Characterisation of seed germination in sea rockets (*Cakile* spp.)

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Abstract

The sea rocket genus (*Cakile*) includes several species; two of these are *C. edentula* and *C. maritima*. Both are invasive and have become widely distributed throughout the world. *C. edentula* was introduced earlier than *C. maritima* in Australia, and quickly expanded. However, after *C. maritima* was introduced to Australia, *C. edentula* disappeared from many regions. This replacement of *C. edentula* by *C. maritima* has occurred in other areas of the world. An exception also exists where the two species show coexistence in climates where the winters are cold and summers are wet.

Several hypotheses have been raised to explain this replacement. In this thesis, a difference in the germination timing of the two species was hypothesised to be of importance in different climates. In order to work towards understanding and predicting germination of *Cakile* species, the germination of *C. edentula* from two different climates was examined in this thesis.

A hydrothermal time model was used to describe seed germination. The results showed that populations from different climates differ in seed germination behaviour. The *C. edentula* population from temperate area showed lower base temperature and smaller hydrothermal time accumulation requirement than that from sub-tropical area. Overall, the sub-tropical population germinated more readily than the temperate population across almost all experimental conditions.

Dormancy was found in *C. edentula* seeds during the germination experiment. Further study therefore investigated methods in relieving dormancy of the two *Cakile* seeds to see whether they responded differently. Two treatments (i.e. damage on seed coat and cold stratification) were applied. The results indicate that *C. edentula* generally had a higher percentage germination than its counterpart in each treatment (including the control group) while *C. maritima* germinated faster than *C. edentula* under most damage levels.

The data generated by this thesis could be used as the basis of comparing germination timing between the two *Cakile* species. For predicting the germination timing more effectively, populations of both species from more climates are required, and dormancy needs to be studied under field conditions.

Keywords: *Cakile edentula*, *Cakile maritima*, hydrothermal time model, germination fraction, germination rate, dormancy.
Declaration

This is to declare that:

(i) The thesis comprises only my original work towards the MPhil.
(ii) Acknowledgement has been made in the text to all other material used.
(iii) The thesis is fewer than the maximum word limit in length, exclusive of tables, maps, bibliographies and appendices.
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Chapter 1 General introduction

The genus *Cakile* (Brassicaceae) consists of several species, including *C. edentula* and *C. maritima*. Both of these species are coastal species with dimorphic fruits, which enables the seeds to disperse to both short and long distances (Rodman, 1974). The deciduous upper fruit segments may be blown down the beach and then be transported by sea currents to other beaches. This well-developed dispersal potential, together with the transport of ships, make the two *Cakile* species widely distributed throughout the world (Maun et al., 1990, Barbour, 1972).

*C. edentula* was first introduced to Australia in Victoria in the 19th century. It expanded very fast and invaded the neighbouring states of New South Wales, South Australia as well as Queensland, and even spread to islands such as Tasmania (Rodman, 1986). However, *C. edentula* disappeared from many of these regions as another *Cakile* species was introduced to Australia. *C. maritima* arrived in Australia in the late 19th century near Perth. The first collections of *C. maritima* from the east coast were from South Australia in 1918, and spread to New South Wales and Tasmania. *C. maritima* has now colonized almost the same regions as *C. edentula* and replaced *C. edentula* in much of southern and western Australia (Rodman, 1986) as well as the North Island of New Zealand (Cousens and Cousens, 2011). Despite the replacement in many places, these two species coexist in northern California, North America and from there into British Columbia where the winters are cold and summers are wet (R. D. Cousens personal communication).

Several hypotheses have been raised to explain this replacement, including differences in life history, genetic variation, hybridisation, susceptibility to disease, response to climate, herbivory and the combination of these possibilities (Ohadi, 2015). One explanation related to life history involves their difference in emergence. *C. maritima* in Tasmania and Victoria can germinate in both autumn and spring, which is two seasons compared with only one germination season (i.e. spring) of *C. edentula*. This gives an advantage to *C. maritima* to dominate the coastline before *C. edentula* emerges. However, this happens only when the winter is warm so that the *C. maritima* seeds emerged in autumn can survive overwinter and flower before the beach gets hot and dry, like in Mexico. When there is a cold winter, like in Alaska, then the seedlings of *C. maritima* will not be able to overwinter and only the spring germinated seedlings will survive (Fig. 1). It can be concluded that, the germination patterns of seeds varied with the particular climates which seeds are exposed to.
Therefore, the aim of this thesis is to characterise germination of these two species in relation to climate. In order to characterise germination timing, a model has been developed. In this project, we use radish at first to develop the methodology and to test the viability of the model, and then use one of the two Cakile species to look at the parameters in the model. The fruits of C. maritima vary highly in form and size geographically, and perhaps also physiologically, related to differences in region of origin. In order to avoid the germination variation caused by the confounding of genetic background and morphological traits, only C. edentula was used in this experiment. Finally, the influence of dormancy on germination will be investigated to see whether the two species differ in dormancy as well as in germination.

Chapter 2 reviews our knowledge on the germination and dormancy mechanisms for seedling emergence and how these mechanisms can be incorporated to characterise germination.

Chapter 3 illustrates how the particular model, based on hydrothermal time, works to describe seed germination in the laboratory. Radish (Raphanus sativus) is utilised to demonstrate the model. This step formed a base understanding of the model as well as the parameters in the model.

Chapter 4 investigates how much the parameters in the model vary when the seeds were collected from two populations occurring in different environmental conditions, and whether the same model is capable of characterising germination.

Chapter 5 looks for any dormancy differences between the two species that can lead to variation in germination. If this is the case, then dormancy needs to be modelled as well as germination. This step involves both C. maritima and C. edentula collected from a similar climatic environment.

Finally, Chapter 6 discusses the differences in germination and dormancy between the two species, and what can be done in the future to ultimately predict the environmental conditions under which C. maritima would have an advantage.
Chapter 2  Literature review

Germination is an irreversible process: once it happens, the seeds will grow or die. Various environmental factors are related to the triggering of germination, e.g. temperature, light, chemical environment and water. These factors can also prevent germination from happening when the environment is unfavourable for seedling establishment. Therefore, many studies have worked on incorporating these environmental factors into predicting germination.

When the environment is favourable for germination, but the probability of seedling survival is low, seeds also will not germinate. This function is known as dormancy, which is to ensure the higher survival rate. Dormancy determines the percentage in a seed population that can germinate (Fenner and Thompson, 2005).

2.1 Temperature effect on germination

Under a seasonal climate, temperature can be a good indicator of the time of a year and therefore is related to germination timing. As an example, Washitani and Masuda (1990) conducted an experiment of seed germination at different temperature intervals, which comes with a relationship between the temperature at which germination initiates and the timing of seedling emergence in the field. Responses to variation in temperature on germination can occur in two different ways – alternating temperatures and constant temperatures.

2.1.1 Alternating temperature

Alternating temperature is known to increase germination rate in many species (Baskin and Baskin, 2001). Some studies show that for faster germination rate to occur, the difference between the minimum and maximum daily temperature should be large, e.g. more than 10 °C (Pons and Schröder, 1986). However, in some species, an amplitude of only 1 °C is enough to stimulate germination (Thompson and Grime, 1983). A critical mean temperature is required for seed germination in some species, while in some species, germination will occur only when the difference between minimum and maximum temperature exceeds a certain value (Ekstam and Forseby, 1999). Murdoch et al. (1989) proposed several variables for seed germination response to alternating temperature, which includes primary variables, e.g., number of cycles, the minimum and maximum temperature, time per cycle below and above mid-temperature, rate of warming and cooling. Also, there are secondary variables, i.e., treatment duration, periodic time, mean temperature, amplitude and rate of temperature change.

Alternating temperature interacts with other environmental factors, such as light. The alternating temperature normally indicates gap detection in woodland or a shallow burial and its effect on germination should be of more significance in relatively small seeded species. Small size seeds are discovered to germinate better in light than in darkness under a species-specific magnitude of diel temperature fluctuation (Pearson et al., 2002). Large gaps can result in rapid drying of surface soil; thus, if small seeded species germinated under these large gaps, they would not be able to develop their roots deep in the soil and be very likely to die.

2.1.2 Constant temperature

Under constant temperature environments, the germination rate of seeds normally accelerates with the increase of temperature within a certain range (Baskin and Baskin, 2001). The rate of germination, which is defined as the reciprocal of time taken for half the population to germinate, forming a linear relationship with temperature (Figure 2.1). Therefore, the temperature accumulated over a given time duration is constant. This constant value, termed
as thermal time, can be used to compare germination in different species, climates and locations (Garcia-Huidobro et al., 1982, Moot et al., 2000, Bierhuizen and Wagenvoort, 1974).

Germination response to temperature can be characterised by three “cardinal temperatures”, i.e. base temperature ($T_b$), optimal temperature ($T_o$) and ceiling temperature ($T_c$). Germination will not occur below the base temperature ($T_b$) or above the ceiling temperature, $T_c$. $T_o$ represents the temperature at which germination is most rapid. If the germination rate, i.e., the inverse of time to germination of a certain percentage of the seed population, is plotted against temperature, an inverted V-shaped always appears (Figure 2.1).

![Figure 2.1 Hypothetical relationship between temperature and germination rate (GR), the slope of the line between $T_b$ and $T_o$ is $\theta_T$.](image)

The linear function between germination rate and temperature can be expressed as:

$$\theta_T(g) = (T - T_b) t_g$$

$\theta_T(g)$ is known as thermal time, i.e. the degree days that a fraction of the population needs to accumulate for germination. $t_g$ is the actual time required for a fraction to germinate. $\theta_T$ for a particular percentage is a constant when the temperature is between base and optimal values. This thermal time model is highly predictive when soil is wet and temperature is between the base and optimum and is effective in comparing or predicting germination time in the field (Baskin and Baskin, 2001).

2.2 Light

The light environment is determined by the seed position, i.e. buried or lying on the soil surface. Small seeded species rely more on the light environment in contrast to large-seed species because the shoots of the former ones will be less likely to reach the surface after germination. Even though the germination of many seeds is affected by light (Fenner and Thompson, 2005), strong light intensity may also result in inhibition of germination. This is because strong light intensity is commonly accompanied with high temperature and drought conditions; the inhibition effect can reduce the probability of seedling death.

Except for intensity, the quality of light, i.e. spectral composition, is another feature of the light environment that is related to germination. When the other environmental conditions such as temperature and water are not limited, the germinability of seeds depends on the ratio of red/far-red (Grime et al., 1981). There is great reduction in red/far-red ratio after sunlight transmits through the vegetation. This is because the red spectrum is more absorbed than the
far-red part. Germination of most photoblastic seeds is inhibited when located under canopy- filtered sunlight, even the negatively photoblastic species also display germination reduction (Górski, 1975). A low red content may indicate the surrounding of vegetation and thus a potential competition around. The sensitivity of seeds to the high or low red/far-red ratio has been interpreted as a gap-detection mechanism (Grime et al., 1981), the ratio required to stimulate germination is species-specific. This sensitivity to light is mediated by the presence of phytochrome in seeds.

Phytochrome is classified to red light absorbing (Pr) and far-red light absorbing (Pfr) forms. These two forms are interconvertible. Pfr can revert to Pr in the dark and this process can help reduce Pfr in buried seeds. The phytochrome family is encoded by five different genes (from A to E), Phytochrome A (phy A) is the most abundant one but degrades rapidly from the Pfr form upon exposure to light. The synthesis of phy B and phy C shows lower rates than phy A; however, they are more stable (Somers et al., 1991). Based on their stability, phytochromes are divided into two types, type I (phy A) and type II (phy B, C, D, E).

Phy A is involved with detecting the light at very low intensities, which is known as very-low-fluence response (VLFR). Under this circumstance, the amount of Pfr after a short pulse of FR is often enough to induce significant germination. On the other hand, phy A is also related to the inhibition of seed germination under high irradiance (Casal and Sánchez, 1998). Phy B is involved in the low-fluence response (LFR), which is the classical red and far-red reversible response, i.e. the effect of R is cancelled by a subsequent pulse of FR. The stimulus of germination by LFR involves weakening the surrounding tissues of embryo, modulating gibberellin metabolism and so on (Arana et al., 2007). Consequently, VLFR enables seeds to germinate under a very brief exposure of light, like during tillage; LFR induces germination after the gap perception under the canopy.

Despite the important relationship between light and germination, using light conditions to predict germination is not easy and needs to be carefully planned. The response of seed germination to phytochrome is tightly related to the status of phytochrome and other environmental variables. The original amounts of phytochrome present in seeds can be very different due to the varied maternal light environment. Seed size can also result in quite different germination under low R/FR ratio. Storage of seeds in the laboratory can show increased germination rate with longer storage time, while in the field, the light and dark effects can interact; seeds can remain ungerminated until a flash of light occurs (Baskin and Baskin, 1992). Also, other environmental conditions, such as gas, water and temperature, will all interact with phytochrome (Casal and Sánchez, 1998). Additionally, germination can be triggered by a flash of light, which is too quick to be measured. In conclusion, predictions using light conditions conducted in the laboratory may not apply well for seeds in the field.

2.3 Chemical environment

The chemical environment of a seed can be considered as the gaseous and liquid substances in the soil, which includes oxygen, carbon dioxide and nitrogen.

Oxygen is necessary for respiration during seed germination for most species, even though some species can germinate in anoxic environments. Some marine species, like Zostera marina, are known to germinate better in a deoxygenated environment rather than aerated conditions (Moore et al., 1993). Due to the varied response of different species to reduced oxygen, it is uncertain how oxygen is involved ecologically in regulating germination.
Carbon dioxide concentration in the soil can differ from levels above ground. It depends on the soil depth, temperature, rate of gas exchange and so on. Normally, the level of carbon dioxide increases with the depth in the soil. Some studies have shown that an elevation of carbon dioxide to 2-5% may increase germination rate or final germination percentage, although a level above 5% will inhibit germination (Baskin and Baskin, 2001). However, this response to different concentrations of carbon dioxide is not applicable in the field, because if the concentration reaches this amount, the seed will be so deep in the soil, i.e. 1.75-4.0 m (Richter and Markewitz, 1995), that seedlings will not be able to reach the soil surface.

Nitrate is one of the most important ions for seed nutrition in the soil and is known to promote germination in some cases. The endogenous nitrate of seeds is easily leached out into the soil; thus, the stimulation on seed germination is mostly reliant on the sensitivity to the external nitrate ions. Increases in germination are observed to be closely related with the flush of nitrate levels in soil (Popay and Roberts, 1970). It is also found that the nitrate levels vary seasonally but no general pattern is associated with different soil types. Therefore, it is not possible to use nitrate levels to predict seasonal changes in germination (Hilhorst and Karssen, 2000).

The level of nitrate in the soil can vary with temperature, soil type and the cover by vegetation or not. For example, nitrate levels in bare soil are higher than those under the canopy in chalk grassland in the Netherlands, because the nitrate present in the latter environment tends to be absorbed by the vegetation around. The nitrate concentrations in bare soil are high enough to promote germination. Therefore, the function of a nitrate response can be interpreted as a gap-detecting mechanism (Pons, 1989). Light and temperature also change simultaneously when gaps occur and thus the peaks of germination under gaps would be contributed by all three factors, not just nitrate itself.

Even though the nitrate can promote germination in many crops, it is not effective for weed species (Baskin and Baskin, 2001). A possible reason is that seeds of many species require both light and nitrate for germination, thus the nitrate effect would be better performed when the seeds are exposed to light at the same time (Hilton, 1984).

2.4 Water

Water is essential for seed germination, and the critical amount of water content required for germination varies among different species. Typically, water uptake occurs in three phases: 1) imbibition, during which water is absorbed rapidly by the embryo (or endosperm); 2) activation, in which the development is activated and the water uptake reaches a plateau; 3) growth – when the seedling emerges and protrudes the seed coat (Bewley et al., 2012). The initial movement of water from substrate to embryo is basically a consequence of matric forces, because the water content of dry seeds is normally very low. After the water inside seeds comes almost to equilibrium with the water outside, there will be gradual or no more uptake of water (Phase II). For dormant seeds, they can remain in this state for months or even years; for non-dormant seeds, the breakage of this phase is indicated by the rapid absorption of water and seedling elongation. Therefore, it is the length of this phase that affects the timing and extent of germination (Bradford, 1995). Imbibition at reduced water potential could lead to a lower seed water content as well as longer time at phase II (Figure 2.2).
The imbibition time for seeds to reach the required water content is also related to seed size. For seeds up to 1000 mg, the amount of water necessary for germination is linearly related to seed mass (Kikuzawa and Koyama, 1999). The smaller sized seeds may have the advantage that they are able to absorb the water more rapidly than larger seeds. Additionally, the smaller size enables them to penetrate the soil, which provides a more benign microenvironment that promotes germination and minimizes desiccation.

In the field, seeds might not receive continuous rainfall during the germination period; they could experience drought or a few wet and dry cycles before germination is completed. Many experiments have been conducted to explore the effect of hydration/dehydration cycles on seed germination. A common opinion is that seeds treated under this cycle will show faster germination than untreated ones when the required moisture is finally applied (Fenner and Thompson, 2005). Baskin (1982) proposed that the effects of imbibition during the hydration/dehydration cycles might be cumulative. The final germination percentage of the seed population is not affected, but the cycle contributes to reducing the time required for germination. The wet/dry cycle may not be so efficient in improving germination when the hydration period is very short and the germination has not proceeded to the crucial stage. Additionally, the long dry period may result in desiccation and damage of seeds (Jansen and Ison, 1994).

Seeds of different species tend to respond differently to the pattern of rainfall, and this behaviour may determine which species will establish under a particular environment. A slow response to rainfall is favourable in the circumstance when the wet period is too short for seedlings to grow and establish eventually (Fenner and Thompson, 2005). The hydration effect that slow germinators receive can be accumulated even if it is interrupted by drought. This drought break is unlikely to cause slow germinators to die, while for fast germinators, it can be fatal. On the other hand, when the length of rain season is long enough to support the seedlings and there is no long-term drought break, fast response could be of advantage. This shows that the frequency and timing of water supply is crucial in determining germination. However, the way by which seeds sense the length or frequency of shower remains unknown.
2.5 Models for predicting germination

Brown and Mayer (1988) summarised that many approaches have been made to analyse germination time course data. In these studies, germination time course was fitted empirically and four parameters were required, i.e., lag period, shape of the cumulative distribution and maximum germination, and germination rate. However, when there was a change in another parameter, such as water potential, they could not predict the effect accordingly. A separate curve was needed for each quantitative level of the factor (Bradford, 1995), as sensitivity to water among seeds had not been incorporated into the equations. Additionally, the empirically fitted models were not easy to ascribe biological meanings.

As mentioned in section 2.4, the shape of the germination time course is primarily determined by the length of Phase II and the sensitivity to water potential among individual seeds. When a seed germinates, it is already in Phase II of germination. This indicates that the initiation of germination is a changing state rather than a steady one, and germination is a bimodal phenomenon, not a continuous process. The empirical models referred to above describing plant growth time courses as well as the cumulative germination time course seems to confound the underlying biological meanings (Bradford, 1995). In order to predict germination, water potential should be incorporated. Also, a model should be able to account for the lag period prior to germination as well as the delay and slowness in germination since water potential is reduced. Additionally, the delay in germination should be related to the inhibition of final germination percentage.

A hydrotime model was proposed by Gummerson (1986) to describe the germination rate as well as germination percentage to the seed water environment. A threshold water potential ($\Psi_b$) for germination is used and the extent to which the water content inside seeds ($\Psi_s$) exceeds $\Psi_b$ is proportional to the time for completing germination. Every single seed among a population can differ in the $\Psi_b$ value. The lower the $\Psi_b$ value is, the faster the seed can germinate. For a population, the distribution of $\Psi_b$ values is generally assumed to be a normal distribution, and the median base water potential is described as $\Psi_b(50)$ (Bradford, 1990). The relationship between the water potential and the time required for germination can be demonstrated in the following equation:

$$\text{Equation 2.2} \quad \theta_H = (\Psi - \Psi_b(g)) t_g$$

where $\theta_H$ is the hydrotime constant, $\Psi$ is the seed water potential, and $\Psi_b(g)$ is the base water potential for a given fraction of seed population. $t_g$ is the time required for germination for that fraction. Since $\theta_H$ is a constant, the time required for radicle emergence in a given population decreases as the difference between $\Psi$ and $\Psi_b(g)$ increases. Unlike the constant $T_b$ mentioned in Section 2.1, $\Psi_b$ is assumed to vary among individual seeds. Consequently, when plotting $GR_s$ against $\Psi$, the curves for different percentages of seeds should be parallel (Figure 2.3).
Figure 2.3 Relationship between germination rates and water potential (Bradford, 2002), the same slope of the three lines is $\theta_H$. The $\Psi_b(g)$ value increases as the germination percentage increases and the $\Psi_b(g)$ values across different percentages is normally distributed.

Further, when both temperature and water are changing, combining these two factors together will make it possible to describe the effect of water and temperature on germination. HTT model can thus be specified as below (Gummerson, 1986):

$$\theta_{HT} = (T - T_b) (\Psi - \Psi_b(g)) t_g$$

where $\theta_{HT}$ is the hydrothermal constant, a unique value for a given seed population. Under constant water and temperature environment, the model assumes that the $g$th proportion of seeds in the population will germinate when they have accumulated sufficient hydrothermal time ($\theta_{HT}$). In the equation, $T_b$ is a constant for any population, while $\Psi_b(g)$ is different for each germination percentile. Different percentiles of seeds accumulate different amounts of hydrotime during a specific time period. Since $\theta_H$ is a constant, the value of $\Psi_b(g)$ is reflected in the $t_g$ values among the population. Therefore, variation of $\Psi_b(g)$ results in differences in germination time of seeds within a population. $\Psi_b(g)$ is assumed to be normally distributed, with $\Psi_b(50)$ being the median base water potential of the population.

Even though the HTT model was found to be useful in predicting the timing of germination in many species (Gummerson, 1986, Meyer et al., 2000), exceptions have also been found. For example, base water potential varies with temperature or the state of after ripening (Alvarado and Bradford, 2002, Del Monte and Dorado, 2011). Apart from this, the HTT model did not fit with the data when temperature exceeded the optimum (Alvarado and Bradford, 2002). Therefore, adjustment of the HTT model is required in some circumstances.

2.6 Dormancy

The interaction of environmental factors and germination demonstrated in Section 2.2 is based on non-dormant seeds. When seeds are dormant, the germination process is more complex. Dormancy is not a characteristic of the environment, but of the seed itself. The degree of dormancy defines the environmental range required for germination (Vleeshouwers et al., 1995).

So far, only temperature has been recognized to alter the degree of dormancy and influence the environmental range for germination; other factors, like light, cannot change germination requirements but have an effect on germination itself and should be treated as a germination trigger.
2.6.1 Classification of dormancy
There are two general kinds of seed dormancy: endogenous and exogenous dormancy. Endogenous dormancy is a result of embryo characteristics which prevent germination, while exogenous dormancy is imposed by the covering structures, such as the seed coat or fruit wall. Based on this, Baskin & Baskin (2001) provided a comprehensive list of dormancy classifications:

1. Physiological dormancy (PD): Seeds with PD are water-permeable, they have a physiological inhibiting mechanism that prevents the radicle emerging from the embryo. Additionally, the structures covering the embryo, such as endosperm, can delay germination. The level of PD varies with respect to the strength of the physiological inhibiting mechanism, response to gibberellic acid (GA) and dormancy breaking requirements. Three levels have been distinguished, non-deep, intermediate and deep. Seeds with the first two level of dormancy produce normal seedlings, and their dormancy can be broken by GA treatment. With deep physiological dormancy, seeds produce abnormal seedlings, and GA does not break their dormancy.

2. Morphological dormancy (MD): The embryo in the seeds of some species is underdeveloped and may be differentiated or not, but they all need time to grow before radicle emergence.

3. Morphophysiological dormancy (MPD): MPD exists in seeds with an underdeveloped embryo, and additionally, they have a physiological component of their dormancy. Thus, a dormancy-breaking trigger is also required to break the physiological dormancy.

4. Physical dormancy (PY): Seeds with water-impermeable layers that require physical or chemical scarification to allow water in.

5. Combinational dormancy (PD+PY): This is found in seeds with water-impermeable coats combined with physiological embryo dormancy.

2.6.2 Physiological dormancy cycle
Dormancy in Cakile species was relieved to some extent by cold stratification (Davy et al., 2006, Payne, 1980); this treatment only affected physiological dormancy (Baskin and Baskin, 2001). Therefore, physiological dormancy is the main focus of this section.

Seeds with non-deep physiological dormancy cannot germinate at any temperature; even if some germination occurs, it is within a very narrow temperature range. Seeds with non-deep physiological dormancy can cycle between dormancy and non-dormancy (ND) several times. If the fresh mature seeds fail to germinate under favourable conditions, they are in a state of “primary” dormancy. The stage between PD and ND is conditional dormancy (CD). Under CD, seeds can germinate over a narrow range of conditions. This range widens and finally seeds can germinate over the full range of conditions under which the population of the species can germinate. Seeds can re-enter dormancy again after becoming ND; this new state is called “secondary” dormancy. This cycle can be repeated several times until seeds encounter a favourable environment for germination (see Figure 2.4). Changes in these states correspond to changes in environmental factors. Seeds with PD can be relieved through cold stratification or dry after-ripening. During these processes, the amounts of ABA or GA inside the embryo are considered to correlate with loss of dormancy. In some cases, seeds are covered by hard, woody structures which is permeable to water, but germination will not occur until receiving a damage on the covering structures.
Temperature is the major environmental factor that is related to dormancy. Temperature determines the environmental range under which germination could occur if other conditions such as water availability or light are suitable, and germination occurs when the field temperature is in this range. For example, in *Persicaria maculosa* (Vleeshouwers et al., 1995), seeds become ND during winter and may germinate in spring if conditions are favourable; if the seeds remain in the dark, the same temperature that induces germination will reduce dormancy. During this cycling period, the range of conditions under which germination can occur widens gradually.

Winter annuals in a temperate environment do not germinate in summer because the maximum temperature for germination is below the habitat temperature. They stay dormant until autumn when the maximum temperature required for germination increases (seeds become CD) and the habitat temperature decreases; once these two temperatures overlap, seeds will initiate germination (Baskin and Baskin, 1985), and vice versa for summer annuals (Figure 2.5).

This is the typical pattern seeds exhibit in temperate areas. Winter annuals require high summer temperatures to become ND, and if the temperature is too high, seeds do not come out of
dormancy until soil temperatures decline at relatively low (15, 20°C) temperatures (Schütz, 2002, Bolger et al., 1999). This delay of dormancy breaking helps ensure that seeds do not germinate in summer when the environmental conditions are unfavourable for seedling survival. For summer annuals, the induction of dormancy or CD is also controlled by temperature. If the seeds which fail to germinate in the growing season are exposed to high temperatures, they will be induced into secondary dormancy. Additionally, in tropical environments, where temperature does not change much over season, the daily alternating temperature is more important than constant temperature. The fluctuation of temperature can enable seeds to determine whether there is a gap in the vegetation or not, and thus stimulates the seeds to break dormancy.

Seeds in the dormant state are continuously perceiving the environment and adjusting the conditions required for germination. For example, Vegis (1973) found that the maximum temperature increases in some species, while in some other species, the minimum temperature decreases for germination during primary dormancy breaking. Additionally, some species germinate at intermediate temperatures, and then the maximum and minimum temperature required for germination increases and decreases, respectively.

Studies have also shown that the dormancy state of seeds has changed when the species spread into new environment (reviewed by Donohue et al., 2010). In comparative studies of native and introduced populations of the same species, the introduced population was more dormant in some cases (Hierro et al., 2009). This was interpreted as an adaptive change, and the introduced population can also show a wider range of germination conditions (Blair and Wolfe, 2004).

As introduced in Chapter 1, *Cakile* species may display varied germination behaviours when spreading to different climates, and the difference in germination timing could be one of the reasons of the replacement of *C. edentula* by *C. maritima*. There are no previous studies on the dormancy variance among different climates of *Cakile* species, also it is unknown how these differences can affect the germination behaviour, or how these differences reflect in the HTT model. These will be discussed in the following chapters.
Chapter 3  Fitting the hydrothermal time model to radish germination

3.1 Introduction
In Chapter 2, hydrothermal time was discussed as a way of predicting germination. Previous studies have shown that it can provide good prediction for germination of crop species. Before implementing a hydrothermal time model (HTT model) for predicting germination of Cakile species, the techniques for obtaining parameters to fit into this model need to be familiarised. Therefore, I did a preliminary study with a crop species, radish (Raphanus sativus cv. Fireball), as unlike wild/weedy species, crops have more uniform germination and their germination behaviour is not complicated by dormancy.

The HTT model, in which base temperature is assumed to be independent from water potential and the base water potential (Ψ) is independent from T, fit well to Gummerson’s data for sugarbeet. However, in a number of studies, Tb and Ψ have been shown to change with water potential and temperature respectively (Kebreab and Murdoch, 1999, Dahal and Bradford, 1994, Fyfield and Gregory, 1989). Bradford (1995) also discovered that if changes in Ψb(g) at supra-optimal temperature range were taken into account, the hydrothermal time model fit better with the actual data than assuming that temperature and water are independent. The hydrotine parameters were also seen to vary when seeds were imbibed in high or low water potential ranges (Dahal and Bradford, 1994). Therefore, the aim of this chapter is to examine the fit of HTT model to radish and to observe whether there is variation in the model parameter values across water potential and temperature.

3.2 Materials and Methods
Radish seeds were purchased from New South Wales (Terranova Seeds Pty Limited). Six different levels of osmotic potential were created for germination, i.e. 0, -0.2, -0.4, -0.6, -0.8, -1.0 MPa. Temperature control was realized by five incubators set at constant 5, 10, 15, 20 and 25 °C, respectively. There were four replicates for each treatment. Polyethylene glycol (PEG 8000) was used to establish the water potentials (Hardegree and Emmerich, 1990). PEG concentrations for each temperature were different. To achieve the required water potential under each temperature, the following equation for calculating PEG for each level was used (Michel, 1983):

\[
\text{Equation 3.1} \quad [\text{PEG}] = \frac{[4 - (5.16\Psi T - 560\Psi + 16)]^{0.5}}{(2.58T - 280)}
\]

where Ψ and T represent water potential and temperature, respectively. Filter papers (Whatman No.1) were initially soaked with the appropriate PEG concentration. After moistening, two layers of filter paper were placed in each plastic Petri dish (90 mm in diameter). Fifty seeds were placed on the filter paper for each treatment. Petri dishes were sealed with Parafilm M® film to minimize water loss. During the experiment, additional PEG was added to prevent the filter paper from drying out (and hence from changing the water potential of the seeds). Germination, defined as a radicle > 2mm, was counted every day. Germinated ones were removed at each count. Germination was regarded as finished when there was no more germination for 7 successive days after 20 days of counting.

3.3 Data analysis
Analysis of germination data consisted of two stages. First, the hydrotine model was fitted to data separately for each temperature. This step was to check whether the value of median base
water potential varied with temperature and to obtain initial values for parameters when fitting the full HTT model. Second, the hydrothermal time model was fitted to the full data set.

3.3.1 Hydrotime
Hydrotime was defined in Chapter 2, in which the value of \( \Psi_b \) is known to vary among different percentages \( g \). Therefore, the model assumes that variation in times for germination among seeds in a population is due to the differences in base water potentials within each seed percentage. The distribution of \( \Psi_b \) was assumed to closely approximate a normal distribution (Bradford, 1990, Gummerson, 1986); therefore, a cumulative normal distribution function (cdf) was used to describe germination course (Bradford, 1990):

\[
G(t) \sim \Phi(\psi_{b(g)}, \psi_{b(50)}, \sigma_{\psi_b})
\]

where \( \psi_{b(50)} \) is the base water potential within the median percentage of the population. \( \sigma_{\psi_b} \) is the standard deviation of \( \psi_{b(g)} \) values in the population. Based on Equation 2.2, \( \psi_{b(g)} \) can be represented as:

\[
\psi_{b(g)} = \psi - (\theta_H/\theta_g)
\]

For the analysis in SAS, \( \psi_{b(g)} \) in the cdf function was replaced by Equation 3.4 (see codes in Appendix 3.1). The initial values or ranges for \( \psi_{b(50)} \), \( \sigma_{\psi_b} \) and \( \theta_H \) were required to run the SAS program, which were chosen to be -0.4 MPa, 0.2 and 1 to 10, respectively. These parameters were estimated from literature data on other Brassicaceae species (Tribouillois et al., 2016, Köchy and Tielbörger, 2007).

3.3.2 Hydrothermal time
When taking the temperature effect on germination into consideration, the thermal time accumulated for germination can also be incorporated into modelling germination. As introduced in Chapter 2, based on Equation 2.3, \( \psi_{b(g)} \) can be represented as:

\[
\psi_{b(g)} = \psi - \theta_{HT}/(T - T_b)\theta_g
\]

\( \psi_{b(g)} \) in Equation 3.2 can thus be substituted using Equation 3.4. The SAS code for fitting this model is given in Appendix 3.2. The initial values for \( \psi_{b(50)} \) and \( \sigma_{\psi_b} \) were obtained from the hydrotime results, while \( T_b \) and \( \theta_{HT} \) were taken from the studies on other Raphanus species (Young, 2001).

3.4 Results
3.4.1 Hydrotime
It was clear that \( \psi_{b(50)} \) did not remain constant at different temperature environments (Figure 3.1). \( \psi_{b(50)} \) showed a decreasing trend with temperature increasing, and increased when temperature exceeded 25 °C. This result indicates that the conventional hydrothermal time model which assumes a single \( \psi_{b(50)} \) value across different temperatures may not fit the original germination course very well.
3.4.2 HTT model

The parameters of the fitted HTT model are shown in Table 3.1. With the RMSE (root-mean-square error) and AIC value presented in the table, the smaller value of RMSE and AIC means the better fit of model.

Table 3.1 Estimated HTT model parameters for radish

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_b$</td>
<td>2.69</td>
<td>0.120</td>
</tr>
<tr>
<td>$\psi_b(50)$</td>
<td>-1.02</td>
<td>0.019</td>
</tr>
<tr>
<td>$\theta_{HT}$</td>
<td>21.67</td>
<td>0.990</td>
</tr>
<tr>
<td>$\sigma_{\psi_b}$</td>
<td>0.44</td>
<td>0.019</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.21</td>
<td>0.005</td>
</tr>
<tr>
<td>AIC</td>
<td>-302.00</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Based on the parameters obtained above, the germination time course can be graphed against temperature at different water conditions (Figure 3.2). Obvious systematic lack of fit appeared at almost all levels of temperature except at 20 and 30 °C. The model generally overestimated the germination rate at 10 and 15 °C, while at 25 °C the fitted germination was lower than the actual data over all water potentials. These deviations indicated that the model did not fit very well with the actual data. Therefore, adjustment of the HTT model was necessary for a better result in fitting the germination data.
3.4.3 Adjusted HTT model 1

An adjustment to base water potential in the full HTT model was considered first due to its obvious variance across temperature. An equation was used for calculating the corresponding base water potential at each temperature:

\[ \Psi_b(50)_T = \Psi_b(50) - k(T - T_b) \]

where \( \Psi_b(50)_T \) is the temperature-adjusted \( \Psi_b(50) \), and \( k \) is the slope coefficient which shows how fast \( \Psi_b(50) \) decreases with temperature. In this equation, \( \Psi_b(50) \) is the median base water potential at the base temperature. The value of \( \Psi_b(50) \) in the original model was replaced by \( \Psi_b(50)_T \); the CDF function was therefore expressed as:

\[ G(t) \sim \Phi(\psi_{b(g)}, \psi_{b(50)_T}, \sigma_{vb}) \]

The HTT parameters obtained by fitting the HTT_adjusted1 model are shown below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>Standard Error</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_b )</td>
<td>-7.29</td>
<td>1.990</td>
<td>-11.20</td>
</tr>
<tr>
<td>( \Psi_b(50) )</td>
<td>0.03</td>
<td>0.120</td>
<td>-0.21</td>
</tr>
<tr>
<td>( \theta_{HT} )</td>
<td>35.08</td>
<td>3.050</td>
<td>29.09</td>
</tr>
<tr>
<td>( \sigma_{vb} )</td>
<td>0.39</td>
<td>0.013</td>
<td>0.36</td>
</tr>
<tr>
<td>( k )</td>
<td>0.04</td>
<td>0.002</td>
<td>0.04</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.15</td>
<td>0.003</td>
<td>0.15</td>
</tr>
<tr>
<td>AIC</td>
<td>-893.80</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
The RMSE value of the HTT_adjusted1 model was improved compared to the original HTT model. However, the $T_b$ value of -7.29 °C was unrealistic. Normally, the $T_b$ of Brassicaceae species is around 0 °C or a little higher (McCormick et al., 2014). Therefore, a further adjustment of the HTT model was made.

3.4.4 Adjusted HTT model 2

Apart from the temperature effect on base water potential, the way heat sum is accumulated also varies with increasing temperature (Alvarado and Bradford, 2002). In Chapter 2, the concept of cardinal temperatures was introduced, i.e., $T_b$, $T_o$ and $T_c$. Heat accumulates when the temperature is between $T_b$ and $T_o$; while when temperature exceeds $T_o$ (below $T_c$), no more heat is accumulated. Equation 3.5 therefore becomes:

$$\psi_{b(g)} = \psi - \frac{\theta_{HT}}{(T-T_b) t(g)}, \text{ if } T < T_0; \psi_{b(g)} = \psi - \frac{\theta_{HT}}{(T_o-T_b) t(g)}, \text{ if } T \geq T_0$$

where $T_o-T_b$ is a constant. The new expression for $\psi_{b(g)}$ was therefore incorporated into Equation 3.7 for estimating germination. Comparing this to the HTT_adjusted1 model, a new parameter $T_o$ was added. The resulting parameter values estimated from SAS are shown in Table 3.3.

Table 3.3 Estimated HTT_adjusted2 model parameters for radish

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>Standard Error</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_b$</td>
<td>-5.65</td>
<td>1.40</td>
<td>-8.410</td>
</tr>
<tr>
<td>$T_o$</td>
<td>20.00</td>
<td>0.010</td>
<td>19.980</td>
</tr>
<tr>
<td>$\psi_{b(50)}$</td>
<td>0.04</td>
<td>0.087</td>
<td>-0.130</td>
</tr>
<tr>
<td>$\theta_{HT}$</td>
<td>28.86</td>
<td>2.080</td>
<td>24.780</td>
</tr>
<tr>
<td>$\sigma_{\psi_{b}}$</td>
<td>0.38</td>
<td>0.013</td>
<td>0.360</td>
</tr>
<tr>
<td>$k$</td>
<td>0.04</td>
<td>0.002</td>
<td>0.039</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.15</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>AIC</td>
<td>-912.60</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The RMSE and AIC values of HTT_adjusted2 were similar to those of HTT_adjusted1, which means there was little improvement in the model performance. The value of $T_b$ increased to -5.65 °C, was closer to the $T_b$ values of Brassicaceae species.

3.4.5 Adjusted HTT model 3

In order to achieve better model performance, a further adjustment of the model was made. In this model, the restriction for heat sum was not considered, but a broken stick response was used to describe the relationship between base water potential and temperature.

As shown in Figure 3.1, base water potential decreased before temperature reached the optimum, and increased after that as temperature increased further. Therefore, the equation below was used to describe the relationship:
\[
\begin{align*}
\psi_{b(50)adj} &= 0 & \text{if } T \leq T_b \text{ or } T \geq T_c \\
\psi_{b(50)adj} &= \psi_{b(50)} \frac{t - T_b}{T_0 - T_b} & \text{if } T > T_b \text{ and } T \geq T_o \\
\psi_{b(50)adj} &= \psi_{b(50)} \frac{T - T_c}{T_0 - T_c} & \text{if } T > T_o \text{ and } T < T_c
\end{align*}
\]

Equation 3.8

In the equation above, the value of \( \psi_{b(50)} \) is reached when temperature is at the optimum \( (T_o) \). By using \( \psi_{b(50)adj} \) to replace the \( \psi_{b(50)} \) in Equation 3.3, the new HTT\_adjusted3 model was thus formed. The parameters obtained from fitting this model are shown in Table 3.4.

**Table 3.4 Estimated HTT\_adjusted3 model parameters for radish**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>Standard Error</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_b )</td>
<td>-5.29</td>
<td>0.330</td>
<td>-5.95 - 4.64</td>
</tr>
<tr>
<td>( T_o )</td>
<td>28.18</td>
<td>20.820</td>
<td>-12.68 - 69.04</td>
</tr>
<tr>
<td>( T_c )</td>
<td>39.62</td>
<td>147.80</td>
<td>-250.41 - 329.65</td>
</tr>
<tr>
<td>( \psi_{b(50)} )</td>
<td>-1.42</td>
<td>0.880</td>
<td>-3.15 - 0.31</td>
</tr>
<tr>
<td>( \theta_{HT} )</td>
<td>30.69</td>
<td>1.040</td>
<td>28.64 - 32.74</td>
</tr>
<tr>
<td>( \sigma_{vb} )</td>
<td>0.36</td>
<td>0.012</td>
<td>0.34 - 0.39</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.15</td>
<td>0.003</td>
<td>0.14 - 0.15</td>
</tr>
<tr>
<td>AIC</td>
<td>-1014.00</td>
<td>N/A</td>
<td>N/A - N/A</td>
</tr>
</tbody>
</table>

Using \( \psi_{b(50)} \) and the cardinal temperature parameters from the table above to insert into Equation 3.8, a fitted relationship between base water potential and temperature was obtained (Figure 3.3).

**Figure 3.3 Relationship between \( \psi_{b(50)} \) and temperature for model HTT\_adjusted3 of radish**

In Figure 3.3, the solid red circles are the \( \psi_{b(50)} \) values estimated from the original hydrotime analysis while the blue line shows the fitted \( \psi_{b(50)} \) values from the HTT\_adjusted3 model. The fitted line passed close to all points except for 25 °C.
HTT_adjusted3 resulted in the smallest value of AIC so far (even though the RMSE value remained the same as the previous model), which means that the HTT_adjusted3 fitted better than the previous models although the estimated $T_b$ of -5.29 was still rather low.

Figure 3.4 shows the germination courses fitted by Equation 3.8. Germination rate and final germination percentage were severely affected by the reduction of water potential under a single temperature environment; this reduction was especially obvious at temperatures lower than 25 °C. At 20 °C, germination in pure water (0 MPa) reached 100%, while the germination of seeds imbibed at -1.0 MPa was less than 50%. The reduced water potential affected the germination fraction to different extents, depending on the incubation temperature. At 5 °C, germination rarely occurred when the water potential was lower than -0.6 MPa. The time required for accomplishing germination at each water potential was also shortened as temperature increased when the temperature was below 30 °C. On the other hand, exceptions also existed. When the temperature was at 25 °C, the actual germination fraction and germination rate at -0.4 MPa was the lowest compared to any other water potentials of the same temperature. Even so, the hydrothermal time model accounted for most of the variation in germination time that was contributed by decreasing in water potential.

The model fit well when the water potential was higher than -0.4 MPa, except for the fitting of 0 MPa at 5 °C where the prediction underestimated the germination fraction. Systematic deviations also appeared in the fitted HTT_adjusted3 model, especially when the water potential was lower than -0.4 MPa. For example, the most obvious deviation appeared at -1 MPa of 30 °C where the prediction overestimated the germination.

![Figure 3.4 Germination time course at different combinations of temperature and water potential fitted by HTT_adjusted3 model of radish. The curved lines are the fitted germination time course, the symbols are germination fraction for each replicate.](image-url)
The goodness of fit of the HTT_adjusted3 model can also be presented by plotting the fitted germination fraction against the observed germination fraction (Figure 3.5). The $R^2$ values at temperatures from 5 to 20 °C are 0.90, 0.86, 0.83 and 0.93, respectively; while at 25 and 30 °C, the $R^2$ value falls to 0.68 and 0.75, respectively, showing a poor fit at 25 °C of the HTT_adjusted3 model. At 25 °C, seeds germinated in various water potentials converged on high fractions. Even though the $R^2$ values were not low at 5 ~ 20 °C, the goodness of fit showed a systematic lack of fit to a certain degree. For example, germination fraction was generally underestimated at 5 °C, while it was overestimated at 10 °C.

![Figure 3.5 Goodness of fit between the observed germination fraction and the fitted germination fraction of radish, the lines are the 1:1 equation lines.](image)

3.5 Discussion

The classic HTT model proposed by Gummerson (1986) did not fit the radish germination data very well in this experiment. This was due to the interaction between temperature and water potential. By adjusting the original HTT model in three different ways, the fitness of the model for the actual germination course under each adjustment could be improved but only to a limited extent.

At supra-optimal temperatures, i.e., 30 °C, the germination fraction of seeds under low water potential (< -0.4 MPa) decreased compared to 25 °C (Figure 3.4). This might be due to the very high base water potential at 30°C. This phenomenon was also observed in other species and regarded as a thermoinhibition effect (Rowse and Finch-Savage, 2003, Bloomberg et al., 2009). It is the decline of seed germinability when the soil temperature exceeds the optimum for germination. In the absence of thermoinhibition, seeds germinate rapidly following a summertime “false break”, which will probably induce a high rate of mortality due to the desiccation after that. However, with the effects of thermoinhibition, only the earliest
percentiles without significant thermoinhibition rapidly germinate (Watt and Bloomberg, 2011), and the rest of the seeds can therefore survive. The HTT_adjusted3 model used in this radish study had an adjusted Ψ₆(50) above the optimal temperature to a certain degree, but there were still deviations (Figure 3.4). Watt and Bloomberg (2011) reported in their experiment with four unrelated species that the Weibull distribution may be more suitable for an HTT model in the supra-optimal temperature range than the normal distribution. Mesgaran et al. (2013) also suggested that using other distributions, e.g. the log-logistic, may give better fits to the actual germination time course and various distributions should be tested before implementing the HTT model. Given that the aim of this chapter was to become familiar with the HTT model, only the normal distribution was used.

Unlike the decreasing germination fraction in <-0.4 MPa at supra-optimal temperatures, the germination rate in >-0.4 MPa increased compared to that at 25 ⁰C. Germination rate also generally increased with the increasing of temperature (Figure 3.4). A similar pattern was found in Brassica carinata seeds (Patanè and Tringali, 2011). For B. carinata, seeds showed a faster germination rate at supra-optimal temperatures of any water potential even though the germination fraction was relatively lower than it was at the optimal temperature. High temperatures might reduce the water viscosity of the surrounding medium and thus increase the diffusion of water (Kader and Jutzi, 2002). This mechanism was considered to have a major agronomic impact: seeds are able to germinate fast in rapidly drying soil even though it is a small percentage of that population (Kebreab and Murdoch, 1999). This is regarded as a key benefit for seedling survival in an arid environment. This characteristic of base water potential also emphasizes the importance of allowing for the trend of Ψ₆(50) across temperature when modifying the HTT model.

The HTT_adjusted2 model was based on the model of Alvarado and Bradford (2002). The calculation of heat sum accumulation at sub- and supra-optimal temperatures in different ways was useful in adjusting the HTT model in their experiment for Solanum tuberosum. It also proved to be effective in Pinus radiata seeds (Bloomberg et al., 2009). However, it did not seem to be the best way to describe germination course for radish seeds in this experiment. Alvarado and Bradford (2002) assumed that the heat sum remains constant at ≥ optimum temperature, which is to say that base water potential is the same at optimal and supra-optimal temperatures (Equation 3.7). This assumption clearly did not fit the trend of Ψ₆(50) across temperature in this radish study (Figure 3.1).

The base temperature estimated by HTT_adjusted3 in this Chapter was -5.29 ⁰C (Table 3.4). This value was lower than other Brassica crops, which ranges between 0 ~ 2.1 ⁰C (Tribouilliois et al., 2016, Bierhuizen and Wagenvoort, 1974), and also lower than the winter crop species Brassica napus (4.5 ⁰C) (Trudgill et al., 2000) or the winter annual Rapistrum rugosum (L.) All. (5 ⁰C) (Cousens et al., 1994). Base temperature acquired by the HTT model was through extrapolation beyond the range of temperatures, which means, there are always dubious values. The value of other cardinal temperatures, i.e., optimal temperature (28.18 ⁰C) and maximum temperature (39.62 ⁰C) for germination were within the range of Brassica crops: 28.9 ~ 37.4 for optimum and 35.4~40.7 for maximum (Tribouilliois et al., 2016). The differences in base temperature may be due to differences in the methods, calculation or cultivars used, or can be attributed to the nutrient status of the seeds. On the other hand, the poor fits of the model may be due to the poor data. It is possible that there were operation mistakes of some temperatures, or the incubators did not maintain the temperature stably during the experiment. Above all, this chapter was intended to familiarize the author with the methods and skills in using the HTT
model. In that aim, it was successful; a little deviation of the results from the existing studies did not impact the objective of the chapter.

The application of the hydrothermal time model to cultivated species is straightforward. However, when using this model in predicting germination on wild species, the situation becomes more complicated due to the heterogeneous characteristics of the wild species. Single species collected from locations with different environmental conditions is likely to evolve different germination requirements. Additionally, dormancy is also common in seeds collected directly from the wild plants. The dormancy removing procedures, such as after-ripening may be accompanied with other factors that affect germination or dormancy cycling, e.g., ageing (Neya et al., 2004, Hilhorst, 1998). Hence, the effect of water and temperature on germination as well as dormancy were studied when working on *Cakile* species in the next two chapters.
Chapter 4  Germination of C. edentula

4.1 Introduction
Germination timing is suggested to be one of the reasons for the replacement of C. edentula by C. maritima. During the spread of species from one climate to another, their germination behaviour may experience adaptive changes in new environments (Hierro et al., 2009). The changing of environmental factors, such as day length, temperature and water availability are known to affect germination strategies in numerous species (Gutterman, 2000). Sometimes, the change of environment results in wider range of germination conditions or faster germination rate. Sometimes, seeds can be more dormant (Donohue et al., 2010).

The invasion history of C. edentula showed that it spread from Victoria to South Australia in the west, and New South Wales and Queensland in the east as well in Australia. This indicated that C. edentula seeds had experienced temperate environment, Mediterranean and sub-tropical environment during invasion. As for C. maritima, seeds were introduced to South Australia, and then spread to New South Wales and Tasmania. C. maritima has also invaded Mediterranean and temperate conditions. The replacement has been found in much of the Mediterranean and temperate zones, and now is starting to occur in the tropical area (Queensland).

Seeds of the same species in these different climatic zones could have evolved varied germination strategies, and thus the germination requirements, e.g. temperature and water requirements may change with the environmental conditions they experienced. In this chapter, only C. edentula was used because C. maritima from different locations tend to show highly variable form, size and even physiology (see Chapter 1).

C. edentula from two different climates were selected, and this could give a range of variability in germination. Temperature and water stress are two of the environmental factors that are known to affect a wide range of different species (reviewed by Penfield and MacGregor, 2017, Gutterman, 2000). Therefore, locations with contrasting temperature and water environment are required. The aim of this chapter is to compare the germination behaviour of C. edentula from extremes of environmental ranges.

4.2 Materials and methods
Pure C. edentula now only occurs in a few locations, Queensland (QLD), north New South Wales (NSW) and Tasmania (TAS). QLD is sub-tropical, while TAS has cool temperate environment. The former environment normally shows higher temperature and precipitation than the latter one during the growing season of C. edentula. Seed collection in QLD was made in December 2015, and in TAS it was made in December 2014. The collection sites were 27°26’S, 153°25’E in QLD, and 43°01’S, 147°37’E in TAS respectively.

Plants with matured seeds on them from two locations were collected and dried in drying cabinets for 4-7 days at 38 ºC. A knife was used to open the pods and extract seeds from individual fruits. At least 7200 seeds from each location were extracted.

Previous studies on Cakile species showed C. edentula can germinate when placed on moist filter paper at room temperature (Boyd and Barbour, 1993). To understand germination reaction to a wide range of temperatures, e.g. above and below the optimal temperature, 6 levels of temperature were set, i.e. 10, 15, 20, 25,30 and 35 ºC. As a coastal species, C. edentula is known be tolerant to water stress (Maun et al., 1990); thus 6 water levels were also set: 0, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa. The experiments were carried out from November 2015 to February
The experimental procedures were the same as those for radish germination (see Chapter 3).

4.3 Data analysis
Germination data were analysed using the HTT model (see Chapter 3). The fitted parameters were then used to compare germination behaviours between the two populations. The initial parameter values used in the hydrotim and HTT model were derived in the same way as described in Chapter 3. In order to acquire the most suitable HTT model, the following steps were performed:

1) Use only hydrotim model at first to determine which form of the model fits best among six different distributions (i.e., Gumbel, Log-Normal, Log-logistic, Logistic, Normal and Weibull distribution);
2) Check whether the Ψ₀(50) value acquired through the hydrotim model at each temperature remains the same. If yes, keep on the HTT modelling; if not, an adjustment on Ψ₀(50) is required using the similar method as mentioned in Chapter 3;
3) The adjusted Ψ₀(50) at each temperature is used in the HTT model separately instead of the same Ψ₀(50) value across all the temperatures.

4.4 Results
4.4.1 Hydrotim parameters for *C. edentula* seeds from TAS
The hydrotim parameters estimated at 25 °C by the six distributions of *C. edentula* seeds from TAS and QLD were summarised in Table 4.1 (see the full data in Appendix 4.1). The Log-Normal gave the poorest fit (largest AIC value) to the percentage germination across all temperatures for both populations, even though generally the AIC values were very close for all the six distributions (Table 4.1). The Log-logistic and Weibull function did not seem to be applicable for *C. edentula* from the two locations because of the unrealistic Ψ₀ values obtained from these two distributions. Take the TAS population for example, the Ψ₀(50) value produced by Log-logistic and Weibull was -238.4 and -151.2 MPa at 25 °C, respectively, while it was -43.78 MPa at 30 °C by Log-Normal (Appendix 4.1 & 4.2), which was far different from the water potential range used in this experiment. Therefore, these three distributions were eliminated for the next step of examination.
Table 4.1 Parameter estimates and goodness of fit of the hydrotime model for each distribution at 25 °C for C. edentula

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gumbel</th>
<th>Log-Normal</th>
<th>Log-Logistic</th>
<th>Logistic</th>
<th>Normal</th>
<th>Weibull</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \theta_H ) (MPa d)</td>
<td>2.14</td>
<td>3.05</td>
<td>2.43</td>
<td>2.43</td>
<td>2.41</td>
<td>2.42</td>
</tr>
<tr>
<td>( \Psi_{b50} ) (MPa)</td>
<td>0.07</td>
<td>-20.98</td>
<td>-945.70</td>
<td>0.09</td>
<td>0.12</td>
<td>-293.70</td>
</tr>
<tr>
<td>( \sigma_{\Psi_b} ) (MPa)</td>
<td>0.19</td>
<td>3.05</td>
<td>945.80</td>
<td>0.25</td>
<td>0.46</td>
<td>293.90</td>
</tr>
<tr>
<td>Shape (( \lambda ))</td>
<td>--</td>
<td>0.02</td>
<td>3752.90</td>
<td>--</td>
<td>--</td>
<td>1054.60</td>
</tr>
<tr>
<td>AIC</td>
<td>-3250.53</td>
<td>-3135.34</td>
<td>-3490.12</td>
<td>-3490.16</td>
<td>-3463.52</td>
<td>-3492.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gumbel</th>
<th>Log-Normal</th>
<th>Log-Logistic</th>
<th>Logistic</th>
<th>Normal</th>
<th>Weibull</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \theta_H ) (MPa d)</td>
<td>2.68</td>
<td>3.11</td>
<td>2.53</td>
<td>2.53</td>
<td>2.59</td>
<td>2.47</td>
</tr>
<tr>
<td>( \Psi_{b50} ) (MPa)</td>
<td>-0.09</td>
<td>-20.65</td>
<td>-359.50</td>
<td>0.04</td>
<td>0.06</td>
<td>-196.00</td>
</tr>
<tr>
<td>( \sigma_{\Psi_b} ) (MPa)</td>
<td>0.59</td>
<td>3.03</td>
<td>359.50</td>
<td>0.28</td>
<td>0.49</td>
<td>196.10</td>
</tr>
<tr>
<td>Shape (( \lambda ))</td>
<td>--</td>
<td>0.02</td>
<td>1302.80</td>
<td>--</td>
<td>--</td>
<td>634.60</td>
</tr>
<tr>
<td>AIC</td>
<td>-3720.57</td>
<td>-3622.1</td>
<td>-3815.43</td>
<td>-3815.55</td>
<td>-3782.46</td>
<td>-3816.05</td>
</tr>
</tbody>
</table>

*\( \sigma_{\Psi_b} \) = standard deviation for base water potential, the shape parameter (\( \lambda \)) is only applicable on Log-Normal, Log-Logistic and Weibull distribution.

To sort out the distribution that fits the data best among the remaining three distributions, the predicted germination by Gumbel, logistic and normal distribution was plotted against actual germination (Figure 4.1). The Normal (\( R^2 = 0.92 \)) and Logistic (\( R^2 = 0.92 \)) distribution showed similar fitness and both of them fitted better than the Gumbel distribution (\( R^2 = 0.65 \)) for TAS seeds. The Gumbel distribution (\( R^2 = 0.79 \)) also displayed the worst fit for QLD seeds while the logistic distribution (\( R^2 = 0.92 \)) showed slightly better fit than the Normal distribution (\( R^2 = 0.92 \)). Given that the normal distribution was most commonly used by other studies, it was more convenient to compare the estimated parameters by using the normal distribution.
4.4.2 Adjustment of $\Psi_b(50)$ and HTT model

Based on Equation 3.3 of the HTT model provided in Chapter 3, seed germination was plotted against a single water potential across all the temperature conditions. However, the HTT model clearly did not fit with the original germination data for TAS population (Figure 4.2), which was similar to the results of radish seeds in Chapter 3. The model did converge. The conspicuous poor fit at the predicted value of 0.12 and 0.22 was due to the overestimation of germination at 10°C. Germination hardly occurred at 10°C, while the fitted value resulted in high values of germination. Therefore, an adjustment was required for HTT modelling on TAS population.
Figure 4.2 The goodness of fit between predicted and actual germination value of TAS (a) and QLD (b) population, the lines are the 1:1 equation lines.

The reason for the poor fit of the HTT model could be the interaction of temperature and water potential; thus, the estimated base water potential of the hydrot ime model is plotted against temperature (Figure 4.3 & Figure 1.1). The HTT model assumes that the $\Psi_b(50)$ is the same across all the temperatures while it has also been reported to be sensitive to temperature change in some studies. Generally, in this study, the $\Psi_b(50)$ showed a decreasing trend as temperature increased for both populations, with a particularly sharp decrease found between 10 and 15 °C.
The Ψ_b(50) values at the three temperature conditions mentioned above were actually very close.

![Figure 4.3 Relationship between estimated Ψ_b(50) and temperature for TAS (blue) and QLD (red) C. edentula seeds. Ψ_b(50) value at 10⁰C showed the same value for both populations, and error bars at >10⁰C were too small to be shown, the line represented the moving average of Ψ_b(50) along temperatures of the two populations.](image)

As discussed in Chapter 3, an adjustment on Ψ_b(50) across different temperatures is required. Due to the moving average of Ψ_b(50) with temperature increasing, a “broken stick” method was used to model the structural change of gradient for C. edentula at first. This broken stick model was used for radish seeds in Chapter 3 and provided better fit than other modification models.

### Broken stick model
A broken stick response shape was tried to describe the change in Ψ_b(50):

Equation 4.1

\[
\begin{align*}
\psi_{b(50)} &= 0 & \text{if } T \leq T_b \text{ or } T \geq T_c \\
\psi_{b(50)} &= \psi_{b(50)} + k \ast (T_o - T) & \text{if } T > T_b \text{ and } T \leq T_o \\
\psi_{b(50)} &= \psi_{b(50)} + k \ast (T - T_o) & \text{if } T > T_o \text{ and } T < T_c
\end{align*}
\]

where Ψ_b(50)_adj is the temperature-adjusted Ψ_b(50), and k is the slope coefficient which shows how fast Ψ_b(50) changes with temperature. This equation also takes the thermo-inhibition effect into consideration, i.e., the Ψ_b(50) increases after temperature reaches the optimum (Rowse and Finch-Savage, 2003). It is noteworthy that Ψ_b(50) in the above equation is actually the median base water potential at optimal temperature because when T=T_o, Ψ_b(50)_adj=Ψ_b(50).

As described in Chapter 3, the Ψ_b(50)_adj value was used to replace the Ψ_b(50) in Equation 3.3, and a new HTT model (HTT model 1) was thus formed. The RMSE values of these two new models were 0.03 and 0.04 for TAS and QLD population, respectively, which showed an improvement compared to the original unadjusted model, especially for the TAS population (the lower value in RMSE represents the better fit).

### Heat sum restriction
In addition to the adjustment of Ψ_b(50) as stated above, restriction of heat sum accumulation (see Equation 3.8) above the optimal temperature was also incorporated to construct another HTT model (Alvarado and Bradford, 2002). However, this additional restriction on heat sum did not improve the performance of the model. The RMSE values for the two populations increased compared to HTT model 1 (i.e., 0.32 and 0.43 for TAS and QLD, respectively).
Even though there may be adjustments that can improve the model performance to a better extent, the HTT model was used at this stage. The estimates of seed parameters produced by the HTT model of the two locations are demonstrated as below (Table 4.2), and a t-test has been performed to determine whether the parameter difference between the two populations was significant or not (see the P values in Table 4.2). Test results indicated that the TAS population had a negative estimate of base temperature while the optimal temperature of TAS was higher than QLD. The value of $\theta_{HT}$ demonstrated that the hydrothermal time required for completing germination in TAS was shorter than in QLD. On the other hand, the difference in $\Psi_b(50)$ indicated that the QLD seeds were more sensitive to low water potential, although both of the $\Psi_b(50)$ values were positive. Given that the positive values of water potential are not realistic, and base temperatures for 50% germination often are reported to be positive values, those values displayed in Table 4.2 are merely artefacts of the limited number of treatments and the mathematical procedures (extrapolation) used to estimate the values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TAS</th>
<th>QLD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_b$ (°C)</td>
<td>-2.07</td>
<td>3.57</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>$T_o$ (°C)</td>
<td>32.94</td>
<td>29.45</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>$\Psi_b(50)$ (MPa)</td>
<td>0.022</td>
<td>0.001</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>$\theta_{HT}$ (MPa °d)</td>
<td>44.68</td>
<td>52.22</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>$\sigma_{\Psi_b}$ (MPa)</td>
<td>0.47</td>
<td>0.50</td>
<td>--</td>
</tr>
<tr>
<td>k</td>
<td>0.02</td>
<td>0.013</td>
<td>--</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.03166</td>
<td>0.03999</td>
<td>--</td>
</tr>
</tbody>
</table>

$^*$ $T_b$ = base temperature, $T_o$ = optimal temperature, $\theta_{HT}$ = hydrothermal time, $\sigma_{\Psi_b}$ = standard deviation for base water potential; --, no comparison done. The P value is for the difference between the same parameters from TAS and QLD respectively.

Germination time courses based on these parameters were plotted against water potential at each temperature. However, the germination fractions were very low and concentrated at similar values especially at 10 °C, which makes it hard to distinguish among different water potentials. Therefore, instead of using the full germination data, the mean of the germination was plotted against temperature and water potential (Figure 4.4 & Figure 4.5). Overall, both populations showed low germination, i.e., less than 50% at the end of the experiment across all temperatures and water potentials. The highest germination fraction of the both populations occurred at 35 °C. The germination at 10 °C was so low that it was hard to distinguish the effect of water potential for both populations.

The proportion of seeds germinated basically showed an increasing trend as temperature increased for the TAS population, although the germination fractions were very limited at 10 and 15 °C (Figure 4.4). The time required for seeds to reach the final germination was shortened as temperature increased. As for the effect of water potential, germination of seeds at a single temperature decreased as the water potential became lower while little germination happened when the water potential was lower than -0.4 MPa across all temperature conditions. The effect of reducing water potential on seed germination varied with the experimental temperature. The fitted germination time course generally aligned well with the original data except for a few
deviations. For instance, the predicted data underestimated the germination when seeds were in the conditions of 25°C & -0.2 MPa and 10°C & 0 MPa.

For QLD seeds, germination rarely occurred until the temperature reached 15 °C, and then accelerated as temperature went up to 30 °C. However, a decline in germination was also observed when the temperature was increased to 35 °C (Figure 4.5). Furthermore, more germination was observed at lower water potentials with the increasing of temperature (i.e. lower than 0 MPa). For example, the lowest water potential in which QLD seeds germinated was -0.6 MPa. The fitted germination generally agreed with the actual data except for the germination at 35 °C, where more seeds germinated than predicted at 0 MPa. Additionally, fitted data overestimated germination under the conditions of -0.2 and -0.4 MPa at 35 °C.

Overall, the QLD population germinated more readily than its TAS counterpart across almost all experimental conditions. The only exception occurred when QLD seeds were placed at 35 °C and the water potential was lower than 0 MPa: under such conditions, the germination decreased.

![Figure 4.4 Germination time course at different combination of temperature and water potential for C. edentula seeds of TAS. The curved lines were the fitted germination time course and the symbols are germination means.](image-url)
4.5 Discussion

The temperature responses of a species reflect the environments to which it has adapted (Trudgill and Perry, 1994, Trudgill et al., 2000). Even for the same species, germination behaviour was found to have changed with the differences in temperature environment (reviewed by Donohue et al., 2010). For *C. edentula*, seeds from TAS showed smaller $T_b$ than the QLD seeds (Table 4.2), which indicates that TAS seeds could be more tolerant to low temperatures. This result was consistent with the previous study which concluded that seeds from temperate areas tended to require cooler growing conditions than from tropical areas (Trudgill et al., 2000). On the other hand, the sub-tropical population (QLD) showed lower optimal temperature temperate population (TAS). Such traits of QLD seeds may provide a mechanism to ensure the germination does not happen in a hot environment in which seeds cannot survive.

Germination of both the two populations was markedly affected by water potential (Figure 4.4 & Figure 4.5). The results showed that TAS population had a higher $\Psi_b(50)$ than its QLD counterpart (Table 4.2), indicating that low $\Psi$ had stronger effects on TAS seeds than on QLD ones. Having seeds that can germinate at low water availability is often related to the adaptation to dry environment (Evans and Etherington, 1990, Bochet et al., 2007). Even though the rainfall amount is higher in QLD in average, the water content in the sand may be lower than in TAS. This is because that the high temperature in QLD can increase evaporation and sand does not hold a large amount of water.
The $\theta_{HR}$ values showed that QLD seeds required accumulation of a longer hydrothermal time than TAS ones. However, it is obvious that the seeds in QLD population could reach a higher germination fraction than in TAS population across all the conditions (Figure 4.4 & Figure 4.5). Seeds with higher $\Psi_b(50)$ and $T_b$ and lower $\theta_{HR}$ (i.e., TAS seeds in this study) tend to germinate rapidly when there is no water stress or temperature restriction but are strongly inhibited at low water potentials and temperatures. In conclusion, even though there was less requirement for accumulating hydrothermal time of TAS seeds, the higher thresholds of both temperature and water potential made it germinate slower with a smaller fraction than QLD seeds.

In the QLD population, the final germination under each water potential (except for 0 MPa) at 35 $^\circ$C was lower than that at 30 $^\circ$C, which is probably because it exceeded the estimated optimal temperature (i.e., 29.45 $^\circ$C) for seed germination and a thermoinhibition effect occurred (Bloomberg et al., 2009, Rowse and Finch-Savage, 2003). This is an example of interacting effect between $\Psi$ and temperature: $\Psi_b(50)$ increased correspondingly with temperature increasing above the optimal temperature. Such interacting effects could appear in different ways, which means $T_b$ can also change considerably with decreasing water potential in some species (Larsen et al., 2004, Kebrab and Murdoch, 1999). However, in this study, the effect of $\Psi$ on $T_b$ was not observed. Previous studies have suggested that using separate models at sub- and supra-optimal temperatures should be more accurate in modelling germination (Alvarado and Bradford, 2002, Rowse and Finch-Savage, 2003), which was proved to be useful in this study. Even for QLD population at 35 $^\circ$C where the thermoinhibition occurred, the model generally predicted the germination trend.

As mentioned in Chapter 3, the base temperature for cultivated Brassicaceae family species commonly ranges between 0 ~ 2.1 $^\circ$C; although those of C. edentula in QLD population was higher than the common range, it was still lower than the base temperature for some Brassicaceae weed species, for instance, 6.5 $^\circ$C for Hirschfeldia incana (Steinmaus and Prather, 2000) and 5 $^\circ$C for Rapistrum rugosum (Cousens et al., 1994). Base temperature is always a dubious value due to the extrapolation beyond the range of the data. As for the $\Psi_b$, previous study on the salinity tolerance of C. edentula has concluded that no inhibition was found in seeds even at 10,000 ppm (-0.78 MPa) salt condition (Boyd and Barbour, 1986). However, in this study, seeds from both TAS and QLD populations hardly germinated at <-0.6 MPa. A proportion of the seeds in this study possessed dormancy, which restricted the possible range of water potentials for seed germination (Batlla and Benech-Arnold, 2003, Alvarado and Bradford, 2005). In addition, it should be noted that the germination of QLD seeds under 0 MPa at 35 $^\circ$C did not seem to be inhibited even though the temperature exceeded the optimum. This may also be because that a proportion of the seeds were in dormant state and the high temperature helped relieving dormancy to some extent.

There are several possible explanations for the results that the maximum total germination of both seed lots did not exceed 50% and there was a negative estimate of $T_b$ for TAS population. Dormancy could be one of the reasons. Studies on barley seeds have shown that partial seed dormancy can result in the negative estimate of $T_b$ (Ellis et al., 1987). It is also possible that such results were due to the lack of viability of seeds. Even though a seed viability test should be prepared before the experiment, it was not conducted in this study due to time limitation. However, many of the seeds that did not germinate at the end of the germination experiment had been used in another growing comparison experiment. Such seeds turned out to have showed very good germination after removing the seed coats in the latter experiment. Although not all the remained seeds were used, the assumption of lack of viability should not be the main
cause of the low germination fraction. Previous study has showed *C. edentula* exhibits highest germination at alternating temperatures of 20 °C (14.5 hr) and 10 °C (9.5 hr) under dark conditions, and light inhibits germination rates but not total germination (reviewed by Maun et al., 1990). All the germination tests in this study were performed in constant temperature and alternating light condition (12 hr light/ 12 hr dark), which could be another reason that seeds did not show full germination.

It is documented in many studies that the Ψₜ(50) was found to decrease as after-ripening proceeds, i.e., relieving of dormancy state (Christensen et al., 1996, Meyer et al., 2000). Seeds in different dormancy states would show various tolerance to water stress, and thus lead to different germination percentages. Although dormancy was assumed to exist in the seed lots in this study, it was not easy to predict the proportion of seeds with dormancy due to the interactions among water potential, temperature and dormancy on seed germination (Corbineau and Côme, 2000, Larsen et al., 2004). The dormancy effect on *Cakile* seed germination is discussed in the following chapter. Given partial dormancy may persist in both populations, this study can only provide a rough germination comparison between the two *Cakile* populations; further study with non-dormant seeds is required to see the precise differences between the two populations.
Chapter 5  Dormancy in C. edentula and C. maritima

5.1  Introduction

Dormancy is known to exist in Cakile species (Davy et al., 2006). It was found in the upper fruit segments of C. maritima (Binet, 1960, Ignaciuk and Lee, 1980), and C. edentula also possesses dormancy to a certain degree (Payne, 1980). In Chapter 4 there was poor germination of extracted seeds of C. edentula, which is possibly due to the persistence of dormancy, rather than the lack of seed viability. In an unpublished field study of buried fruit segments by R. Cousens, most C. maritima germinated rapidly while there was no germination at all of C. edentula despite at least some seeds being viable. As introduced in Chapter 1, the replacement of C. edentula by C. maritima may be related to their difference in germination timing. Thus, differences in dormancy of the two species might be partially responsible for the replacement.

As discussed in Chapter 2, the dormancy of seeds can be imposed by the embryo itself, by the covering layers (fruit and/or seed coat) or by the interaction of both. Covering structures may a) reduce the rate of imbibition; b) restrict the movement of oxygen; c) mechanically restrict embryo or seedling growth; or d) contain chemical inhibitors (Bewley et al., 2012). Breakage in the covering structure is likely to improve gas exchange, chemical release or water movement. Cold stratification, another way of breaking dormancy, is thought to be related to a decrease of abscisic acid (ABA) content. Both of these two treatments are known to promote germination to a certain extent in many species (Baskin and Baskin, 2001).

In many Brassicaceae species, the fruit wall can impose a mechanical restriction on embryo growth and thus reduce germination (Lu et al., 2015). The seed coat rather than the fruit wall was shown by Young (2001) to be the primary cause of dormancy in Raphanus raphanistrum, even though the seed disperses in the dried fruit section. In Chapter 4 it was observed that even when C. edentula fruit coats (both upper and lower parts) were removed, seeds still did not germinate with a high percentage. Since no viability test was made at that time, the seed coat could have inhibited germination. Whether the seed coat imposes dormancy on C. maritima is unknown. In the field, seedlings can certainly be observed that have emerged through the ruptured abscission zone of the intact fruit of both species (R. Cousens, unpublished observation), showing that over time any inhibitory effects of the fruit or the seed coat can be overcome.

Prolonged cold stratification has been shown to relieve dormancy in C. maritima (Binet, 1960). As for C. edentula, cold stratification is required to overcome dormancy (Payne, 1980). In temperate North America, the majority of C. edentula was found to require cold stratification at <4 °C for 4-6 weeks to break their dormancy requirements (Maun, 2009). However, temperatures in Australia rarely reach as low as 4 °C and perhaps the conditions for cold stratification may never be met (or only in the south). Unfortunately, the two species from similar regions have seldom been studied under the same conditions.

In order to understand the replacement of C. edentula by C. maritima, experiments are needed for seeds of both species from similar regions and incubated alongside one another. The aim of this chapter is therefore to determine: 1) whether seed coat damage and cold stratification can break the dormancy of Australian population of the two Cakile species; and 2) whether the two species differ in their responses to these two treatments.
5.2 Materials and methods

Mature *C. edentula* and *C. maritima* fruits were collected from Tasmania, Australia in 2012. Since the two species have come into contact in many regions and may have already hybridised, they were collected from two different beaches occupied by phenotypes closely resembling only one species (See Figure 5.1). *C. edentula* was collected from Lighthouse Jetty Beach (43°27’S, 147°08’E), ahead of the advancing *C. maritima* invasion and therefore most likely pure. *C. maritima* was collected from a beach at Ulverstone (41°09’S, 146°10’E) where *C. edentula* phenotypes have not been seen for many generations. The collected seeds were then air-dried and stored in dry conditions under room temperature. Seeds were extracted from the fruits by hand, making sure not to damage the seed coat. A knife was used to slice the fruit down the lateral abscission layer. To investigate the effects of the seed coat, six treatments were applied in a factorial design of +/- cold stratification (CS+, CS-) and three levels of damage (D0, D1, D2). Seeds under cold stratification (CS+) were put on moistened filter paper in Petri dishes and then placed in a refrigerator for two weeks, while seeds in the control treatment remained at room temperature unimbibed. To create two different degrees of damage, seed coat was either given a small nick with a knife, sufficient to just reach the seed surface (D1), or half removed (D2). Treatment D0 was a control with no damage.

![Figure 5.1](image)

*Figure 5.1 The collection sites of *C. edentula* (Lighthouse Jetty Beach, blue dot) and *C. maritima* (Ulverstone, red dot) in Tasmania*

For each treatment, there were four replicates in which each contained 50 seeds. The seeds were put into 90mm Petri dishes lined with two Whatman No.1 filter papers; purified water was added to ensure sufficient water for germination. The Petri dishes were then sealed with Parafilm M® film and placed into an incubator at a constant temperature of 25 °C. The light
environment in the incubator cycled: 12 hr light and 12 hr dark regime. Water in the Petri dishes was checked every day and additional water was added when necessary. During the incubation, germinated seeds were removed once the radicle had exceeded 2mm. Germination count was conducted every day until there was no germination for seven successive days in all Petri dishes. The final germination fraction, expressed as a percentage, was calculated as:

\[ \text{Equation 5.1} \quad \text{Germination fraction} = \frac{\text{Final germination}}{50} \times 100\% \]

Germination rate (GR) was calculated using the equation:

\[ \text{Equation 5.2} \quad GR = \frac{\sum_{i=1}^{k} n_i}{\sum_{i=1}^{k} n_i t_i} \]

where \( t_i \) is the time from the start of the experiment to the \( i^{th} \) day of observation; \( n_i \) is the number of seeds that germinated on the \( i^{th} \) day (this is not the accumulated number, but the number of new germination since the previous day); \( k \) is the last day of observation (Ranal et al., 2009). GR (day\(^{-1}\)) can be referred to as the inverse of the time taken for germination; the faster GR is, the less time is required for germination. A factorial ANOVA was conducted through SAS. Based on diagnostic plots of the GR result, the residuals were not well scattered in the predicted versus observed values plot. Therefore, a transformation on the GR data was required prior to statistical analysis (see diagnostic plots for unlogged and logged data in Appendix 5.1 & 5.2).

5.3 Results

5.3.1 Germination fraction

The effect of damage on seed coats was significant (\( P < 0.05 \)) between the two Cakile species (Table 5.1). The interactive effects of damage and CS also showed significantly different effects on the two species (\( P < 0.001 \)). The only clear effect of cold stratification on germination fraction was shown on C. maritima when there was no seed coat damage (Table 5.2). Overall, the cold treatment had only a slight effect on seeds compared with seed coat damage or removal. C. edentula appeared to benefit more from seed coat removal than C. maritima (the germination fraction of C. edentula was 92% compared to 78% of C. maritima); however, the germination fraction was over 75% for both species.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage</td>
<td>2</td>
<td>2.51</td>
<td>1.26</td>
<td>148.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CS</td>
<td>1</td>
<td>0.03</td>
<td>0.03</td>
<td>3.20</td>
<td>0.0822</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>0.06</td>
<td>0.06</td>
<td>7.11</td>
<td>0.0114</td>
</tr>
<tr>
<td>Damage*CS</td>
<td>2</td>
<td>0.07</td>
<td>0.03</td>
<td>3.91</td>
<td>0.0292</td>
</tr>
<tr>
<td>Damage*Species</td>
<td>2</td>
<td>0.09</td>
<td>0.04</td>
<td>5.18</td>
<td>0.0106</td>
</tr>
<tr>
<td>CS*Species</td>
<td>1</td>
<td>0.02</td>
<td>0.02</td>
<td>2.56</td>
<td>0.1184</td>
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<tr>
<td>Damage<em>CS</em>Species</td>
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<td>0.18</td>
<td>0.09</td>
<td>10.48</td>
<td>0.0003</td>
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<tr>
<td>Residual</td>
<td>36</td>
<td>0.31</td>
<td>0.01</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>3.26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2 Final germination fraction (%) of C. edentula and C. maritima under 6 combinations of seed coat damage and cold stratification.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage</td>
<td>2</td>
<td>0.52</td>
<td>0.26</td>
<td>7.06</td>
</tr>
<tr>
<td>CS</td>
<td>1</td>
<td>4.03</td>
<td>4.03</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>0.72</td>
<td>0.72</td>
<td>19.66</td>
</tr>
<tr>
<td>Damage*CS</td>
<td>2</td>
<td>1.04</td>
<td>0.52</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Damage*Species</td>
<td>2</td>
<td>0.90</td>
<td>0.45</td>
<td>12.36</td>
</tr>
<tr>
<td>CS*Species</td>
<td>1</td>
<td>0.02</td>
<td>0.02</td>
<td>0.42</td>
</tr>
<tr>
<td>Damage<em>CS</em>Species</td>
<td>2</td>
<td>0.10</td>
<td>0.05</td>
<td>1.43</td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
<td>1.28</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>8.61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The pooled standard error of the estimates was 4.6%. The Pr > F is for the separate effect of damage or CS on a single species.

5.3.2 Germination rate

The combination of damage and CS treatment had significant (P < 0.0001) effect on the germination rate, while this effect did not differ with different species (Table 5.3). The effect of different damage levels varied significantly (P < 0.0001) with different species. Cold stratification significantly (P < 0.0001) improved germination rate of both species, especially when there was no seed coat damage (Table 5.4), i.e., the time needed to germinate was shortened more than half (C. edentula) or one third (C. maritima) compared to the control group (CS-D0). Damage on seed coat increased germination rate of C. edentula of both cold stratified and unstratified treatments but not for C. maritima seeds. Even though the results showed significant difference (P < 0.0001) among C. maritima seeds when treated with cold stratification, it could be due to the abnormal high value in D0 & CS+ (Table 5.4).

Table 5.3 ANOVA of germination rate (ln transformed).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage</td>
<td>2</td>
<td>0.52</td>
<td>0.26</td>
<td>7.06</td>
<td>0.0027</td>
</tr>
<tr>
<td>CS</td>
<td>1</td>
<td>4.03</td>
<td>4.03</td>
<td>110.24</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>0.72</td>
<td>0.72</td>
<td>19.66</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Damage*CS</td>
<td>2</td>
<td>1.04</td>
<td>0.52</td>
<td>14.17</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Damage*Species</td>
<td>2</td>
<td>0.90</td>
<td>0.45</td>
<td>12.36</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CS*Species</td>
<td>1</td>
<td>0.02</td>
<td>0.02</td>
<td>0.42</td>
<td>0.5228</td>
</tr>
<tr>
<td>Damage<em>CS</em>Species</td>
<td>2</td>
<td>0.10</td>
<td>0.05</td>
<td>1.43</td>
<td>0.2528</td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
<td>1.28</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>8.61</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C. maritima generally germinated faster than C. edentula at all damage levels, except for D2 (Table 5.4). This was also reflected in Table 5.3 where there were differences between the two species, depending on the levels of damage (P < 0.0001).

Table 5.4 Back-transformed germination rate (1/day) of C. edentula and C. maritima under 6 different treatments.

<table>
<thead>
<tr>
<th></th>
<th>C. edentula</th>
<th></th>
<th></th>
<th></th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0</td>
<td>D1</td>
<td>D2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS-</td>
<td>0.1449</td>
<td>0.1634</td>
<td>0.2461</td>
<td>0.0018</td>
<td></td>
</tr>
<tr>
<td>CS+</td>
<td>0.3237</td>
<td>0.2270</td>
<td>0.4004</td>
<td>0.0014</td>
<td></td>
</tr>
<tr>
<td>Pr &gt; F</td>
<td>&lt;0.0001</td>
<td>0.0203</td>
<td>0.0015</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C. maritima</th>
<th></th>
<th></th>
<th></th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0</td>
<td>D1</td>
<td>D2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS-</td>
<td>0.2100</td>
<td>0.2315</td>
<td>0.2313</td>
<td>0.7327</td>
<td></td>
</tr>
<tr>
<td>CS+</td>
<td>0.6527</td>
<td>0.3342</td>
<td>0.3297</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Pr &gt; F</td>
<td>&lt;0.0001</td>
<td>0.0183</td>
<td>0.0220</td>
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<td></td>
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</tbody>
</table>

*The pooled standard error of the estimates was 9.6%. The Pr > F is for the separate effect of damage or CS on a single species.

5.4 Discussion

It is obvious that even slight damage to the seed coat could result in a significant increase in germination fraction for both species. This effect was much greater than the effect of cold stratification in C. edentula (Table 5.2); the only significant effect of stratification seemed to be for C. maritima in the absence of damage. Of the two factors, seed coat damage was therefore the most effective stimulator of seed germination.

During the experiment, it was observed for both species in the control group (CS-D0) that seeds could imbibe water, since they swelled after placement on the moistened filter paper. Water-permeable seed covering structure is a general feature of species in the Brassicaceae family. For instance, Raphanus raphanistrum seeds with very thick fruit walls still imbibed (Young, 2001). Studies on the dormancy of six different Brassicaceae species also showed that seeds absorbed water within the fruits (Lu et al., 2015).

Restriction of oxygen from reaching the embryo can be caused by: 1) fixing the oxygen by oxidation in phenolic compounds in the embryo-covering layers; or 2) maintaining a continuous wet layer around the embryo, and thus forming a barrier for oxygen diffusion (Corbineau and Come, 1995). Dormancy caused by interference with gas exchange should be gradually relieved as the severity of damage increased, while in this experiment, there was a rapid increase in germination fraction for both species from no damage (D0) to small damage (D1). This result indicated that a small damage could cause a major change in germination, which did not comply with the dormancy caused by gas exchange inhibition.

A mechanical impediment to the radicle would mean that the growth potential of the embryo was not sufficient to break through the seed coat. Clearly, scarification or removal of the seed coat could decrease the force required for perforating the covering structure and thus break dormancy. Germination percentage thus increased as the damage level increased. Studies on R. raphanistrum showed that the seed coat mechanically delayed seed germination (Young, 2001). A mechanical inhibition by the covering structure was also found in other Brassicaceae species, e.g. Lachnoloma lehmannii (Mamut et al., 2014) and Isatis violascens (Zhou et al., 2015).
Consequently, it is possible that the dormancy of these two species was caused by a mechanical restriction and reduction in embryo growth potential.

Chemical inhibition of germination resulting from the covering structure include 1) prevention of inhibitors leaching from the embryos, and 2) germination inhibitors in the seed coat preventing embryo development (Baskin and Baskin, 2001). The greater surface area exposed would thus lead to a greater amount of leachable inhibitor. Therefore, the same gradual increase as oxygen inhibition is expected if germination is inhibited by chemical inhibition. Due to the rapid increase between D0 and D1, an inhibitor present in the seed coat was unlikely to be the reason for dormancy in the two Cakile species.

In summary, although based on only weak evidence, it is possible that the main cause of dormancy on the two Cakile species was due to mechanical restraint by the seed coat. This mechanism has also been found in many Brassicaceae species. For example, testa rupture released dormancy to a large extent for Sisymbrium officinale (L.) (Toorop, 2015). Additionally, the endosperm as well as testa restrained the embryo growth of Arabidopsis thaliana and Lepidium sativum (Müller et al., 2006, Bethke et al., 2007). Further studies on the mechanism of dormancy of Cakile are clearly required.

In this study, damaging of the seed coat significantly improved germination percentage of both species, and under each level (except for CS+D0), more C. edentula seeds germinated than C. maritima seeds. However, the difference between the two species at the same treatment could be due to the differences in seed viability, which unfortunately was not assessed. C. maritima, had a faster germination rate than C. edentula (Table 5.3, except at D2). Thus, within the same period, more C. maritima seeds could germinate and emerge than C. edentula. These two Cakile species both grow on coastal sand dunes, which is a very unstable environment. The freshly produced seeds may be washed away by storms before the seeds are ready to germinate. Therefore, during the short period of a relatively stable coastal environment, the species that germinates faster will be more likely to generate more seedlings. Compared to C. edentula, C. maritima benefits more from the same damage extent of the seed coat and then produces more seedlings in a short period. In the natural environment, alternating temperature as well as the hydration and dehydration cycles is able to affect seed integrity (Egley, 1995). This advantage could finally enable C. maritima to dominate the coastal area.

For both species, cold stratification did not significantly improve the percentage germination, except for C. maritima seeds in D0. On the other hand, germination rate at any damage level was improved by cold stratification (Table 5.2 & Table 5.4). This difference of germination rate and percentage may be because during the cold stratification period, seeds have begun to develop even when the temperature was only 5 °C. These seeds were therefore “primed” before the germination experiment began. This “priming” could make seeds germinate fast but is an artefact of the methods.

Clearly, damage and cold stratification are only two aspects of the control of timing and abundance of emergence in the real world and Petri dishes are a very artificial environment. Other environmental conditions or the traits of the seed itself could affect the germination requirements. For example, in the natural environment, seeds germinate at the same time could have varied ages, i.e., some seeds have been buried in the seed bank for a few years while other seeds are relatively fresh. Ageing is known to affect seed dormancy or emergence timing in many species (Rice and Dyer, 2001, Valleriani and Tielbörger, 2006); Additionally, many species with two segments showed differed germination requirements between the basal and distal part of
seeds (Baskin and Baskin, 2001); however, whether this occurs in *Cakile* species remains unknown. Besides, the ratio of hormone content in seeds also affects dormancy directly (Hilhorst and Karssen, 1992, Kucera et al., 2005); furthermore, studies on the embryo covering layers have also suggested that the ratio of Abscisic Acid (ABA) and Gibberellic acid (GA) is related to the testa or endosperm weakening (Müller et al., 2006, Debeaujon and Koornneef, 2000). In consequence, further studies are required to better understand the germination characteristics of the two *Cakile* species.
Chapter 6  Final Conclusions

The aim of this thesis was to work towards predicting germination of the two Cakile species in relation to climate. Specifically, the experiments examined the germination of C. edentula from two different climates, i.e. QLD and TAS. Factors governing germination, e.g. dormancy, was also investigated on the two Cakile species. The major findings were listed as follows:

- Percentage germination of C. edentula was affected by both temperature and water.
- The temperature and water requirements for germination of C. edentula from potential two climates varied significantly. Seeds from the sub-tropical area displayed higher Tb and lower Ψb for germination.
- Dormancy of the two Cakile species was perhaps caused by a mechanical impediment.
- Percentage germination as well as germination rate was significantly improved by breakage of the seed coat of the two Cakile species.

The findings of this thesis have implications for understanding the replacement of C. edentula by C. maritima. This chapter will discuss the questions arising from the results.

6.1 Changes in germination in different climates

In Chapter 1, it was introduced that differences in germination timing may be one of the reasons that contribute to the replacement of C. edentula by C. maritima. C. maritima usually germinates in the early spring when it is located at middle and northern latitudes; however, this species is also found to germinate in autumn and survive the winter in California, North America. At sub-tropical latitudes, it was suspected that Cakile demonstrated an annual growth cycle (reviewed by Rodman, 1974). Given the varied seed germination strategies evolved in different climates, it is necessary to determine the climate effects on germination before comparing the two Cakile species.

Temperature, water and light are all major factors that affect seed germination. In this thesis, a hydrothermal time model was used to show the combined effect of temperature and water on germination. Past studies using HTT model were mainly working on crop species. The application of this model on weed species is more complicated due to the more heterogeneous characteristics on weed species. In this thesis, adjustments on the model were made based on the interactive effect between temperature and water potential. Even though this adjustment improved the model performance compared to the original HTT model, there is still systematic lack of fit (e.g. Fig 4.3). Temperature and water potential were found to interact with each other by other researchers (Fyfield and Gregory, 1989, El Sharkawi and Springuel, 1977, Akeson et al., 1980), while the HTT model did not always give a very good fit with the actual germination. Kebreab and Murdoch (1999) suggested that the HTT model was invalid for their study, and they came up with a modified thermal time model which accounted for a higher rate of variation than the HTT model. This model developed by Kebreab and Murdoch (1999) could be fitted with the Cakile data in the future. In addition, the HTT model is perhaps inadequate and further work on a new model is required to predict the Cakile germination.

The results of Chapter 4 imply that the C. edentula seed populations from different climates (i.e. sub-tropical and temperate) did show differences in germination rate as well as germination requirements regarding to temperature and water. This indicates that adaptive changes or drift in germination occurred during the invasion of C. edentula. Temperature requirements and germination rate changed accordingly in some species when they were introduced into different climates (Kudoh et al., 2007, Beckmann et al., 2011). However, studies on comparing water
requirement of populations in different habitats are rare. There is only one study that works on the comparison of species in the same genus from different habitats, showing that the habitat had no effect on seed germination response to water stress (Hu et al., 2015), which is not consistent with the results of this thesis. This may be because seed populations in this study were from the same species instead of different species of the same genus.

Causes of the adaptive changes or drift in the environment where seed population developed and matured can be divided into two aspects, i.e. genetic and maternal effects. Normally, the genetic components of a seed are more from maternal than paternal plants since the seed coat is totally contributed by the maternal plant. Besides, about two thirds of the endosperm and half of the embryo is inherited from its maternal parent (Young, 2001). Environmental effects (e.g. temperature or water stress), both during seed development and maturation, are correlated to germination in a wide range of species (Fenner, 1991). To achieve a deeper understanding on the germination differences in different habitats, the extent to which variance is due to environmental factors, or genetic should be studied. Therefore, future work should focus on distinguishing between genetic effect and maternal effect.

6.2 Dormancy difference between Cakile species
The percentage germination did not exceed 50% for either of the C. edentula populations from the two locations. Dormancy was thus assumed to persist in seeds. Damage on seed coat and cold stratification was used to relieve dormancy in Chapter 5. The results showed that scarification on seed coat was more effective in improving germination fraction than cold stratification.

It was concluded that the dormancy in Cakile species was due to the lack of strength of the embryo to pierce the seed coat. This mechanistic dormancy was also found in other Brassicaceae species (Toorop, 2015). Cakile seeds in the natural environment are also covered by fruit coat, which is also known to impose dormancy on many Brassicaceae species (Lu et al., 2015). In this thesis, fruit coat was removed before experiment. Therefore, to gain a full understanding of the dormancy in Cakile species, studies on fruit coat is required in the future.

C. edentula was discovered to show higher germination fraction than C. maritima under most treatment conditions. This indicates that there was a wider environmental range for germination in C. edentula. Some researchers have found that width of germination niche was related to the ecological range of the species (Brändle et al., 2003, Grime et al., 1981). However, in some cases, a wider germination range did not correspond to a wider ecological range (Baruah et al., 2009, Berger et al., 2005). Therefore, there is no clear pattern of associations between germination conditions and ecological range. As shown in this thesis, the results did not indicate that C. edentula had wider ecological ranges than C. maritima.

6.3 Conclusions
This thesis has demonstrated that the germination behaviour differed between populations of the same species from different climates as well as between the two Cakile species. Together with the preliminary prediction of C. edentula germination, this thesis has made it closer to the objective of the prediction of Cakile germination.

This thesis also helped us further understand how C. edentula was replaced by C. maritima although not giving a direct answer. In fact, none of the researchers so far have completely answered this question. This study makes a step forward in the understanding the germination characteristics as well as the dormancy of Cakile species. In order to acquire a more
comprehensive understanding on germination, further studies of maternal effect on seed germination as well as comparison of germination performance between *C. edentula* and *C. maritima* are required. Such step by step studies will eventually help understand the replacement of *C. edentula* by *C. maritima* thoroughly, presenting valuable implications on the success of plant invasion and consequently how to tackle invasion-related ecological dilemmas.
References


Appendices

Appendix 3.1 SAS codes for hydrotime analysis of *Raphanus sativus*.
data radish;
input water temp rep time germination germin;
cards;
......
;
Run;

proc sort data=radish;
by temp;
run;

proc nlin data=both maxiter=3000;
parms
wb=-0.4
theta= 1 to 100
sigma= 0.2;
wbg = water-(theta/time);
model germin = cdf('normal',wbg,wb,sigma);
ods select ParameterEstimates;
ods output anova=anov_Normal;
ods output ParameterEstimates =parm_wb;
output out=pred p=fit;
by temp;
run;

Appendix 3.2 SAS codes for hydrothermal time analysis of Raphanus sativus.

title "This the HTT model with no adjusment for base water potential";
proc nlmixed data=radish maxiter=3000;
parms
Tb = 0
wb_50 = -0.5
Theta_HT= 4
sigma= 0.35
rmse = 0.04;
wbg = water-(Theta_HT/((temp-Tb)*time));
p =cdf('normal',wbg,wb_50,sigma);
model germin ~normal(p,rmse**2);
predict p out=model_withOut_Adjust;
run;

proc nlmixed data=radish maxiter=3000;
  parms Tb = 0
         wb_50 = -0.5
         Theta_HT= 4
         sigma= 0.35
         k = 0.03
         rmse = 0.04;
wbg = water-(Theta_HT/((temp-Tb)*time));
wb_50_T = wb_50 - k*(temp-Tb);
p =cdf('normal',wbg,wb_50_T,sigma);
model germin ~normal(p,rmse**2);
predict p out=model_with_Adjust;
run;

title "This the HTT model with adjustment for base water potential";

proc nlmixed data=radish maxiter=3000;
  parms Tb = 0
         Top = 25
         wb_50 = -0.5
         Theta_HT= 4
         sigma= 0.35
         k = 0.03
         rmse = 0.04;
wbg = water-(Theta_HT/((temp-Tb)*time));
if temp < Top then
  wb_50_T = wb_50 - k*(temp-Tb);
else if (temp > top and temp <= top)
  then wb_50_T = wb_50*((temp-tb)/(top-tb));
run;

proc nlmixed data=radish maxiter=3000;
 parms Tb = 0
         top=25
         Tc = 35
         wb_50 = -0.5
         Theta_HT= 4
         sigma= 0.35
         rmse = 0.04;
if temp <= tb then wb_50_T = 0;
else if (temp > tb and temp <= top) then wb_50_T= wb_50*((temp-tb)/(top-tb));
else if (temp >= top and temp < tc) then wb_50_T = wb_50*((tc-
temp)/(tc-top));
else wb_50_T = 0;
wbg = water-(Theta_HT/((temp-tb)*time)); /* as above*/
p =cdf('normal',wbg,wb_50_T,sigma);
model germin ~normal(p,rmse**2);
predict p out=model_Qi;
ods output ParameterEstimates=parm_broken;
run;

Appendix 4.1 Parameter estimates and goodness of fit for each distribution used in hydrotime for C. edentula from TAS (T means temperature)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gumbel</th>
<th>Log-Normal</th>
<th>Log-logistic</th>
<th>Logistic</th>
<th>Normal</th>
<th>Weibull</th>
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</thead>
<tbody>
<tr>
<td>T</td>
<td>Parameter</td>
<td>Gumbel</td>
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<td>Logistic</td>
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Appendix 4.2 Parameter estimates and goodness of fit for each distribution used in hydrotimes for *C. edentula* from QLD (T means temperature)

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Appendix 5.1 Diagnostic Panel of original germination rate data of *Cakile* spp.
Appendix 5.2 Diagnostic Panel of logged germination rate data of *Cakile* spp.
Observations: 47
Parameters: 12
Error DF: 35
MSE: 0.0366
R-Square: 0.8513
Adj R-Square: 0.8046