potential for expression of hybrid vigour was considered low.

In the 18°C treatment of swede, the phyllochron was 114°Cd/leaf at the 0 week duration and 137°Cd/leaf at the 12 weeks duration (Table 4.2). An explanation for this could be that in warm conditions, large leaves shade leaf primordia that are therefore not activated and do not produce visible leaves. This then decreases the rate of leaf appearance. Davies and Thomas (1983) demonstrated this in grasses through the concept of site filling for tiller production. The authors reported that plants with larger leaves did not have all tiller buds develop into visible tillers due to shading at the base of the plant that inhibited the activity of the buds. This could be another example of the limitation of counting visible expanded leaves versus initiation of leaf primordia, as described above.

5.2 Vernalisation

All data presented were based on observations until the 28th of September of 2024. The duration of experiment 2 was 113 days (Figure 3.2, Table 3.3). In experiment 1, buds continued to emerge beyond 208 days from sowing. It is likely that some plants in the 4°C and 18°C treatments, as well as a number of plants in the 8.1°C and 12°C treatments will develop buds. However, they had not done so by the final data collection date. Wei et al. (2022) and Su et al. (2018) reported that bud development in *Brassica* is a quantitative trait regulated by multiple loci which leads to variability in the vernalisation requirement within a population of plants. Assuming a normal distribution, the data presented only includes the fastest portion of the bell curve. However, Stewart (2002) reported that one large off-

type plant occupying 1 m^2 within a field could cause a 1 in 10,000 contamination rate. Off-types within a crop can provide greater risk of contamination than nearby crops. If the practical implication of flowering is accounted for, only one contaminant plant is required to release pollen and result in hybridisation. For this reason, providing analysis of the earliest developing plants is still meaningful and it was the approach adopted here.

In this study, 'Mainstar' rape and 'Hawkestone' swede did not differ in their responses to vernalisation (Figure 4.1 and Figure 4.2, Table 4.7 and Table 4.8). No buds developed from the 0 week treatment after 208 days (Table 4.7 and Table 4.8). These crops may have an obligate vernalisation requirement. The *B. napus* species is highly polymorphic and the literature predominantly addresses oilseed rape, which is a variety of *B. napus* cultivated for the extraction of oil from its seeds (Gupta & Pratap 2007). In *B. napus*, adaptation to different agroecological environments has led to winter types with a vernalisation requirement and spring types that will flower without exposure to cold (Wang et al. 2011a). Rape and swede did not produce buds without exposure to cold which is consistent with many authors reports of an obligate vernalisation requirement in winter type cultivars of *B. napus* (Tommey & Evans 1991; Habekotte 1997; Chalhoub et al. 2014; Mendham & Robertson 2016; Robertson & Lilley 2016).

In the 4°C treatment, 20.8 and 8.33% of rape and swede plants, respectively, developed buds at 3 weeks, and all plants developed buds at 6 weeks or more (Table 4.7 and Table 4.8). This suggests that the vernalisation requirement for 100% of plants with buds is more than 3 weeks but less than or equal to 6 weeks at 4°C. Robertson and Lilley (2016) reported that the range of vernal inductive temperatures for winter type oilseed rape is 0 to 15°C. This suggests that a longer duration of 8.1°C or 12°C may allow rape and swede plants to accumulate VDD to produce buds, although they had not done so by the final data collection date. The authors also reported that the vernalisation requirement is saturated with 25 days at 2°C. Wiebe (1990) reported that the vernalisation requirement of swede is saturated with by 2 to 4 weeks at 7°C. Tommey and Evans (1991) reported that the optimum temperature for vernalisation of winter type oilseed rape is between 6 and 9°C. The development of 100% of rape and swede plants with buds after 6 weeks or more at

4°C (Table 4.7 and Table 4.8) is consistent with this literature (Wiebe 1990; Tommey & Evans 1991; Robertson & Lilley 2016).

When vernalised with 6 weeks or more at 4°C, rape and swede plants produced buds in 95.3 days (± 3.40 days) and 102 days (± 5.01 days), respectively (Figure 4.1a and Figure 4.2a). However, the Tt to bud decreased from the 6 weeks duration to the 9 and 12 weeks durations in rape and swede by 412°Cd and 460°Cd, respectively (Figure 4.1b and Figure 4.2b). The data from the 4°C temperature treatment at the 3 weeks duration was not included in the analysis as only two rape plants and one swede plant had buds at the end of the period of assessments. These plants took 164 days, equivalent to 2520°Cd, and 182 days, equivalent to 2840°Cd, respectively, to produce a bud (Figure 4.1 and Figure 4.2). Whish et al. (2020) reported that four winter type *B. napus* cultivars had an obligate vernal requirement of 12 vernal days where one VDD was accumulated when the daily mean air temperature was 2°C, and less VDD was accumulated between 0 and 15°C. After the obligate accumulated cold had been achieved, a facultative vernal response was observed between 12 VDD and 25 VDD. This was characterised by a linear ($p<0.0001$, $r^2=0.89$) decrease in Tt to flowering of 29°Cd per VDD. Nanda et al. (1996) also reported that the Tt from plant emergence to bud in *B. napus* reduced at 21.9^{o}Cd/^{o}C as mean temperature decreased from 24°C to 12°C. Figure 4.1b and Figure 4.2b for rape and swede, respectively, demonstrate a response consistent with the literature for winter type *B. napus* (Nanda et al. 1996; Whish et al. 2020).

In 'Firefly' kale, no plants developed buds from the 0 week treatment (Table 4.9). The same was observed at the temperatures of 8.1, 12, and 18°C and for the duration of 3 weeks at 4°C (Table 4.9). This indicates that 'Firefly' kale may have an obligate vernalisation requirement. Wiebe (1990) reported that kale had an obligate vernalisation requirement that is saturated with 3 to 5 weeks at 6°C and inductive vernalisation temperatures ranged from 0 to 13°C. Mero and Honma (1984) reported that 100% of 'Siberian' kale plants developed buds after 6 weeks or more of 5°C exposure, but none developed buds after 5 weeks of the same temperature treatment. This is consistent with the response presented in Table 4.9 for kale.

In the 4°C treatment, 56.9% (± 34.2%) of kale plants developed buds at 6, 9 and 12 weeks durations (Table 4.9). The last kale plant to develop a bud did so on the 30th of July of 2024, which was 60 days before the final data collection date. Bradshaw (2021) tested the number of forage kale plants that developed buds after being vernalised for 15 weeks at 10°C/4°C (day/night) treatments, with 0, 5, 10 and 15 true leaves at the time they were transferred to the vernalisation treatments. The treatment that started with an average of 4.5 true leaves had 50% of plants with buds, while the treatment that started with 10.2 leaves had 100% of plants with buds. Wiebe (1990) reported that *B. oleracea* spp. *sabellica* required a minimum of 4 true leaves greater than 2 cm before the plant was receptive to vernalisation. However, the author also reported that for *B. oleracea* spp. *botrytis* and *B. oleracea* spp. *capitata,* the minimum true leaf number for vernalisation receptiveness could be 12 or 15 for each subspecies, respectively. As presented in Section 3.1, plants were placed into the vernalisation treatments with an average of 4 true leaves, but some individual plants did not reach this number. Incomplete bud development in the 4°C treatment at the 6, 9 and 12 weeks durations (Table 4.9) could be attributed to kale plants that may not have exited the juvenile phase at the time when the vernalisation treatment started.

Kale plants that developed buds took 90.2 days (± 7.67 days), or 1190°Cd (± 127°Cd) (Figure 4.3). Murphy and Scarth (1994) reported a vernal response pattern in some cultivars of *B. napus* where vernalisation is required for bud development however there was no response from additional vernalisation beyond the minimum requirement. This is consistent with the data presented on Figure 4.3 for kale.

In 'Endurance' radish, all plants from the 0 week duration at the 4 and 18°C vernalisation treatments developed buds (Table 4.10). The last plant to develop a bud from these treatments took 171 days from sowing. Plants in the 8.1 and 12°C treatments had 113 days from sowing until the final data collection date which may explain the lower 66.7 and 45.8% of plants with buds in the 0 week duration of these temperature treatments, respectively (Table 4.10). At the 4 and 18°C temperature treatments, the 0 week duration plants developed buds after 147 and 141 days respectively (Figure 4.4), during which they were under a 20 hour photoperiod. Kaymak and Güvenc (2010) reported that five cultivars of

radish flowered under long days of 16 hours without vernalisation, but did not flower under short days of 8 hours without vernalisation. Similarly, Han et al. (2021) reported that accessions of radish from northern Japan that did not go through vernalisation flowered under long days (natural photoperiod between April to July), but did not flower under short days (natural photoperiod between October to January). Therefore, 'Endurance' radish may have a facultative vernalisation response, as long as the photoperiod requirement is fulfilled.

A facultative vernalisation response for radish was demonstrated in the reduction of days to bud and Tt to bud with long durations of cold temperature (Figure 4.4). The days and Tt to bud for the 4, 8.1 and 12°C temperature treatments at the 3 week duration was 72.2, 54.4 and 62.1 days, or 1280, 1153 and 1370°Cd respectively. At the same duration, the 18°C treatment had a higher requirement at 157 days, or 2870°Cd (Figure 4.4). Kaymak and Güvenc (2010) reported that cold exposure was not required for flowering in five cultivars of radish in Turkey, but flowering occurred faster after cold treatment. Wiebe (1990) reported that the facultative vernalisation response is saturated by 1 to 4 weeks at 5 to 8°C and a vernal inductive range between 0 and 15°C. The 18°C treatment did not cause any reduction in Tt to bud for radish, which is consistent with this inductive range. The results in Figure 4.4 confirm the optimal range proposed by Wiebe (1990) because only the 4°C treatment showed a further reduction in Tt to bud after the 3 weeks duration.

The Tt between sowing and bud development for the 0 week duration was 2700°Cd (± 117°Cd) for experiment 1 radish plants, and 1470°Cd (± 104°Cd) for experiment 2 radish plants (Figure 4.4, Section 4.2.4). Experiment 1 plants reached the end of the juvenile period on the 26th of March where the monthly average air temperature in the glasshouse was 19.6°C (± 4.13°C), compared with experiment 2 radish plants which reached it on the 8th of July where the air temperature was 16.5°C (± 2.75°C) (Table 3.2). Wiebe (1990) reported that the inductive temperatures for vernalisation of radish are between 0 and 15°C. The author also reported that devernalisation occurred at temperatures above 20°C. Experiment 2 plants may have accumulated VDD in the glasshouse, and temperatures did not exceed the threshold required to reset the requirement through devernalisation.

In ‘Hunter’ leafy turnip, no plants from the 12°C treatment, nor from the 0, 9 or 12 weeks durations at 8.1°C developed buds (Table 4.5). At 8.1°C, 16.7% of plants at the 3 weeks duration and 70.8% of plants at the 6 weeks duration developed buds between the 13th and 28th of September. All plants in the 18°C treatment developed buds between the 2nd and 28th of September, and all plants from the 0 week duration at 4°C developed buds between the 7th and 28th of September (Table 4.5). The number of plants with buds in leafy turnip was limited by the length of the experiment. *B. rapa* exhibits broad morphological diversity in the form of leafy vegetables, turnips and oil crops (Zhang et al. 2014). Su et al. (2018) reported that *B. rapa* has two main flowering types, one requires no vernalisation and the other requires vernalisation for flowering. Plants from the 0 week treatment developed buds so leafy turnip may have a facultative vernalisation response under long days, which is consistent with the first flowering type described.

The facultative vernalisation response in leafy turnip was demonstrated in the reduction of days to bud and Tt to bud with long durations of cold temperature (Figure 4.5). The days to bud and Tt to bud at the 0 week duration for the 4 and 18°C temperature treatments was 174 and 168 days, or 2500 and 2450°Cd to bud. In the 4 and 8.1°C treatments, the Tt to bud at the 3 weeks duration was 1330 and 1110°C, respectively. In the 4°C treatment, the Tt to bud further decreased to 583°Cd (± 80.0°Cd) at 6, 9, and 12 week durations, while in the 8.1°C treatment the Tt to bud decreased to 886°Cd at the 6 weeks duration (Figure 4.5). Wiebe (1990) reported that the vernalisation requirement of turnip is saturated with 2 to 4 weeks at 7°C. As the Tt to bud in ‘Hunter’ leafy turnip continued to decrease until the 6 weeks duration in the 4 and 8.1°C treatments (Figure 4.5), it may have a higher cold requirement for vernal saturation that that proposed by Wiebe (1990). Takada et al. (2019) tested the vernalisation response of nine lines of *B. rapa* after 4 weeks at 4°C and reported significant variation among genotypes, including two lines where all plants flowered and two lines where no plants flowered. The difference in vernalisation required to maximise the vernal response by ‘Hunter’ leafy turnip and the turnip reported by Wiebe (1990) may be due to different genotypes. Wiebe (1990) also reported a range of inductive vernal temperatures between 0 and 18°C. The lack of reduction of Tt to bud from longer durations at the 18°C treatment in leafy turnip (Figure 4.5) is consistent with the maximum inductive temperature for vernalisation reported.

In the 4°C treatment of 'CC Pallaton' raphanobrassica, 50% (± 17.5%) of plants developed buds, independent of the duration (Table 4.12). At the 18°C temperature treatment, 66.7% of plants developed buds after 0 and 6 weeks, while 33.3% developed buds after 3 weeks, 8.3% after 12 weeks, and none after 9 weeks. Raphanobrassica may not have an obligate vernalisation requirement, as 33.3 to 66.7% of plants developed buds without a vernalisation treatment, and cold exposure did not increase the percentage of plants with buds (Table 4.12).

In the 18°C treatment of raphanobrassica, the Tt to bud was 3100°Cd (± 107°Cd), independent of duration (Figure 4.6). In the 4°C treatment, the Tt decreased from 2970°Cd (± 149°Cd) at 0 and 3 weeks to 2370°Cd at 6 weeks, and then to 1610°Cd (± 149°Cd) at 9 and 12 weeks (Figure 4.6). Wiebe (1990) reported that the upper temperature margin for most brassica species is between 10 and 14°C which is consistent with the absence of a reduction of Tt to bud from the 18°C treatment. Raphanobrassica may have a facultative vernalisation response, shown by the reduction in Tt to bud by 6 weeks or more at 4°C (Figure 4.6).

The duration of the vernalisation treatment had a significant effect on the Tt to bud but not the days to bud in the 4°C temperature treatment for rape (Figure 4.1), swede (Figure 4.2) and raphanobrassica (Figure 4.6), as well as the 8°C treatment for leafy turnip (Figure 4.5). The days to bud increased as the duration of the vernalisation treatment increased in the 18°C treatment for radish (Figure 4.4) and leafy turnip (Figure 4.5). Luo et al. (2018) conducted a controlled environment study in oilseed rape to assess the influence of temperature on floral initiation. They reported that the transition from vegetative to reproductive growth in plants with a vernalisation response is delayed at high temperatures by the slow accumulation of cold requirement, but also delayed at low temperatures by the slow accumulation of leaf production and dry matter accumulation. The lack of or reduced effect of vernalisation treatments on days to bud (Figure 4.1a to Figure 4.6a) compared with Tt to bud (Figure 4.1b to Figure 4.6b) is consistent with this observation.

In the 4°C temperature treatment for rape and swede, the 3 weeks duration caused 20.8 and 8.33% to develop buds, respectively (Table 4.7 and Table 4.8). Genetic variation in paralogs of *FLC* has been found to be associated to the vernalisation requirement for *B. rapa*, *B. oleracea* and *B. napus* (Schiessl et al. 2019). The reference genome of *B. napus* has nine annotated copies of *Bna.FLC* (Chalhoub et al. 2014). Akter et al. (2023) reported that the total expression of *FLC* paralogs in pre-vernalised plants and the degree of suppression of expression in each *FLC* paralog after vernalisation are two of the most important factors that cause intraspecific variation in the vernalisation requirement. The authors also reported that loss of function of one *FLC* paralog results in a decrease in the total expression and therefore a reduction in the vernalisation requirement, and if one *FLC* paralog is not reduced in expression after vernalisation, the vernalisation requirement will be higher. Genetic variation may have caused different vernalisation requirements in individual rape and swede plants, where some bud development occurred at the vernalisation treatment of 3 weeks at 4°C (Table 4.7 and Table 4.8).

The lowest Tt to bud values for each species was observed across multiple durations of the 4°C temperature treatment. For rape, it was 698°Cd (± 44.9°Cd) at 9 and 12 weeks (Figure 4.1). For swede, it was 1060°Cd (± 78.6°Cd) at 9 and 12 weeks (Figure 4.2). For kale, it was 1190°Cd (± 127°Cd) at 6, 9 and 12 weeks (Figure 4.3). For radish, it was 910°Cd (± 47.4°Cd) at 6, 9, and 12 weeks (Figure 4.4). For leafy turnip, it was 583°Cd (± 80.0°Cd) at 6, 9, and 12 weeks (Figure 4.5). For raphanobrassica, it was 1610°Cd (± 149°Cd) at 9 and 12 weeks (Figure 4.6). Nanda et al. (1996) reported that *B. campestris, B. juncea, B. napus* and *B. carinata* each had a minimum Tt between emergence and bud. The presence of a maximum vernalisation response, or a minimum Tt to bud for species in this study (Figure 4.1 to Figure 4.6) is consistent with these findings.

5.3 Thermal time between bud and flower

The Tt between bud and flower was consistent across durations of the 4°C temperature treatment in kale (Table 4.15), leafy turnip (Table 4.17), and raphanobrassica (Table 4.18) at 303, 163 and 275°Cd, respectively. This interval was also consistent for raphanobrassica at the 18°C treatment which took 331°Cd (Table 4.18), and the 8.1°C treatment for radish (Table 4.16) which took 255°Cd. Robertson et al. (2002) reported that this period was

250°Cd in oilseed rape. Murphy and Scarth (1994) reported that days between bud and flower in oilseed rape plants exposed to 42 days of 4°C ranged from 23.7 to 28.1 days while the non vernalised control varied from 32.8 to 42.7 days. Hodgson (1978) and Habekotte´ (1997) reported that vernalisation affected the duration of *Brassica* plant development prior to the bud stages but had no effect on subsequent stages. This is consistent with observations in other plants such as winter wheat (Steinfort et al. 2017) and winter field pea (Trevino & Murray 1975). Nanda et al. (1996) also reported that the length of developmental stages after bud development is determined solely by temperature, but the length varied across different species of *Brassica*. These authors support the constant Tt between bud and flowering observed in kale (Table 4.15), leafy turnip (Table 4.17) and raphanobrassica (Table 4.18).

The duration of the vernalisation period had a significant effect on the Tt between bud and flower in the 4°C treatment of rape (Table 4.13), swede (Table 4.14) and radish (Table 4.16), as well as the 18°C treatment of radish (Table 4.16), which contrasts with the literature. As described in Section 3.1.1.1, temperature measurements in the glasshouse were recorded at a height of 2 m from the ground. As *Brassica* plants transition to reproductive development, the stem elongates which meant that during the period from bud to flower the plants height was taller than the sensor placement. The temperature experienced by the plant could have been different to the temperature measured. Hence, this could have contributed to the observed variation in Tt. Furthermore, as described in Section 3.3, plants were assessed for buds and flowers twice a week. There could have been a three days delay between the actual date that a bud or flower appeared and the date that was recorded. Accounting for the bud date and flower date, this could result in a total inaccuracy of up to six days in the Tt between bud and flower calculation.

5.4 Application for isolation areas and seed production

In this experiment, rape, swede, and kale had obligate vernalisation requirements (Table 4.7 to Table 4.9) which restricts their sowing times to early Autumn to ensure adequate crop cover before winter frosts, but also ensure adequate VDD is accumulated through winter to promote flowering and subsequent seed production. Radish, leafy turnip and raphanobrassica did not require vernalisation for plants to develop buds (Table 4.10 to

Table 4.12). Ream et al. (2014) reported that *Brachypodium* species flowered after 6 weeks of vernalisation at 5°C followed by a 20 hour photoperiod, but all plants remained vegetative when followed by an 8 hour photoperiod. In this experiment, all plants were placed under a 20 hour photoperiod after their vernalisation treatment. This may have caused some plants to bud, even though this would not have happened under a lower photoperiod, like 16 hours. Further studies should assess the interaction between vernalisation and photoperiod in these crops.

The minimum Tt to bud observed in this experiment was similar for swede (Figure 4.2), kale (Figure 4.3) and radish (Figure 4.4) at 1060°Cd (± 78.6°Cd), 1190°Cd (± 127°Cd) and 910°Cd (± 47.4°Cd) respectively. Rape (Figure 4.1) and leafy turnip (Figure 4.5) required 698°Cd (± 44.9°Cd) and 583°Cd (± 80.0°Cd) respectively. Raphanobrassica had a higher minimum Tt requirement (Figure 4.6) than all other crops at 1610°Cd (± 149°Cd). Robertson and Lilley (2016) reported that the duration between flowering and maturity is a function of Tt only. Mendham and Robertson (2016) reported this period was 600 to 700°Cd in Australian cultivars of *B. napus*. If all crops were sown at the same time and their photoperiod and vernalisation requirement was saturated, defined as when the Tt to bud is no longer reduced by additional photoperiod or vernalisation, raphanobrassica would not have a period of overlapping flowering time with rape or leafy turnip. Under these conditions, raphanobrassica could be sown without isolation from rape or leafy turnip.

In all crops, longer durations of the 18°C temperature treatment did not cause a reduction in Tt to bud (Figure 4.1 to Figure 4.6). Wiebe (1990) reported that the maximum temperature for vernalisation was between 10 and 14°C for most vegetable species, though there were exceptions such as winter cultivars of cauliflower (*B. oleracea* spp. *botrytis*) at 16°C and turnip (*B. rapa* spp. *rapa*) at 18°C. The lack of reduction in Tt to bud at durations of 18°C for all crops in this experiment indicates that 18°C is at or beyond the maximum temperature for VDD accumulation.

Further research should examine intermediate temperatures to complement this completed dataset which will enable the determination of cardinal vernalisation temperatures, and the vernalisation requirement in VDD for each of these crops. With this

information, the *B. napus* (rapeseed) APSIM model (Robertson & Lilley 2016) could be parameterised to predict development of these crops, determine risk of overlapping flowering times, and optimise the land available for seed multiplication.

5.5 Conclusions

- The phyllochron was determined for six forage brassica crops. Each crop had a range of phyllochron values, with shorter phyllochron values observed under lower vernalisation temperatures and longer durations of cold exposure. Phyllochron values ranged from 35.2 to 88.4°Cd/leaf for 'Mainstar' rape, 55.8 to 137°Cd/leaf for 'Hawkestone' swede, 75.2 to 178°Cd/leaf for 'Firefly' kale, 48.3 to 133°Cd/leaf for 'Endurance' radish, 22.0 to 80.8°Cd/leaf for 'Hunter' leafy turnip, and 116 to 149°Cd/leaf for 'CC Pallaton' raphanobrassica. It is difficult to determine when a leaf is fully expanded in brassicas, which may have led to inaccurate phyllochron estimates when the temperature affected leaf size.
- Rape and swede had an obligate vernalisation requirement, followed by a facultative period where additional vernalisation reduced the Tt to bud. Genetic variation may explain a smaller vernalisation requirement in some plants.
- Kale had an obligate vernalisation requirement. No vernalisation treatment caused all plants to develop buds which may be due to some plants not having exited the juvenile phase.
- Radish, leafy turnip and raphanobrassica had a facultative vernalisation response as plants developed buds without a vernalisation treatment, and Tt to bud decreased with vernalisation. However, these crops may have an obligate vernalisation requirement under short days.
- The 18°C temperature treatment did not reduce the days or Tt to bud in any crop, which suggests this temperature is beyond the range of inductive temperatures for vernalisation.
- Vernalisation reduced the Tt to bud, but it also slowed the accumulation of Tt. This resulted in a lack of, or smaller reduction, in the number of days to bud.
- The Tt between bud and flower was consistent among durations within the same vernalisation treatment in kale, leafy turnip and raphanobrassica. Variation was

observed in rape, swede and radish and may be attributable to the location of temperature measurements and the frequency of data collection.

- The minimum Tt to bud for swede, kale and radish was 1060, 1190 and 910°Cd respectively. Rape and leafy turnip required 698 and 583°Cd respectively, and raphanobrassica required 1610°Cd. When the vernalisation and photoperiod requirements are saturated, there is potential for raphanobrassica to be sown without isolation from rape or leafy turnip due to an asynchrony of the flowering period.

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