MODELLING THE GERMINATION OF *BUDDLEIA DAVIDII* UNDER CONSTANT CONDITIONS WITH THE HYDROTHERMAL TIME CONCEPT

A Thesis

Submitted in partial fulfilment

For the degree of

Master of Science

In

Forestry Science

By

Julien P.A. Jay

University of Canterbury

New Zealand

2006

ACKNOWLEDGMENTS

Studying for this Masters thesis taught me much more than just Forestry skills. Along the years I realized that carrying out experiments, pondering about hypothesis or interpretation of data gain in accuracy and interest as we speak to other people. Different views and opinions are always interesting in order to break the limits of our own understanding. That's why I can say with no doubt that this thesis couldn't have been done without the help of few people who enthusiastically shared with me their knowledge and skills.

First, I would like to thank my main supervisor, Dr Euan Mason (School of Forestry), who is so much committed and filled with passion that working with him was an honour as well as a pleasure. Modelling was new for me and Euan spent hours with me looking at my modelling data and responding to my questions about mathematics. From the beginning Euan has been encouraging, willing to help when I was confused, and his confidence and satisfaction are gifts which always pushed me further in this work. So, Euan, thank you for being a comprehensive man before being a judging supervisor.

I am indebted to Dr Michael Watt (Ensis) for helping me with statistics, mostly with SAS, using the data to find the most relevant transformations until significance has been – or has not been – found out. Michael was doing in a few hours what I would have done in a few weeks, and he also spent quite a bit of time reading this thesis in order to correct and comment it. I thank him for having shared with me his professionalism and for all his critics which enriched this work.

I wish to thank Dr Brian Richardson (Ensis) without whom I wouldn't be here today. The financial help received was salutary for me to keep on doing this study, and I also thank him very much for his acceptance of my philosophical doubts concerning my life at one stage of this Masters.

I was fortunate to receive the contribution of a few other people: Dr Darren Kriticos, Marc Bloomberg and his useful experimental advices, Stefan Gous who spent a while collecting *Buddleia* flowers, Dr Graeme Buchan, Marc Merlin Thomas, Tom Belton from the DOC in Hokitika and Ross Jackson from Timberlands West Coast for their help in finding *Buddleia* stands, and last but not least Dr Anne Miller from England who previously made a large and most interesting PhD thesis about

Buddleia davidii, which allowed me to have the clearest understanding about *Buddleia* at the start of my study.

Lots of experiments were involved in this thesis, therefore I would like to thank a lot Paul Fuller, Marco Casamassima, Nigel Pink, Nicole Lauren Manuera, David Clark, Vicky Wilton and Dave Conder for being really helpful with whatever laboratory device or computer help I needed. Their contribution in providing or fixing tools and the friendly talks we shared made this thesis easier and more appreciable. A special thanks to Jeanette Allen for all the administrative work she's doing with an appreciable efficiency.

This research would not have been possible without Canterprise who funded this Masters thesis. Complementary financial help has been provided by Brian Richardson and Euan Mason. I thank all of them for making possible my living in this beautiful country of New Zealand. Thanks to them my life gained an experience which will remains within my heart forever.

During these years of study I've been surrounded by great people, among who Javier Leon, who made me see through our metaphysical conversations that universal harmony might be just an utopia, Benoit Duchemin who has been here for me not to lose my French language and my memory of huge diners, Jean-Pierre Lasserre, who has been like a comprehensive father to me, Horacio Bown, with who it was always a pleasure to mix French and Chilean humour, Sina Hustedt and her hugs which were the best way to start a working day, Helge Dzierson and our laughs, and so many other pleasant people.

Finally, I'd like to send my thanks and my love to the people who made New Zealand a home for me, who made me feel surrounded by a family: my friends Ben and Melissa, and a beautiful Pixie: my love Debra. Thank you for making my heart feeling so warm. You will remain forever softly nestled within my soul.

To all of you,

Merci

TABLE OF CONTENTS

ABSTRACT

Buddleia davidii is a weed naturalized in New Zealand. It invades radiata pine plantations and causes major growth reduction and economic losses. Modelling its germination for predicting its occurrence will help foresters minimise its influence in forest plantations. Germination experiments have been carried out in laboratory to assess the influence of seed origin, defoliation, temperature and water stress on germination.

Defoliation treatments did not significantly affect germination. The pattern of germination for seeds from four different places within New Zealand revealed so little difference that there is no need to define different models according to the site considered. However this similarity in germination pattern is limited to New Zealand and cannot be generalised to other countries where germination appears to be significantly different.

The germination of *Buddleia davidii* seed appeared to be a function of hydrothermal time. The base, optimum and ceiling temperatures for *Buddleia* are respectively 9, 25 and from 30 to 35°C, and *Buddleia* seed germinate between 0 and approximately -6 bars.

In constant conditions, the predicted germination for *Buddleia davidii* with the thermal time model was limited to sub-optimal temperatures and the hydrotime and hydrothermal time models described well the germination pattern at any temperature and water potential. The modified hydrothermal time model proposed by Alvarado and Bradford (2002) most accurately predicted germination although it tended to overestimate the asymptotes.

Overall the hydrothermal time model allowed prediction of actual timing of germination with much accuracy. This threshold model can therefore be used for modelling the germination of *Buddleia davidii* subjected to constant temperature and water potential conditions.

LIST OF SYMBOLS

Symbol	Description	Units
a	Constant - sigmoid parameter	-
b	Constant - sigmoid parameter	-
c	Constant - sigmoid parameter	-
g	Percentage of germinated seeds	%
GR	Germination rate	Seeds $hour^{-1}$
K	Population constant at sub-optimal temperatures	-
K_s	Population constant at supra-optimal temperatures	-
k_T	Hydrothermal coefficient	-
PEG	Polyethylene glycol	-
r_{inf}	Rate at inflexion	Seeds $hour^{-1}$
T	Temperature	ºC
T_b	Base temperature	ºC
T_c	Ceiling temperature	ºC
t_g	Time taken for g percent of the final germination	Hours
t_{inf}	Time at inflexion	Hours
T_o	Optimum temperature	ºC
θ_2	Thermal time constant at supra-optimal T	ºC $hour^{-1}$
θ_H	Hydrotime constant	Bars $hour^{-1}$
θ_{HT}	Hydrothermal constant	Bars ºC $hour^{-1}$
$\theta_T(g)$	Thermal time constant for a given g	ºC $hour^{-1}$
σ	Standard deviation	-
Σ	Sum function	-
Ψ	Water potential	bars
$\Psi_b(g)$	Base water potential of percentage g	bars

INTRODUCTION

Origin of *Buddleia*

Buddleia davidii, from the family *Buddleiaceae*, is a large shrub native of Hupeh and Szechwan, hilly regions in Central and Western China. *Buddleia* has over ninety species distributed in Africa, Asia, and the Americas (Wallick *et al*, 2001). It was discovered and sent to Europe at the end of the 19th century by the French missionary and explorer Père David, and since then it has been largely imported through other continents and widely grown as an ornamental (Owen and Whiteway, 1981). As a large shrub with colourful fragrant flowers, and due to its capacity to attract a wide range of insects, like butterflies and bees, *Buddleia davidii* is commonly called butterfly bush or summer lilac.

After introduction as an ornamental plant, *Buddleia* escaped from gardens and spread into natural areas. Its seeds are very small (less than 1 millimetre wide) and can be carried away by the wind quite easily and over long distances. Also, its readiness to grow on most soil types facilitated *Buddleia* planting on open areas and it has become a pest in several places where it is cultivated, such as Great Britain, France, Australia, the United States and New Zealand (Binggeli 1998). In the wild, *Buddleia* quickly forms monotypic thickets that can out-compete and inhibit the growth of other seedlings.

Its development as a weed

Buddleia davidii reached New Zealand early in the last century, and nowadays it is abundantly naturalised in open or disturbed places in the North Island and northern South Island (Webb, Sykes and Garnock-Jones, 1988).

Observations of its development showed that after about fifteen years of colonisation by *Buddleia* on previously bare new surfaces, typical lowland forest floor vegetation appears and can finally replace *Buddleia* if it is left undisturbed. Even though *Buddleia* is capable of accelerating successions back to high forest on disturbed sites, it is likely to continue spreading over New Zealand and will persist indefinitely in many places like lowland catchments subject to frequent flooding and alluviation (Smale, 1990a). Forest managers first voiced concern about *Buddleia*'s weed status in 1977.

Context and economic interests in New Zealand

Buddleia must not be seen as a weed without uses, just to be eradicated from New Zealand. It has some positive effects when quickly stabilising new flood deposits, and it can be replaced by native trees and shrubs after several years. However, it is not a native plant nor closely related to any, and ousts those which would otherwise colonise fresh sediments (Smale, 1990b).

Buddleia is a problem weed in protected areas like Te Urewera National Park in the North Island, where it is widely naturalized. However the National Parks Act of 1980 specifies that parks are to be maintained in their natural state and that indigenous plants are to be preserved. In addition, *Buddleia* makes access along riverbeds difficult or impossible in places because of its low branching habit. As a result *Buddleia* is now the subject of several control projects by the Department of Conservation.

In plantation forests, *Buddleia* is a major competitor for light and can cause growth losses and even increased mortality of seedlings (Richardson *et al*., 1996). Controlling its spread and growth is therefore of economic interest. It is thought that *Buddleia* is a problem in the Central North Island in 10% of the 600,000 ha of plantations. Growth benefits from *Buddleia* control may be equivalent to 1-4 years of extra growth for *Pinus radiata* (Richardson and West, 1993) or an annual economic benefit of between approximately $0.5 million and $2.9 million (Richardson and Kimberley, 2002, unpub. data).

Controlling *Buddleia*

There are different ways to control the spread or at least the growth of *Buddleia davidii*. The first one is a physical control which implies cutting, burying or hand picking the plants, if possible at an early stage. However this technique requires a lot of work, time and money. In addition physical control is not always effective and cut plants may sprout. This has been demonstrated by plants buried by river sediment or knocked down by wind storms which continue to grow by sending up new shoots from the base (Smale 1990). Through increasing disturbance hand picking may increase population spread of *Buddleia davidii*.

Chemical control is also possible, and a specific herbicide mix has been defined by Veitch (1997). Although some phytoproducts are known to be efficient against *Buddleia davidii*, there is pressure to reduce herbicide use. One solution is biological

control, a more sustainable method that can provide long-term control of specific targeted weeds. Biocontrol in other species has a good success rate, especially when exotic plants are the target (Julien and Griffiths, 1998).

Study area

Biological control was the control method selected for study by Ensis Ltd. and the School of Forestry at the University of Canterbury. In order to have a global view of this project, studies in several complementary areas are being undertaken: *Cleopus japonicus*, a potential biological control agent for *Buddleia davidii*, is currently under investigation at Ensis Ltd. in Rotorua, while studies of germination and defoliation are undertaken at the University of Canterbury and Landcare Research at Lincoln.

This thesis is focused on the germination pattern of *Buddleia davidii* in order to create a model representing the effects of climatic variables on *Buddleia*'s germination. When integrated into a population dynamics model, these findings may help foresters or researchers determine the most effective way of managing *Buddleia*.

The model has then the double advantage of being a long-term method of control and providing deeper knowledge of *Buddleia*, and to some extent about plants related to it. This model, bringing to light the pattern of one of the most important life stages of the plant, is part of a bigger project aimed at reducing the use of agrichemicals while maintaining biosecurity and crop protection standards, economically viable production levels and international cost competitiveness.

Thesis structure

First, a literature review summarises current knowledge about *Buddleia davidii*, its sensitivity to climatic conditions and factors most influencing seed germination.

The second Chapter details a comparative survey, analyzing the germination rate for four different locations within the South and the North Island. This assesses the general applicability of the future model.

In Chapter 3, the influence of defoliation on germination is examined. This provides greater understanding of how potential biocontrol agents which remove leaves (such as *Cleopus japonicus*) might influence seed germination rates.

As described in Chapter 4, germination with *Buddleia* seeds were tested under constant temperatures for establishing the limiting temperatures affecting germination and to provide a preliminary test of an existing thermal time model.

Chapter 5 introduces the effect of water potential. Germination was tested under constant temperatures and water potentials, then the hydrotime and hydrothermal time models were used to describe the germination pattern of *Buddleia*.

Finally a conclusion summarizes the findings and highlights which areas still need to be studied to extend the validity of a *Buddleia* population dynamics model

CHAPTER 1 – LITERATURE REVIEW

Seed production, dispersal and viability

In many instances, *Buddleia davidii* starts flowering and fruiting one year after germination. The flowering season lasts approximately from summer to mid-autumn. Flowers are long panicles comprising hundreds of capsules, each one containing dozens of seeds. An adult plant, about 2 m high with a canopy of approximately $1m^2$, produced about 2-3 million seeds per year in one study (Miller, 1984).

As capsules do not form on inflorescences from which insects have been excluded, one can infer that *Buddleia* has no self-pollination mechanism. This absence of self-pollination could explain the heavy allocation of resources to insect attractants (flower colour, scent and nectar) in *Buddleia* species. By attracting a great proportion of nectar feeders, *Buddleia* might have an impact on the pollination and seed production of the less attractive species in the same area (Miller, 1984).

The seed capsules remain closed under wet conditions, but open in dry ones, enabling seeds to drop out if there is sufficient air movement. *Buddleia* seeds are small, winged and adapted for mobility, capable of remaining airborne for prolonged periods. Thus releasing seeds in the presence of wind is an excellent dispersal strategy. The highest density of seed rain is directly beneath the parent shrub. However, only a small proportion of the total seed output falls beneath the parent plant. Of the estimated three million seeds produced per plant, only 150 thousand have been recorded within 10 m of the shrub, with over 95% of the seed deposited on sites 10m or further from the parent (Miller, 1984).

In wet weather the capsules rapidly close, preventing the seeds from being shaken out in conditions where wind dispersal is not effective. Since it takes a lot longer to dry out and re-open than it does to shut, the actual shedding of seed may take place over a prolonged period.

Viability of *Buddleia* seed declines more rapidly under moist and warm conditions than in cool, dry environments. It is unlikely that there is a total loss of viability within one year, but probably few would persist after 2 years, and it is very likely that no seed would germinate after 3 years (Geddes, pers. Comm.; Miller, 1984).

Bearing in mind that seeds can be “primed” by a temporary set of temperature and humidity conditions, they may germinate in conditions unfavourable for survival,

such as mid-summer, when water evaporates rapidly from the soil, particularly in open areas or drained land.

***Buddleia*, a pioneer species**

The more a weed is tolerant to stress and to any disruption, the more it will efficiently spread and grow in a wide range of conditions. *Buddleia davidii* shows a remarkable tolerance to many environmental factors, which allows it to germinate and grow quickly under conditions unfavourable for many other species, even pioneer species. This tolerance is outlined below.

There is neither innate dormancy nor a light requirement for germination of *Buddleia* seeds. These will germinate equally well in acid and in alkaline environments (at least for pH ranging from 4 to 11), and low oxygen concentrations are not restrictive until they fall below 5% of the total atmosphere, which is unlikely to happen in field conditions. Soil oxygen rarely falls below 19%, although this may be lower in the atmosphere immediately around a seed, due to microfloral and root activity (Karssen, 1982).

Buddleia is not very susceptible to ammonium toxicity and can maintain leaf area irrespective of the nitrogen form taken up by the plant (Humphries and Guarino, 1987). These two features confer a competitive advantage on *Buddleia* over other species in soils with low nitrogen levels and those with high ammonium levels where for example fertilisers were applied. Also, *Buddleia* benefits from calcium rich soils, without being an obligate calcicole (Smale, 1990; Gillmann *et al*., 1988).

Buddleia can establish on almost every kind of soil. It mostly colonises fresh sediments exposed by slips or deposited by rivers and streams in flood. It germinates on sandy soils, gravel or loam, on a pile of rubble, a heap of limestone chippings, sea cliffs, woodlands, shingles, or sand dunes (Owen and Whiteway, 1981). However *Buddleia* tends to appear earlier on coarser gravely or stony substrates than on silty substrates (Smale, 1990a). Fine silty sediments are likely to be colonised by grasses and dicotyledonous herbs, which resist woody invasion.

As *Buddleia* plants grow and form closed canopies, the amount of exposed sediment progressively declines and there is a corresponding increase of the cover of leaf litter, mosses, ferns, sedges and seedlings of woody plants. In the long term, physical weathering and the growth of lichens and mosses over sites that previously supported germination make the surface unsuitable for further colonisation.

Main factors affecting *Buddleia* germination

Water stress

Lack of water is probably the main factor limiting germination of *Buddleia* seeds and the survival of young seedlings. Previous research shows a progressive decline in the total number of seeds germinating and the rate of germination with increasing water stress, between -2 and -6 bars. At -4 bars, germination was reduced by over 50% and at -6 bars or lower, germination does not occur. (Miller, 1984).

Water stress remains a main issue for *Buddleia* in the first weeks after germination. When *Buddleia* reaches an age of approximately four weeks after seedling emergence, it then becomes drought tolerant and can survive even in really dry conditions, resisting also an excess of water by flooding (Smale, 1990b). Bearing in mind that when *Buddleia* gains this high tolerance to disruptions it becomes hard to eradicate, one may consider that the most efficient way of limiting the spread of *Buddleia* is probably by intervening at the earliest stages of *Buddleia*'s life history.

Temperature

Buddleia seeds can germinate over a range of at least 14°C. Seeds at 9°C are too cold to be metabolically active. The rate is faster at higher temperatures, which reflects the thermal control of metabolic rates. The optimum temperature appears to be around 24°C.

Thresholds for germination differ depending on whether temperature is constant or fluctuating. Although seeds do not germinate lower than a constant 10°C, they will germinate under fluctuating day/night temperatures down to 4°C (Miller, 1984). The final percentage of germinated seeds was over 90% in both high and low temperature treatments, although the rate was predictably slower in the cooler treatment.

Depth of burial of seeds

Buddleia seeds are sensitive to burial, which can be achieved by human activity or natural phenomena like rain, compression by animals or the activity of invertebrates, for example. The rate of germination decreases with the depth of burial. Results obtained by Anne Miller (1984) suggest that no seed successfully emerges from depths below 2 cm.

Burial affects not only germination, it also induces light sensitivity. Even if seeds don't require any light to germinate, for those which have been buried below a certain depth, germination will then take place only in the light, which suggests for those buried seeds the induction of a secondary dormancy. This is due to the presence of a gaseous inhibitor in the soil atmosphere, which is not carbon dioxide, arising from the seeds themselves (Wesson and Wareing, 1968).

Competition

As *Buddleia* exhibits rapid early growth, it can overtop and suppress many other species, of which almost none can compete with this weed if no vegetation was present at the beginning. Only Tutu (*Coriaria arborea*) has been found in mixed stands with *Buddleia* in some places (Bellingham *et al.*, 2005), sometimes replacing it definitively. But from all observations, it can be said that from a bare ground subject to frequent disruptions, like a riverbed, it is likely that *Buddleia* will be the first plant to develop, and will remain the only one for a while.

However, oversowing of grasses and clovers can dramatically reduce the spread of *Buddleia* on cleared areas (Geddes, pers. comm.). No *Buddleia* seed can germinate under dense stands already installed, even under *Buddleia*. As no autotoxicity has been noticed for *Buddleia* species during Miller's experiments (1984), and as germination doesn't require any light, it is likely that competition for water is mainly responsible for preventing germination.

Ecotypic differentiation

The literature suggests that according to the climate under which plants grow, a differentiation can occur in plant phenology, leading to variations in germination pattern. McWilliams (1966) demonstrated that germination of *Amaranthus retroflexus* L. seeds from Northern populations was significantly higher than that from Southern populations at the same temperature. In addition, populations from drier areas tended to show greater dormancy. It has been concluded that variations in germination response were a result of ecological differentiation. Furthermore, New (1958) observed germination polymorphism in *Spercgula arvensis*. Comparative germination of *Pinus strobus* from northern and southern sources showed a quicker germination for seeds from the north (Mergen, 1963). However, no significant variation in germination response had been noticed in *Propisis* (McMillan, 1965).

Difference in seed weight from one location to another seems to be one of the main features affecting germination response, as well as seed dormancy (McWilliams *et al.*, 1967). Dormancy is affected by storage condition and length of time in storage, as well as by the environment where seeds developed (Amen 1963, Heslop-Harrison, 1964).

No data concerning possible ecotypic differentiation for *Buddleia* are available, thus combining this thesis' data with English ones will enable a comparison of germination responses at national and international scales.

Biocontrol and defoliation

Herbivores represent the greatest hope for biocontrol. Releasing host-specific, phytophageous insects or fungi has already shown its efficiency (Julien and Griffiths, 1998; McFadyen, 1998).

The expected efficiency of a biocontrol against *Buddleia* depends on the goal of management: either avoiding harmful competition in *Pinus radiata* plantations or suppressing *Buddleia* from protected areas like National Parks. In this latter instance, the prospects for successful biocontrol of *Buddleia* will probably require the release of a second biocontrol agent, the stem borer *Mecyslobus erro* which is currently under investigation. Most weed biocontrol projects require more than one agent in order to improve their chances of success (Brockerhoff *et al*., 1999).

For a plant which is mass flowering as *Buddleia* does, most of the seeds will be genetically similar. The long-term consequences of this small gene pool will be similar to those which apply to clonally reproducing individuals, that is, in the event of an attack by insects or disease, there is little variation in the range of plant defences to be overcome, and the outcome of a successful attack may be catastrophic for the population.

Buddleia is subjected to grazing by many herbivores where those are present. *Buddleia* leaves are palatable to slugs, snails and other polyphagous insects such as the aphid *Myzus persicae*, *Tetranychus urticae*, the red spider mite, *Trialeuroides vaporariorum*, the glass-house whitefly, and to other oliphagous species. The shrub is a food resource for the larvae of *Verbascum verbasci*, the mullein moth. Other feeders found on *Buddleia* were the weevil *Gymnaetron tetrum*, *Amaromyza verbasci* which is a dipteran leaf miner and *Campyloma verbasci*, a leaf bug (Owen and Whiteway, 1981).

Brockerhoff *et al*. (1999) assessed the impact of the coleopteran *Cleopus japonicus* as a defoliator of *Buddleia*. Grazing resulted in a significant reduction in main stem height, total stem and branch length, and dry weight of roots and shoots. Thus it had been concluded that following permission to release and successful establishment, *C. japonicus* would probably suppress *Buddleia* growth.

However this assumption has to be taken with caution. Recent experiments demonstrated that after severe defoliation, *Buddleia* showed a higher rate of leaf area growth than the undefoliated control. Although leaf area exhibited overcompensation, defoliation reduced seed production per plant by 24%, relative to the control (Thomas, pers. Comm.). Germination of seeds from defoliated and control plants are compared in this thesis.

Hydrothermal time model

The physiological process of germination depends on environmental factors such as temperature, water potential, light and nutrients. Light is not necessary for germination of *Buddleia* seeds and nutrients are not considered in this thesis. Therefore the germination model will take into account temperature (T) and water potential (Ψ).

Among the environmental factors affecting seed germination, temperature is a primary regulator. It affects both the capacity for germination by regulating dormancy and the rate or speed of germination in non-dormant seeds (Bradford, 2002).

The description of germination according to temperature is dependent on three cardinal temperatures: minimum, optimum and maximum. T_b or base temperature is the lower threshold under which no germination occurs. T_o or optimum temperature corresponds to the fastest rate of germination, independent of total germination. T_c or ceiling temperature is the upper threshold above which germination is prevented. Different equations are required for the temperature and water potential effects below or above the optimum temperature.

At sub-optimal temperatures, heat units are used, which allows inferring a thermal time for germination (Bierhuizen and Wagenvoort, 1974). This method uses additional heat over the base temperature multiplied by the time to a given percentage of germination, which leads to $\theta_T(g)$, a thermal time constant for this seed population and for this percentage.

This constant is described by the following equation,

$$\theta_T(g) = (T - T_b)t_g \text{ or } GR_g = \frac{1}{t_g} = \frac{(T - T_b)}{\theta_T(g)} \quad \text{(Eqn. 1.1)}$$

where $\theta_T(g)$ is the thermal time constant for a given g, T is the temperature of the germination substrate, T_b is the base temperature, t_g is the time taken for g percent of the final germination and GR is the germination rate.

The same approach is used for expressing the timing of germination according to water supply above a threshold allowing germination. Considering the difference of timing of germination for particular seed fractions, the germination rate must be expressed according to a particular percentage (g). Gummerson (1986) defined the hydrothermal time constant as,

$$\theta_H = (\psi - \psi_b(g))t_g \text{ or } GR_g = \frac{1}{t_g} = \frac{(\psi - \psi_b(g))}{\theta_H} \quad \text{(Eqn. 1.2)}$$

where Ψ is the seed water potential, $\Psi_b(g)$ is the base allowing germination of percentage g and θ_H is the hydrothermal time constant

At supra-optimal temperatures, GR_g decreases linearly with increasing temperature (Covell *et al.*, 1986). Temperatures over T_o also reduce the total germination of the seed population, as a consequence of dormancy. Thus seeds of the same lot show differential reactions to an increased T, confirming the statement that different fractions of the seed lot are sensitive to different T_c values.

Ellis and Butcher (1988) modelled this differential response to temperature by the following equations,

$$\theta_2 = (T_c(g) - T)t_g \text{ or } GR_g = \frac{1}{t_g} = \frac{(T_c(g) - T)}{\theta_2} \quad \text{(Eqn. 1.3)}$$

where θ_2 is the thermal time constant at supra-optimal T and T_c is the ceiling temperature.

The previous equation doesn't take in account the fact that above T_o there appears a decrease in GR and percentages due to an increase in the $\Psi_b(g)$ thresholds for germination.

Taking in account the fact that $\Psi_b(g)$ shifts positively (becomes more positive) as T increases, Alvarado and Bradford (2002) defined the relationship between $\Psi_b(g)$ and T at supra optimal temperatures,

$$\psi_b(g)_{T>T_o} = \psi_b(g)T_o + k_T(T - T_o) \quad \text{(Eqn. 1.4)}$$

where k_T is the slope of the relationship between $\Psi_b(g)$ and T.

Finally, Alvarado and Bradford (2002) combined the previous equations to obtain a modified hydrothermal time model that can describe both germination timing and percentage at any T and Ψ within the range at which germination occurs,

$$\theta_{HT} = (\psi - \psi_b(g) - [k_T(T - T_o)])(T - T_b)t_g \quad \text{(Eqn. 1.5)}$$

where $[k_T(T\text{-}T_o)]$ applies when $T > T_0$. At supra optimal T, $\Psi_b(g) = \Psi_b(g)_{To}$ and $T\text{-}\ T_b = T_o\text{-}\ T_b$

This model has been validated at sub-optimal temperatures by Gummerson (1986), Dahal and Bradford (1994) and at supra-optimal temperature by Alvarado and Bradford (2002) and by Rowse and Finch-Savage (2003).

Research Objectives

The objective of this research is to model the germination of *Buddleia davidii* subjected to a range of constant temperatures and water potentials. This is accomplished by testing germination in a laboratory and by analysing the fit and accuracy of different population threshold models. More specifically, the possibility to model *Buddleia* germination using the hydrothermal time concept is investigated and parameters specific to this species are defined to allow predictions of timing of germination.

The study also analyses the influence of seed origin and impact of defoliation on germination in order to determine whether adjustments of the hydrothermal time model are required in field conditions.

CHAPTER 2 – INFLUENCE OF LOCATION ON *BUDDLEIA DAVIDII* ALLOMETRICS AND GERMINATION

Introduction

Within the same species, many plants situated in different environmental conditions may differ in morphological or life-history traits, a pattern consistent with environmentally induced ecotypic differentiation (Chapin and Chapin, 1981; Grant and Wilken, 1988; Macdonald and Chinnappa, 1989). Ecotypic differentiation is due to differences in abiotic or biotic effects of the environments as well as the selection effects that the climate induces in different life-history stages of the plants (Volis, 2002). Physiological, morphological or resource allocation traits are characters varying according to the phenotypic plasticity in plants (Taylor and Aarssen, 1988; Lortie and Aarssen, 1996). In addition, temporal and spacial isolation of flowering contribute to differentiation between adjacent populations and even adjacent plants (Levin and Kester, 1968). Over a longer period, evolutionary divergences can occur within a species, influenced mainly by natural selection and genetic drift (MacDonald and Chinnappa, 1989).

However, these phenotypic differences from a plant to another may be more related to the climate under which they grow than to their genotype (Gerrit and Platenkamp, 1991). Most precisely, the state of the seeds depends on environmental conditions (Stanton, 1984; Lalonde and Roitberg, 1989) and influences the fitness of the adult plants (Choe *et al*., 1988). Thus ecotypic variation is also dependant on the reproductive features.

Increasing productivity in respect to rainfall favours high reproductive biomass and large seeds, a large fraction of germinating seeds and a good vigour of seedlings (Volis, 2002).

Another adaptive response of plants to their environment affects germination. Delayed germination through persistence in the seed bank is considered as an adaptive strategy against the effects of temporally varying environments (Cohen, 1966; Venable and Lawlor, 1980; Brown and Venable, 1986). In some studies, ecotypes from high rainfall regions had a shorter dormancy period than ecotypes from drier regions (Hacker, 1984; Hacker and Ratcliff, 1989).

The objectives of this chapter are: (1) to compare germination rate and total germination of seeds originated from several places within New Zealand, and (2) to assess the validity of the germination model nationally and internationally.

Method

Experimental design

Seed samples were collected from four climatically differing regions within New Zealand: Rotorua in the North Island, and Hokitika, Queenstown and Akaroa in the South Island (see maps in annexe). *Buddleia* flowers were collected in March 2005, after flowers faded and turned into capsules within which seeds are produced.

For each of the selected sites, 15 plants covering the range in plant sizes were selected and measured for height, width and basal diameter at 10 cm above the ground. Three flowers (small, average and large) from each plant were randomly collected, their length and width were measured as well as the number of capsules on the inflorescence.

Germination was tested in a laboratory, in the dark and under optimum temperature (25°C). For each of the 15 plants of each four sites, 50 seeds were placed in 8.5 cm plastic Petri dishes, arranged regularly on three sheets of filter paper. 4ml of distilled water was poured in Petri dishes and the latter were monitored daily for water loss and refilled with distilled water as necessary to provide full water supply.

As the first hours of germination include the faster rate of germination, seeds were checked, counted and removed at increasing intervals as following: 3h, 3h30, 4h, 4h30, 6h, 8h, and every next 8 hours.

The minimum criterion of germination – and thus the removal from the Petri dish – was when the radicle appeared to be 1 mm long.

Data analysis

Cumulative germination in each Petri dish, y, was modelled as a function of the number of hours, t, after being placed in the dish by a Weibull function as,

$$y = M(e^{-(\frac{t}{b})^c}) \qquad \text{(Eqn. 3.1)}$$

where y is the cumulative germination at time t, M is the number of seeds tested, that is 50 for this experiment, b is the rate of increase and c is a shape parameter. These parameters were determined in SAS, using a non linear mixed model with code written by Dr Euan Mason.

Results

Overall, the pattern of germination shows little variation from a place to another within New Zealand. The same percentage of germination, which is around 98%, is achieved at all places over approximately the same period of time. Starting times at the four locations are approximately 52 hours after imbibition and cover a range of four hours, which at the scale of a field is considered as pretty much simultaneous. However it was noted that seeds from Hokitika and Rotorua were the first to germinate, and these two locations have the highest annual rainfall. Total germination differed slightly from a place to another, with a difference covering a range of 1.5%.

The germination frequency was plotted against time for each location (Figure 2.1). The skew is obvious, demonstrating that the time of germination of *Buddleia* seed population is not normally distributed, justifying the use of the Weibull transformation.

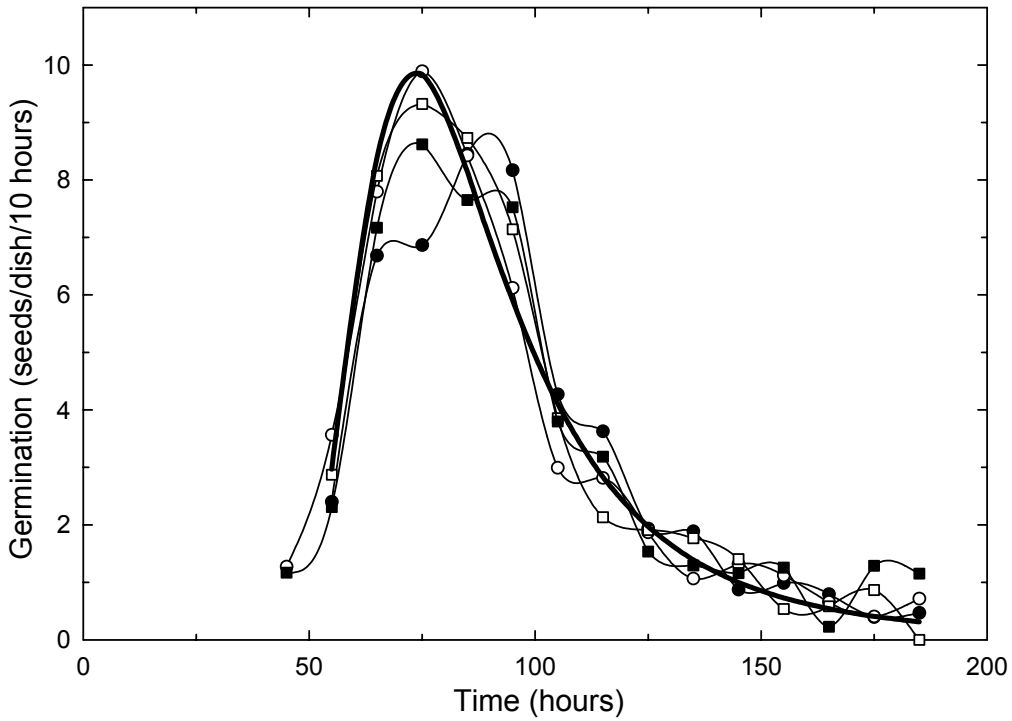


Figure 2.1: Mean germination frequency within ranges of 10 hours for *Buddleia* seeds from Akaroa (●), Hokitika (○), Queenstown (□) and Rotorua (■). The bold line represents the modelled germination using a Weibull model.

Although germination was statistically different from a location to another, the difference is only counted in hours, which at the scale of a plantation remains negligible.

Discussion

While time of germination of a seed population is often considered as normally distributed, the residuals obtained by a probit transformation of the cumulative germination of *Buddleia* suggested a skew representing the delay of germination of late seeds. For this reason the Weibull transformation was adopted and tended to fit with much accuracy the germination frequency of *Buddleia davidii*. The Weibull transformation has been widely and successfully used for modelling germination (Brown, 1987; Dumur *et al*., 1990; Dias, 2001).

The germination pattern of seeds from Akaroa, slower than the three other ones, is mainly responsible for the significant differences between locations. At this place, the stands were visibly smaller than at other places, and particularly, *Buddleia* flowers were much smaller than usual, although this feature did not affect seed size.

After comparison of mean annual rainfall over the 40 past years with the starting time of germination, it appears that the more it rains over a region, the faster germination is initiated, or the easier seed dormancy is broken. This observation can be related with Hacker (1984) and Hacker and Ratcliff's (1989) studies which demonstrates a negative correlation between the length of seed dormancy and the amount of rain over a region, and with Hacker (1984) and McWilliams *et al.* (1967) who found that within a same species, plants from high rainfall regions had shorter dormancy compared to plants from low rainfall regions.

Miller (1984) carried out germination experiments with *Buddleia davidii* seeds in Oxford, England. Although the protocols were the same, there is an important discrepancy between both countries regarding the duration of germination time course as well as the starting time of germination: in optimum conditions, germination starts around 50 hours for New Zealand seeds and 150 hours for English seeds, and while germination lasts approximately six days in New Zealand, it takes more than ten days in England. And this difference is even bigger when comparing germination subjected to water stress.

This difference cannot only be accounted by the climate, as both England and New Zealand have a temperate climate. It is possible that humidity during storage may have caused this difference. Another reason for such a discrepancy in germination pattern is likely to originate from evolutionary divergence of populations influenced by natural selection and genetic drift, as noted by McDonald and Chipanna (1989) and Wood and Degabriele (1985). This implies that the germination model for

Buddleia seeds from New Zealand cannot be used as a reference for *Buddleia* plants in other countries.

In addition, germination patterns from one location to another within New Zealand vary very little, the same percentage of germination being reached in a range of a few hours. At the scale of a forest plantation, this difference is negligible, thus the same model can be used for modelling germination of *Buddleia davidii* at all locations within New Zealand, albeit with a slight bias.

Therefore the use of threshold models applied to *Buddleia davidii* with the parameters defined in this study is limited to both the North and South Islands of New Zealand.

CHAPTER 3 – THE EFFECT OF DEFOLIATION ON GERMINATION

Introduction

Plants subjected to stress, like grazing or defoliation, often show a reduction in seed production and/or seed size (Lee, 1988, Obeso, 1993). This has been confirmed for *Buddleia davidii* by Thomas (unpublished data) who demonstrated that flower length and numbers declined after defoliation treatment and that seed production decreased by 24% due to treatment effects. In addition, the seed weight per flower declined significantly in flowers on main shoots.

In the literature, the effect of seed size and/or seed weight is subjected to controversial findings, and the influence of the resource status of the parent plant on seed characteristics and germination is still unclear. Some studies state that variance in seed size is associated with traits such as probability and timing of germination (Shaal, 1980 ;Winn, 1988 ; Biere, 1991 ; Platemkamp and Shaw, 1993). The timing of germination for *Lobelia inflata* was also dependent on seed size for Simons and Johnston (2000), and a positive relationship between seed size and germination rate and frequency has been found by Mogie *et al*. (1990), Bell *et al*. (1991), Quesada *et al*. (1995). In contrast, Eriksson (1999), found that the probability and timing of germination was not influenced by seed size. In addition, Apollo *et al*. (1998) demonstrated that grazing history of Indian ricegrass had little effect on seed germination**,** and Koptur *et al*. (1996) showed that seeds produced by defoliated and control plants have similar percentages of germination and time to germination. Defoliation did not significantly affect the number of germinated pollen grains of *Alstroemeria aurea* (amancay), nor did it affect germination in the field (Aizen and Raffaele, 1997).

The objective of this chapter is to assess the impact of defoliation on germination of *Buddleia* seeds taken from both main and side shoots.

Method

Experimental design

Buddleia seeds used for this experiment were obtained from plants grown in a defoliation experiment at Landcare Research, Lincoln. Eighty seedlings were planted into 20 blocks with four plants per block. Within each block two plants were randomly selected for defoliation, while the remaining two were allocated to the

undefoliated control. On the defoliated plants 60% of new foliage was removed every month from January to April 2005. After flowering commenced, mesh bags were placed over the main and side flowers of average length on each plant to prevent seed loss. These flowers were then removed in August, dried at ambient temperatures, and seeds were collected by shaking the open capsules along the inflorescences.

From each of the 160 flowers, 50 seeds were placed in as many plastic Petri dishes, on top of three filter papers. 5 ml of distilled water was poured in each Petri dish. The Petri dishes were then placed into a growth cabinet set at 25°C (optimum temperature), in the dark, where they were organized into blocks corresponding to the blocks used in the field experiment. During the experiment, water was added as required to provide a full water supply. Seeds were counted and removed on the same basis than for the previous experiment.

Data analysis

The same analysis than in the previous Chapter was undertaken. Cumulative germination in each Petri dish, y, was modelled as a function of the number of hours, t, after being placed in the dish by a Weibull function as,

$$y = M(e^{-(\frac{t}{b})^c}) \qquad \text{(Eqn. 3.1)}$$

where y is the cumulative germination at time t, M is the number of seeds tested, that is 50 for this experiment, b is the rate of increase and c is a shape parameter.

These parameters were determined in SAS, using a non linear mixed model with Dr Mason's code. The plant was set as a random effect and repeated measures by plants were carried out. The side and main shoots as well as the defoliation treatment were set as fixed effects.

Results

Treatment and shoot type had very little influence on either the rate or timing of germination. The germination frequency was plotted against time for each location (Figure 3.1). The interaction between defoliation and position was statistically significant, however the difference is so little that there is no practical significance in regard of the model.

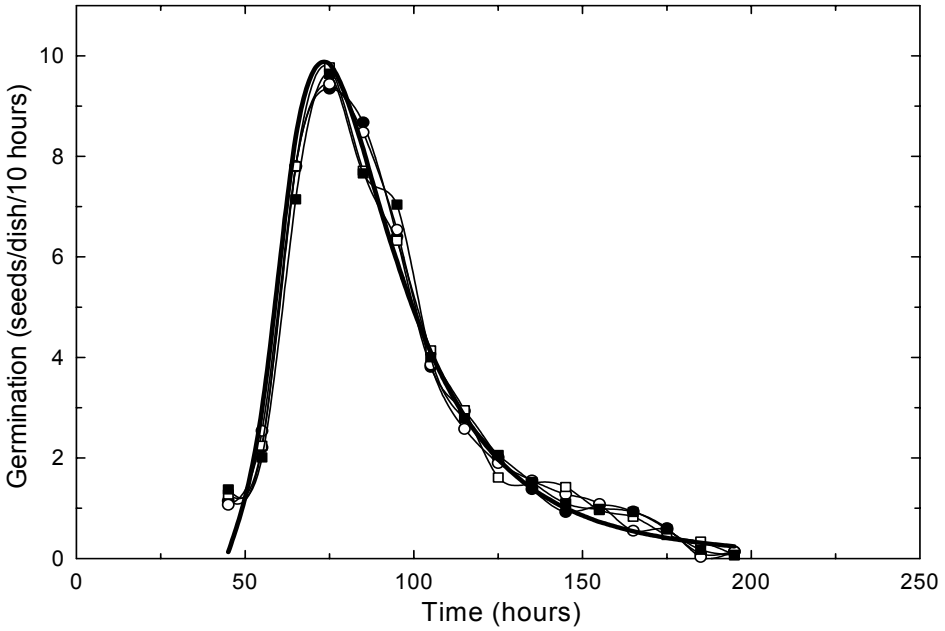


Figure 3.1: Mean germination frequency within ranges of 10 hours for *Buddleia* seeds from main shoot (●) and side shoot (○) of defoliated plants; and seeds from main shoot (□) and side shoot (■) of undefoliated plants. The bold line represents the modelled germination using a Weibull model.

Discussion

Defoliation did not significantly influence the germination of *Buddleia davidii*. Reaching conclusions about the influence of seed weight on germination is difficult because of the way seeds were randomly chosen. However, considering that seeds weight was significantly lower for defoliated plants, and as only largest seeds were selected for germination tests, it is very likely that the bias was the same across treatments, therefore accounting for a slight difference in seed weight.

Either way, these data suggest that the same germination model can be used for defoliated and undefoliated plants.

Although the Weibull function is appropriate for modelling the germination of Buddleia davidii, probit transformations will be used in the next chapters. Firstly, the hydrothermal time has been developed with the use of probits, therefore this transformation is the most relevant to compare results with previous studies; secondly, probit matches to the normality of the distribution of base water potentials; and lastly, applying the Weibull function to the concept of hydrothermal time model has not yet been done, and considering that such a function does not use standard deviations of its parameters, it would be a challenge to find a way to account for the variation in parameters such as the thermal time constants, the base water potential or the ceiling temperature which vary according to the fraction of seed.

CHAPTER 4 – THERMAL TIME MODEL FOR GERMINATION UNDER CONSTANT TEMPERATURES

Introduction

Among the environmental factors affecting seed germination, temperature is a primary regulator. It affects the capacity for germination by regulating dormancy, it influences seed deterioration through ageing and modifies the rate and total germination.

Different definitions of germination can be found, but it is generally accepted that this process starts by the uptake of water by the seeds, or imbibition, and finishes at the emergence of the radicle (Roberts, 1988). Labouriau (1970) was one of the first to demonstrate a positive linear relationship between germination rate and constant temperatures at sub-optimal temperatures. In addition, a negative linear relationship was shown to occur at supra-optimal temperatures. These results were confirmed by Bierhuizen and Wagenvoort (1974) in 31 vegetable species, by Washitani and Saeki (1986) in *Pinus densiflora* Sieb., by Garcia-Huidobro *et al*. (1982) in *Pennisetum typhoides* (pearl millet), by Ellis *et al*. (1986) in several grain legumes and by Alvarado and Bradford (2002) in potato (*Solanum tuberosum*) seed populations.

Germination of a seed population depends on three cardinal temperatures: the base temperature, or threshold below which no germination is possible, the optimum temperature at which germination is fastest, and ceiling temperatures above which germination is prevented.

At sub-optimal temperatures, germination can be described as a combination of temperature and time expressed in thermal time or heat units (Bierhuizen and Wagenvoort, 1974). This approach predicted successfully phenological stages for several crop and weed species (Alm *et al*., 1991). The base temperature is usually the same for all seeds in a population (Garcia-Huidobro *et al.*, 1982; Dahal *et al*., 1990; Kreabab and Murdoch, 2000), but exceptions had been noted in some studies (Fyfield and Gregory, 1989; Grundy *et al.*, 2000). A linear relationship between germination rate and temperature has often been described (Labouriau, 1970; Garcia-Huidobro *et al.*, 1982; Finch-Savage *et al.*, 1998; Alvarado and Bradford, 2002).

At supra-optimal temperatures ($T_o<T<T_c$), the heat sum used for germination purposes does not increase as temperature increases, and $\theta_T(g)$ remains constant in this range. In addition, a linear decline in germination rate related with an increase in

temperature has been observed (Labouriau, 1970, Covell *et al.*, 1986, Alvarado and Bradford, 2002) .

A thermal time model describing germination time courses over a range of temperatures have been developed (Garcia-Huidobro *et al.*, 1982; Ellis and Butcher, 1988) and proved its efficiency to predict germination under constant temperatures conditions. To assess the accuracy of the thermal time model, germination time courses at different temperatures can be linearised and compared to the model by calculation of the coefficient of determination (Bradford, 1990).

The objectives of this chapter are: (1) to assess the cardinal temperatures for *Buddleia* seed germination and, (2) test the hypothesis that the thermal time model fits the germination pattern of *Buddleia* at sub-optimal and supra-optimal temperatures.

Method

Experimental design

The seeds tested originated from *Buddleia davidii* plants grown in Rotorua. 50 seeds placed on top of three filter papers were placed into 8.5 cm plastic Petri dishes. Full water supply was provided and later on, distilled water was added as necessary. Plastic Petri dishes were placed in the dark into incubators respectively set to 13, 17, 21, 24, 25, 26, 29, 33 and 37°C. Each treatment was repeated three times.

Seeds were counted according to the following intervals, starting from the first germinated seed observed: 3h, 3h30, 4h, 4h50, 6h, 8h, and every following 8 hours until germination clearly reached a plateau. When a seed presented a radicle of 1 mm long, germination was considered to be achieved and seed was removed.

Data analysis

The thermal time model (Eqn. 4.1) defined by Garcia-Huidobro *et al*. (1982) was used to test the hypothesis that *Buddleia* seed respond to heat sum at sub-optimal temperatures.

$$\theta_T(g) = (T - T_b)t_g \text{ or} \qquad \text{(Eqn. 4.1)}$$

$$GR_g = \frac{1}{t_g} = \frac{T - T_b}{\theta_T(g)} \qquad \text{(Eqn. 4.2)}$$

The relation between germination rate and temperature was plotted using equation 4.2 and in order to define the cardinal temperatures for *Buddleia*.

The base temperature being usually the same for all seeds in a population at sub-optimal T, all the variation in germination rate is linked to the variation of $\theta_T(g)$. The latter had been calculated using all available data and alongside with cardinal temperatures, these parameters were used in the model proposed by Roberts (1988) for modelling germination below T_o,

$$GR = \frac{T - T_b}{(probit(g) - K)(\sigma(\theta_T))} \qquad \text{(Eqn.4.3)}$$

were probit(g) is the probit transformation of the percentage of germinated seed at T_g, $\sigma(\theta_T)$ is the standard deviation of the thermal time constant and K is a constant specific to a species, which can be calculated by transforming Equation 4.3 into,

$$K = probit(g) - \frac{(T - T_b)t_g}{\sigma(T_c)} \qquad \text{(Eqn.4.4)}$$

were $\sigma(T_c)$ is the standard deviation of the ceiling temperatures.

At supra-optimal temperatures, the heat sum used for germination purposes does not increase as temperature increases, and $\theta_T(g)$ remains constant in this range. To consider the fact that different fractions of the seed population do not germinate above different ceiling temperatures, the model developed by Ellis and Butcher (1988) was used to calculate the thermal time constant at supra-optimal T.

$$\theta_2 = (T_c(g) - T)t_g \qquad \text{(Eqn. 4.5)}$$

were θ_2 is a thermal time constant and $T_c(g)$ are the ceiling temperatures specific to a seed fraction. With these parameters, the germination rate can be estimated as followed,

$$GR_g = \frac{1}{t_g} = \frac{T_c(g) - T}{\theta_2} \qquad \text{(Eqn. 4.6)}$$

For modelling germination at supra-optimal T, Roberts (1988) proposed the following equation,

$$GR = (K_s - probit(g))(\sigma_{Tc}) - \frac{T}{\theta_2} \quad \text{(Eqn.4.7)}$$

The constant K_s can be calculated by transforming Equation 4.7 into,

$$K_s = probit(g) + (\frac{1}{t_g} + \frac{T}{\theta_2})(\frac{1}{\sigma_{Tc}}) \quad \text{(Eqn.4.8)}$$

If germination is responding to heat accumulation at sub-optimal temperature, it is possible to reduce the time courses at all temperature to a single curve calculated using the following equation (Bradford, 1990),

$$t_g(0) = (1 - \frac{T}{T_b})t_g(T) \quad \text{(Eqn.4.9)}$$

where $t_g(0)$ is the time to germination in water, and $t_g(T)$ is the time to germination under any suboptimal T. This normalization reduces all curves to a single one from which the timing of germination at any sub-optimal temperature can be predicted. The coefficient of determination between the model and actual data indicates the accuracy of the model.

Results

I. Cardinal temperatures:

The germination time courses and total germination between repetitions were very similar. Their mean values for each treatment are represented on Figure 4.1. No germination occurred at 37°C, therefore 37°C is higher than the ceiling temperature for *Buddleia davidii*.

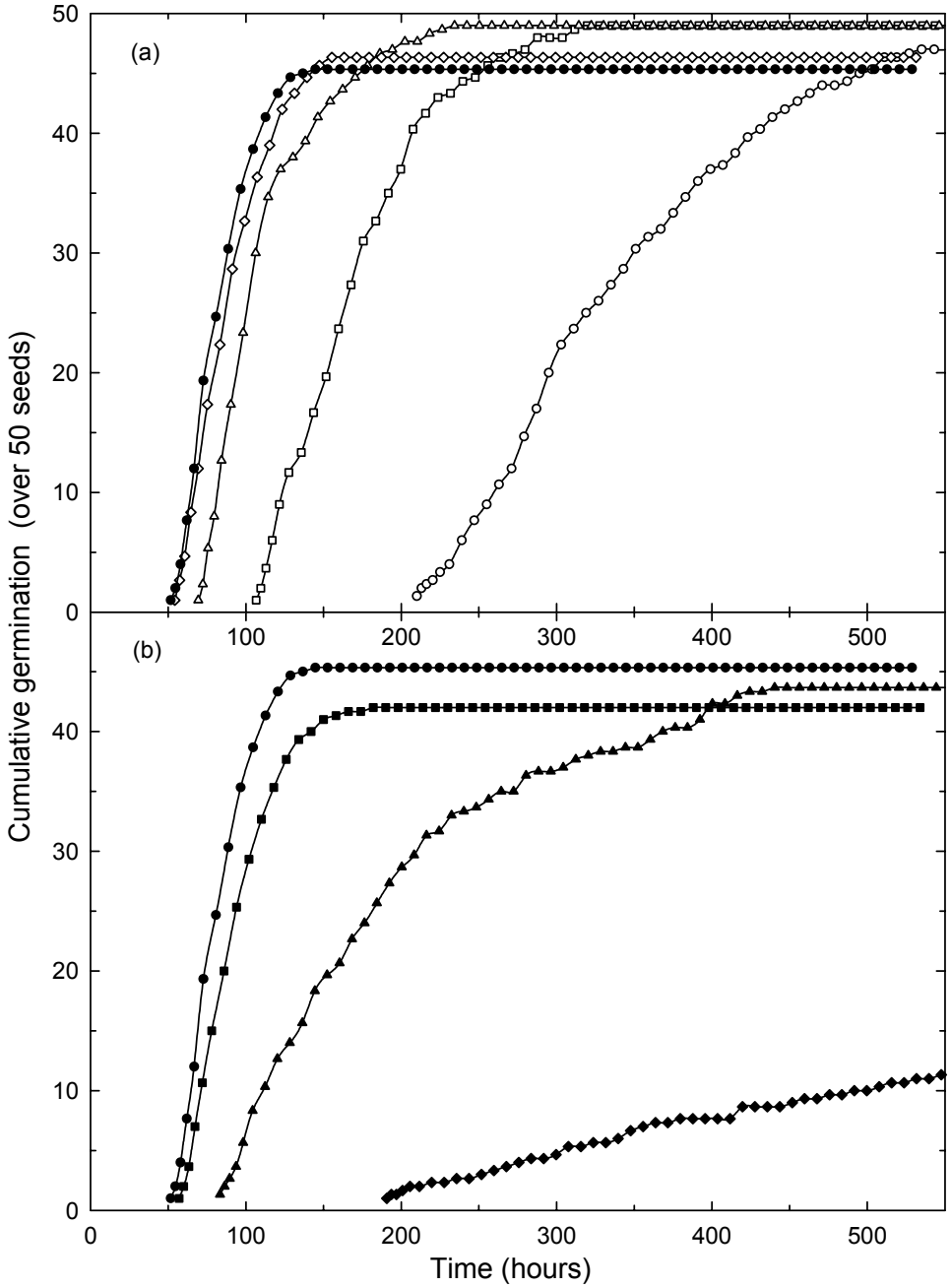


Figure 4.1: Germination time courses of *Buddleia davidii* at (a) sub-optimal temperatures of 13°C (○), 17°C (□), 21°C (△), 24°C (◇) and (b) supra-optimal temperatures of 26°C (■), 29°C (▲) and 33°C (◆). The optimum temperature, 25°C (●), appears on both graphs for comparisons with treatments at lower and higher *T* values.

The starting time for germination was the shortest at 25°C with a mean value of 50 hours. In addition, the rate of germination at this temperature was the highest. Thus it can be concluded that 25°C is the optimum temperature. Above and below this temperature, the germination rate is slower but the total germination differs according to the treatments: at sub-optimal temperatures, more seeds germinate, which demonstrates that at optimum temperature a small seed fraction is already subjected to dormancy due to high temperatures. This limitation in total germination does not affect seed germination at or below 21°C. At supra-optimal temperatures, the total germination decreases but no linear relationship can be inferred between temperature and total germination. Seeds which did not germinate at supra-optimal temperatures germinated when placed in optimum conditions and reached comparable total germination values than at T_o. This shows that high temperatures favour a delay of germination of seed fractions without affecting viability of seeds, at least in a short term.

After collecting the t_g values (time to reach a certain percentage of germination) from Figure 4.1, the germination rates ($1/t_g$) were calculated and plotted against temperature for each percentage of germination (g):

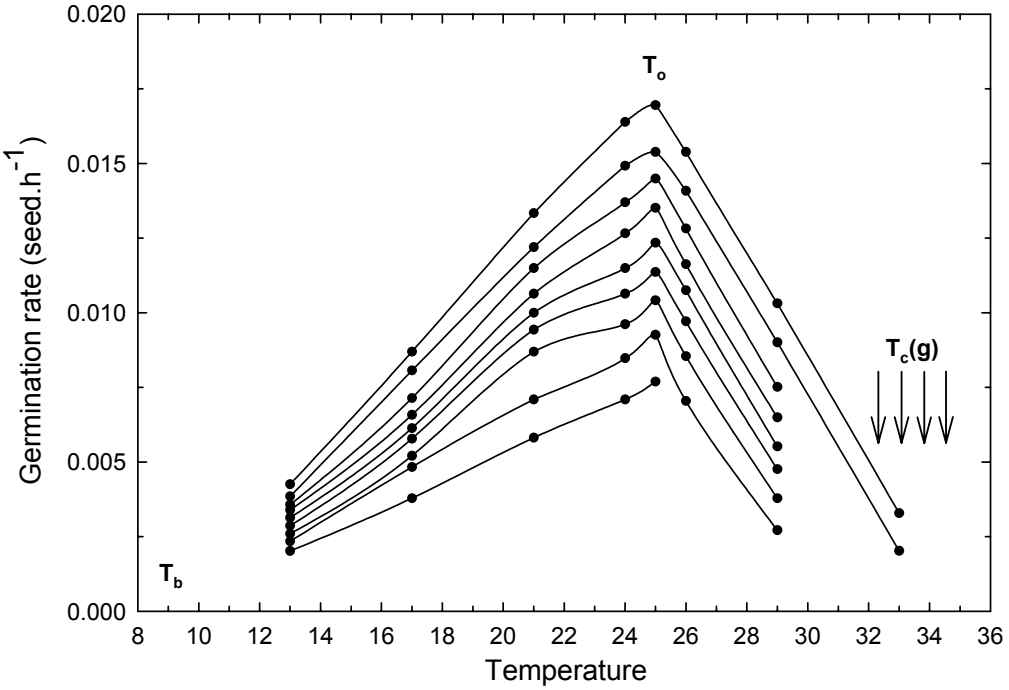


Figure 4.2: Cardinal temperatures for germination of *Buddleia davidii*. From the highest to the lowest lines appear the 10th, 20 th, 30 th, 40 th, 50 th, 60 th, 70 th, 80 th, and 90 th percentiles. T_b and T_c are the intercept on the x axis of the extrapolated lines. While T_b is the same at all fractions, T_c differs consistently from one seed fraction to another.

The positive linear relationships at sub-optimal temperature are consistent and all the lines converge to a similar intercept on the x axis which is the base temperature (T_b). Here, T_b value is 8.9°C with a standard deviation of 0.15. For reference, English data collected for *Buddleia davidii* grown in England Miller (1984) showed that T_b *was* between a constant 9 and 10°C.

The slight discrepancy of the germination rate at 21°C shown in Figure 4.2 and 4.3 is likely to find its origin in temperature variation in the growth cabinet used for the experiment. Variations in temperature were observed, generally ranging between + and - 1°C. In the case of the 21°C treatment, the incubator is likely to have provided for a prolonged period a slightly higher temperature than required.

In the sub-optimal range of temperatures, the rate of germination depends on the amount of heat received by the seeds. This accumulation of heat can be expressed in degree-day and corresponds to the sum of heat received over the base temperature. Between T_b and T_o, the higher the energy perceived, the faster the metabolic processes achieved, thus the faster germination occurs. The thermal time constant $\theta_T(g)$ was calculated according to Equation 4.1 and plotted against seed fractions (Figure 4.3):

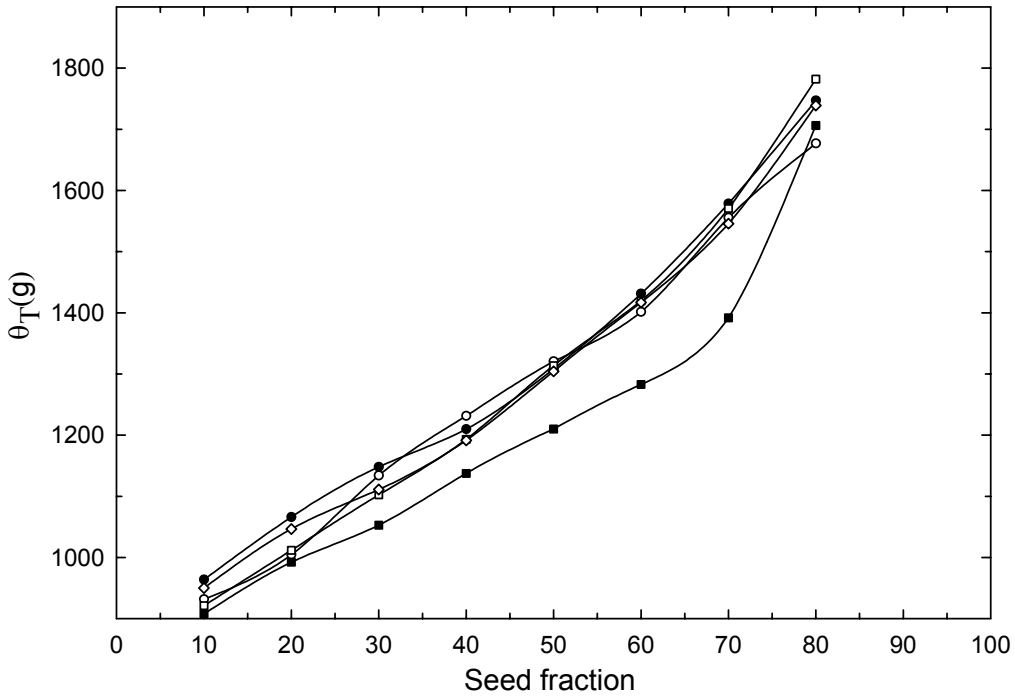


Figure 4.3: Thermal time constant $\theta_T(g)$ at sub-optimal temperatures for each seed fraction. Temperatures tested are 13°C (●), 17°C (○), 21°C (■), 24°C (□), 25°C (◇). The mean trendline gives a slope of 10.95.

$\theta_T(g)$ remains constant at sub-optimal temperatures for a specific percentage of germinated seeds.

At supra-optimal T, a negative linear relationship appears for all fractions (Figure 4.2). This shows that seed fractions are sensitive to different ceiling temperatures $T_c(g)$. Determination of the y intercept provides values of $T_c(g)$ for each fraction. Plotting $T_c(g)$ values against seed fractions reveals a linear relationship (Figure 4.4), although this linear relationship is limited to this range. Plotted against relative frequency in a population, $T_c(g)$ appear to follow a normal distribution.

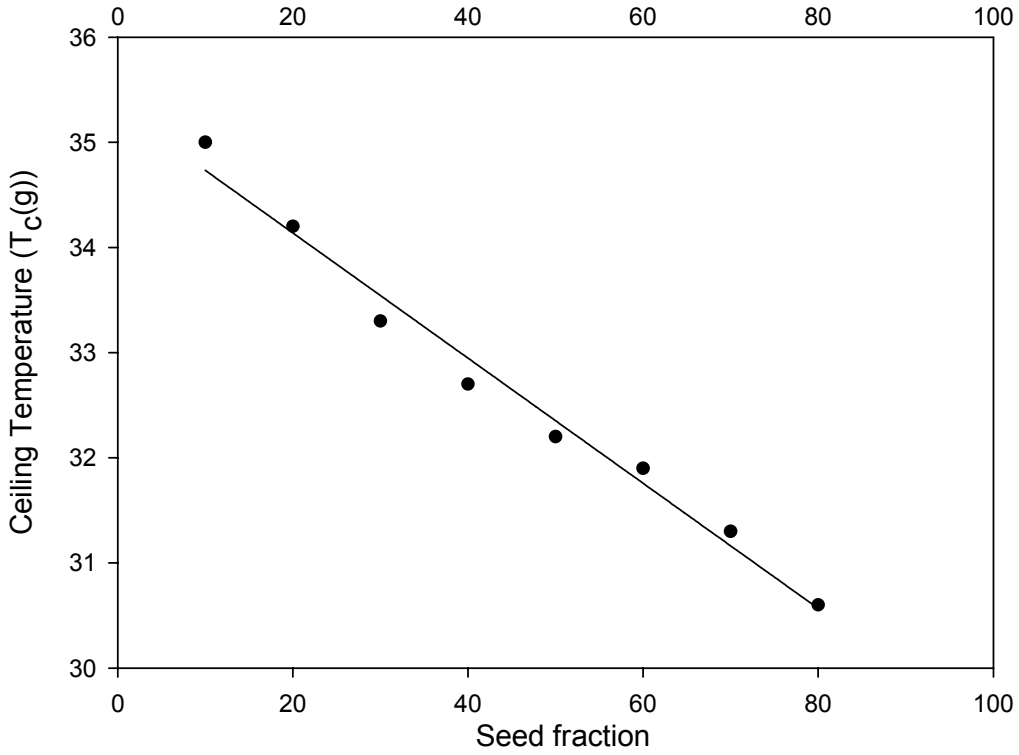


Figure 4.4: Relationship between seed fractions and ceiling temperatures. The trendline drawn through the data has an intercept on the y axis at 34.2°C.

Above T_o, the thermal time constant θ_2 was calculated according to Equation 4.5. θ_2 remained constant at all supra-optimal T and for each seed fractions because in this range a single value of extra heat above T_o is considered. The time for a certain percentile to germinate, t_g, depends on the ceiling temperature for this fraction. Calculation of θ_2 gives a mean value of 592 with a standard deviation of 22.5.

Cardinal temperatures for *Buddleia davidii* are now defined and the following values are used for further calculations: T_b is 8.9°C, T_o is 25°C and T_c(g) ranges between 30 and 35°C with a standard deviation of 1.47.

II. Model:

Equation 4.4 allowed the calculation of a mean K value of –4.15 with a standard deviation of 0.48. At supra-optimal, the constant K_s used in the thermal time model was calculated with Equation 4.8. Results demonstrated that for *Buddleia* seeds, K_s values are not constant and no prediction can be made with this single model.

Using Equation 4.3, the germination rate at sub-optimal temperature can be modelled and compared with actual data (Figure 4.5).

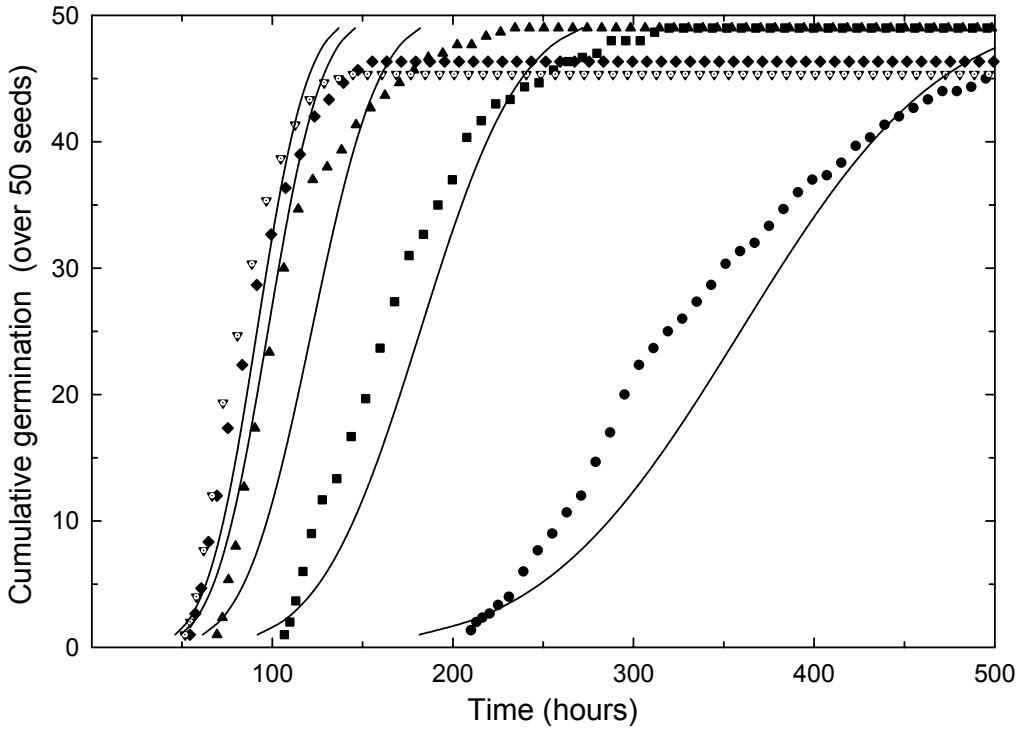


Figure 4.5: Modelled (lines) and actual (dots) germination time courses of *Buddleia* seeds at sub-optimal temperatures of: 13°C (●), 17°C (■), 21°C (▲), 24°C (◆) and 25°C (▼).

The thermal time model proposed by Roberts (1988) does not describe the germination of *Buddleia davidii* adequately. Although less seeds germinate at 24 and 25°C, the difference in asymptote is not taken into account in this model. At all T, estimations of the cumulative germination over time fits better the data at the beginning and the end of the germination time courses. The discrepancy observed for the middle seed fractions, which appears as wide as 50 hours at 13°C, can be reduced by modifying the base temperature. This allows closer fit of the model to intermediate seed fractions, however the discrepancy then affects timing of the beginning and the end of germination.

A last step for testing the relevance of the thermal time model at sub-optimal temperatures was to plot all germination time courses on a normalised time scale (Figure 4.6). Actual t_g values for each seed fraction at all temperatures were transformed using Equation 4.9. Normalised curves were plotted with predicted germination calculated with the thermal time model parameters.

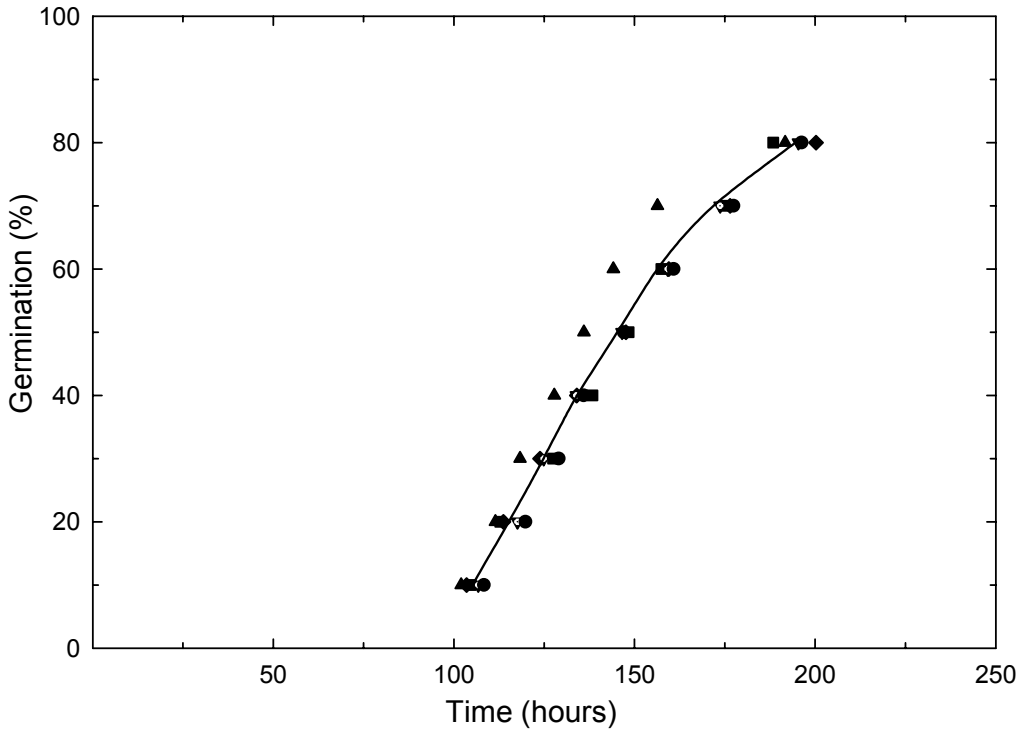


Figure 4.6: Normalised sub-optimal thermal time model (line) and normalised germination time courses at temperatures of 13°C (●), 17°C (■), 21°C (▲), 24°C (◆) and 25°C (▽).

Apart from the discrepancy of the 21°C data, the normalised germination time courses at sub-optimal temperatures fitted the normalised thermal time model, demonstrating that the model accurately accounts for the observed germination pattern. The high value of the coefficient of determination (0.96) confirms that between T_b and T_o, *Buddleia davidii* seed is responsive to an accumulation of heat over time above a threshold temperature.

Discussion

The cardinal temperatures for *Buddleia davidii* had been assessed, however these parameters should not be considered as definite values in all conditions. The base temperature calculated under constant conditions is often higher than the actual base temperature of seed populations which is lowered by temperature cycles (Lang, 1965;

Thompson, 1973). Miller (1984) demonstrated that *Buddleia davidii* seeds which did not germinate in constant conditions just below the T_b were germinating when placed into a varying environment of lower temperatures. Therefore cardinal temperatures defined in this chapter are likely to be valid only under constant temperature conditions.

The thermal time model described by Roberts (1988) fitted the data poorly. At sub-optimal temperatures, predictions of germination time courses made through this single model would led to a discrepancy of as much as 50 hours between predicted and observed germination. This could be a result of the high value of $\sigma(\theta_T)$. While values of θ_T varied little when calculated from a single percentile, their high variation from a percentile to another provoked a large rise in their overall standard deviation, leading to a broader discrepancy of the predictions. However $\sigma(\theta_T)$ is a single parameter used for this thermal time model and cannot be set differently for each percentile.

Gathering the germination time courses on a normalised time scale, as described by Bradford (1990), the sensitivity of *Buddleia* seed population to an accumulation of heat over the base temperature had been revealed. However this is limited to the sub-optimal temperature range, as a decrease in both rate and total germination is observed at higher temperatures. At high temperatures, interaction with water stress becomes inevitable (Bonhomme, 2000), and seed germination behaviour in this temperature range is the consequence of the sensitivity of germination to the water potential Ψ (Alvarado and Bradford, 2002). The importance of water on germination is well known, as well as its interaction with temperature, therefore the impact of water stress on *Buddleia* germination is detailed in the next chapter.

CHAPTER 5 - HYDROTHERMAL TIME MODEL FOR GERMINATION UNDER CONSTANT MOISTURES AND TEMPERATURES

Introduction

For germination to occur, a seed must reach a critical water content. The germination process is triphasic (Bewley and Black, 1978): (1) imbibition, or rapid water uptake from the soil according to the osmotic gradient between the soil and the seed; (2) germination – corresponding to a plateau in the water uptake, when germination activates, and (3) seedling growth – when the radicle starts elongating and emerges from the seed coat while water uptake is largely increased.

As most seeds have a low seed water potential when in a dry state (Roberts and Ellis 1989), lower water content does not affect the duration of Phase 1, but reduces the rate of germination by extending the time in Phase 2. Previous studies showed that both the rate of germination and total germination decrease with a decreasing water potential (Doneen and MacGillivray, 1943, Fyfield and Gregory, 1989, Gummerson, 1986, Alvarado and Bradford, 2002) and that the median germination time, t_{50}, or time for 50% of seeds to germinate, was linearly related to water potential Hegarty (1976). Several studies confirmed this results, such as those of Gummerson (1986), Romo and Haferkamp (1987), Fyfield and Gregory (1989), and Bradford (1990).

It is well known that germination is not possible below a certain threshold (or base) water potential specific to a species (Hunter and Erikson, 1952). In addition, the base water potential Ψ_b differs according to seed fractions. Among a seed population, the variation of $\Psi_b(g)$ is often described by a normal distribution, and its mean as well as its standard variation are important parameters for the hydrotime and hydrothermal time model.

In the germination process, water and temperature interact and are primary determinants of seed germination pattern (Finch-Savage *et al*., 1998). At supra-optimal temperatures, the distribution of $\Psi_b(g)$ has been shown to shift positively with an increased temperature (Bradford and Somasco, 1994; Rowse and Finch-Savage, 2003; Larsen *et al.*, 2004). Above T_o, a decrease in *GR* and total germination due to an increase in the $\Psi_b(g)$ thresholds appears.

The hydrothermal time concept developed by Bradford (1990), Dahal and Bradford (1994), Finch-Savage *et al*. (1998) led to a modified hydrothermal time model Alvarado and Bradford (2002) accounting for the variation in $\Psi_b(g)$ at supra-optimal temperature, allowing to extend the ability of the hydrothermal model to

model germination at supra-optimal temperatures and to account for reduction in both rate and total germination. This model has proven its efficiency at sub-optimal temperatures by Dahal and Bradford (1994), Gummerson (1986), and at supra optimal temperature by Alvarado and Bradford (2002) and by Rowse and Finch-Savage (2003).

This chapter investigates the relevance and the accuracy of the hydrotime and hydrothermal time models to describe the interaction between *T* and water potential on germination of *Buddleia davidii* under constant conditions.

Method

Experimental design

The seeds tested were taken from *Buddleia davidii* flowers in Rotorua. They originated from the same population as seeds used in the thermal time experiment.

Six constant temperatures and eight constant water potentials were tested, leading to a 6x8 factorial design. Temperatures and water potentials tested were respectively: 13, 17, 21, 25, 29 and 33°C, and 0, -2, -3, -4, -5, -6, -7, -8 bars. Three replicates of 50 seeds each were placed in 8.5 cm plastic Petri dishes, arranged regularly on three sheets of filter paper.

In order to obtain the required water potentials, solutions of polyethylene glycol (PEG) 6000 were used according to Equation 5.1. 5 ml of the prepared PEG 6000 solutions were poured in each Petri dish, except for controls which only received distilled water. Furthermore the two lower filter papers were replaced daily and 2 ml of PEG solutions (or distilled water for controls) were prepared daily and added in order to keep the water potentials balanced.

According to Michel (1983), between a temperature of 5 to 40°C and over the concentration range of 0 to 0.8 gram PEG per gram H_2O (range which include the conditions of this experiment), actual bars Ψ are best predicted by Equation 5.1. Michel (1983) states that with this equation, errors seem unlikely to exceed 5%.

$$\psi = 1.29[PEG]^2 T - 140[PEG]^2 - 4[PEG] \text{ or}$$

$$[PEG] = \frac{4 - (5.16\psi T - 560\psi + 16)^{0.5}}{2.58T - 280} \qquad \text{(Eqn. 5.1)}$$

After adding the solutions, Petri dishes were placed in the dark into incubators at selected constant temperatures. As the experiment lasted several weeks, the top filter paper was replaced when becoming easily broken. Petri dishes were sealed with tape in order to minimize variation of the water potential due to evaporation.

Intervals between observation and counting are the same than used in previous chapters. Seeds were removed from Petri dishes when germinated, and a seed was considered germinated when the radicle was 1mm long.

Data analysis

The hydrotime concept proposed by Gummerson (1986) is comparable to the thermal time concept as it considers a water potential above a threshold favourable to germination. This idea is summarized into the following equation,

$$\theta_H = (\psi - \psi_b(g))t_g \text{ or} \qquad \text{(Eqn. 5.2)}$$

$$GR_g = \frac{1}{t_g} = \frac{\psi - \psi_b(g)}{\theta_H} \qquad \text{(Eqn. 5.3)}$$

where Ψ is the seed water potential, $\Psi_b(g)$ the base allowing germination of fraction g of the seed population, t_g is the time to germination of percentage g, and θ_H is a hydrotime constant.

For calculating θ_H, estimations at all temperatures were first made using Equation 5.2 and the recorded t_g values for each available percentile. Then, mean θ_H was calculated and used into Equation 5.2 to calculate individual values of $\Psi_b(g)$ for each percentile. The normal distribution of $\Psi_b(g)$ was linearised by a probit transformation and the final θ_H was determined by maximisation of the coefficient of determination.

Taking in account the positive shift of $\Psi_b(g)$ with an increased T at supra-optimal temperatures, Alvarado and Bradford (2002) defined the relationship between $\Psi_b(g)$ and T at this range of temperatures,

$$\psi_b(g)_{T>T_o} = \psi_b(g)_{T_o} + k_T(T - T_o) \qquad \text{(Eqn. 5.4)}$$

where k_T is the slope of the relationship between $\Psi_b(g)$ and T in the supra-optimal T range and $\Psi_b(g)_{To}$ is the base water potential at optimum T. Combining this equation with the hydrotime model for sub-optimal T represented by Equation 5.2, Alvarado and Bradford (2002) defined a probit transformation able to predict the number of germinated seeds at Ψ,

$$probit(g) = \frac{\psi - k_T(T - T_o) - \frac{\theta_H}{t_g} - \psi_b(50)}{\sigma(\psi_b)} \qquad \text{(Eqn. 5.5)}$$

were $k_T(T\text{-}T_o)$ applies only at supra-optimal T, and when $T>T_o$, θ_H is the hydrotime constant value at T_o.

Germination subjected to water stress can be normalised to the time course recorded in full water supply (Bradford, 1990). If by this transformation the different time courses at different Ψ are gathered along a single curve, germination is clearly responding to hydrotime. Bradford (1990) derived the relationship between the time to germination in water ($t_g(0)$) and the time to germination at other Ψ ($t_g(\Psi)$) as following.

$$t_g(0) = (1 - \frac{\psi}{\psi_b(g)})t_g(\psi) \qquad \text{(Eqn. 5.6)}$$

To express variation in germination according to a combination of water stress and temperature, Equations 4.1 and 5.2 were combined into a hydrothermal time model defined by Gummerson (1986) and Bradford (1995),

$$\theta_{HT} = \{\psi - \psi_b(g)\}(T - T_b)t_g \qquad \text{(Eqn. 5.7)}$$

where θ_{HT} is the hydrothermal time constant.

Once mean θ_{HT} had been estimated using Equation 5.7 with recorded t_g values from the laboratory experiments, this θ_{HT} value was used to calculate $\Psi_b(g)$ for each percentile. The normal distribution of $\Psi_b(g)$ was linearised by a probit transformation and θ_H was determined by maximisation of the coefficient of determination. The inverse of the slope of the regression line inferred from the relation between $\Psi_b(g)$ and probit(g) is the standard deviation of $\Psi_b(g)$, which is one of the required parameters

for the hydrothermal time model. Then the parameters θ_{HT}, $\Psi_b(g)$ and $\sigma(\Psi_b)$ were maximised using the function proc nlin into the SAS software.

As seen before, the base water potential is modified with high temperatures. Combining the modified base water potential (Eqn. 5.4) and the hydrothermal time model (Eqn. 5.7), Alvarado and Bradford (2002) define a modified hydrothermal time model which allows prediction of germination at any temperature and at any water potential.

$$\theta_{HT} = \{\psi - \psi_b(g) - (k_T(T - T_o)\}(T - T_b)t_g \quad \text{(Eqn. 5.8)}$$

were $k_T(T\text{-}T_o)$ applies only at supra-optimal T, and in this range, $\Psi_b(g)$ is set to its value at T_o and $T\text{-}T_b$ equals $T_o\text{-}T_b$.

A probit transformation of this equation, gives a model able to predict germination rate as well as total germination at any T and any Ψ (Bradford, 2002).

$$probit(g) = \frac{\psi - k_T(T - T_o) - \dfrac{\theta_{HT}}{(T - T_b)t_g} - \psi_b(50)}{\sigma(\psi_b)} \quad \text{(Eqn. 5.9)}$$

The final check for testing the relevance of the parameterised hydrothermal time model is to use data from all T and Ψ treatments, and using Equation 5.10, data is plotted on a normalized time scale.

$$t_g(0) = (1 - \frac{\psi}{\psi_b(g)}) * (1 - \frac{T}{T_b}) * t_g(\psi) \quad \text{(Eqn. 5.10)}$$

The fit between data and the modelled prediction identified by a coefficient of determination reveals if the model parameters accurately account for the sensitivity of the seed population to Ψ and T.

Results

I. Hydrotime model:

Cumulative germination of *Buddleia* seeds was plotted against time. At all temperatures, a decrease in water potential induced a decline in both germination rate and total germination (Figure 5.1). At all water potentials, germination rate decreased at higher or lower temperatures than T_o.

These graphs confirm that 25°C is the optimum temperature, having the shortest starting time and the highest rate of germination. The further the temperature from the optimum, the longer the time to start germination.

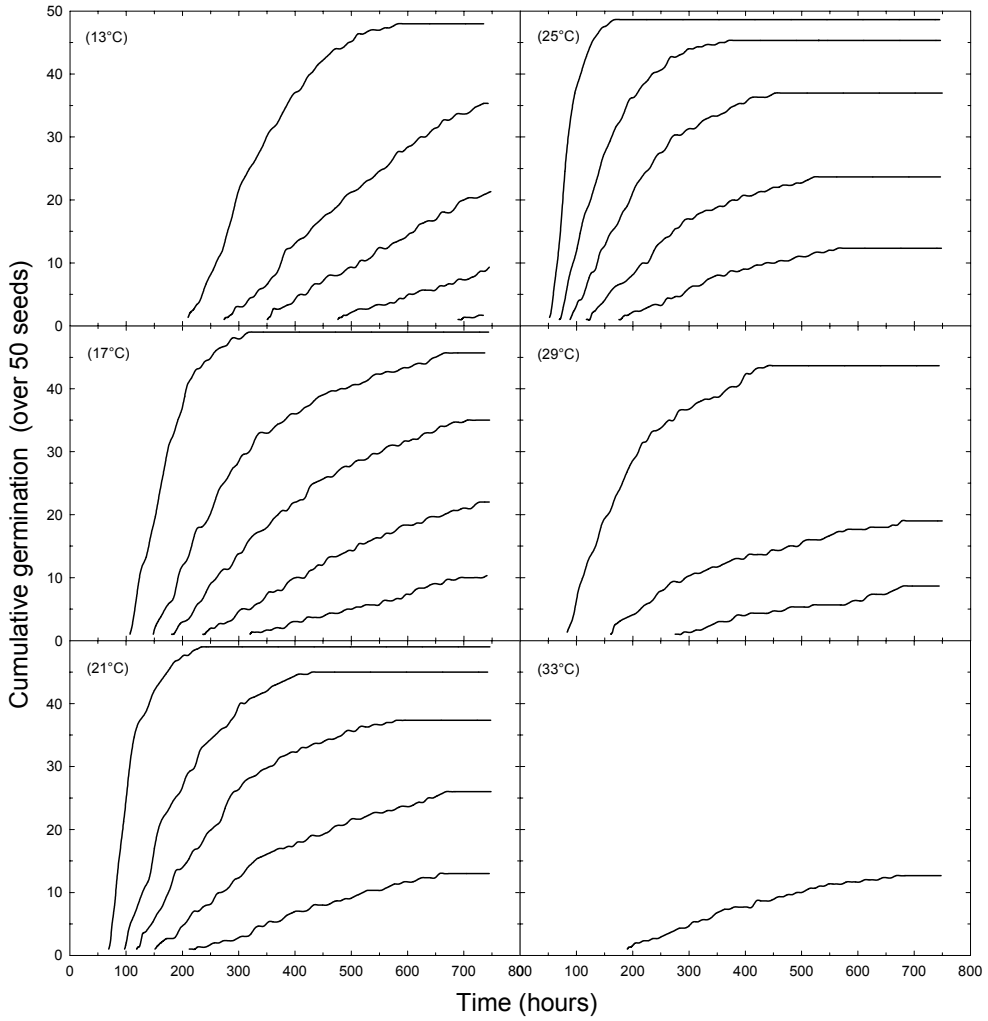


Figure 5.1: Germination time courses of *Buddleia davidii* at temperatures ranging from 13 to 33°C and subjected to five water stress treatments (from top curve to bottom curve within a graph): 0, -2, -3, -4 and –5 bars. Treatments at –6, -7 and –8 bars are not shown because no germination occurred at these potentials.

At supra-optimal temperatures, sensitivity to water potential is increased, as shown by the last two treatments, which corresponds to an increase in the base water potential of the seed population. No germination occurred under –3 bars at 29°C. At 33°C, only the control treatment germinated. Having a single curve for 33°C, no more information can be extracted from this temperature treatment for the hydrotime model as it is the combination of at least two times to reach a similar percentage of germination that allows calculations. Thus analysis of supra-optimal temperatures can only be undertaken with the 29°C data.

The difference in moisture sensitivity of the different seed fractions is a feature at all temperatures. At any T, the base water potential $\Psi_b(g)$ allowing germination is different for each seed fraction considered. This leads to different germination rates according to the fraction considered (Figure 5.2).

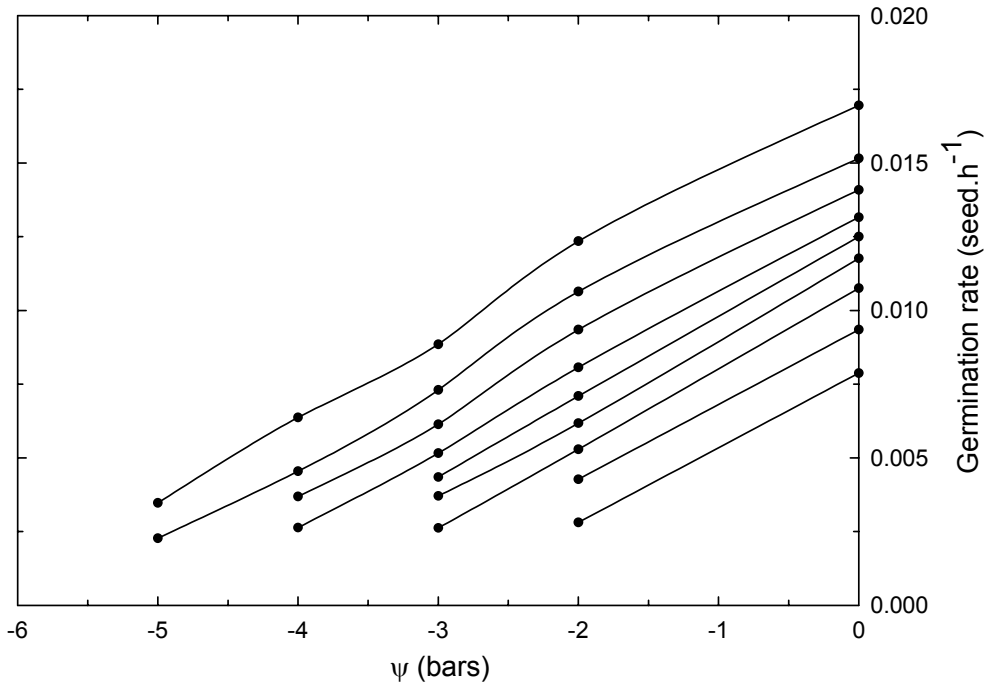


Figure 5.2: Germination rates versus water potential at 25°C for respectively (from top to bottom) the 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th}, 70^{th}, 80^{th} and 90^{th} percentile. All the lines have a similar slope of approximately -0.0026.

At each temperature, results have demonstrated a linear relationship between GR and Ψ. The slopes of these lines, which are all parallel at a specific temperature, equal $1/\theta_H$. $\Psi_b(g)$ is estimated for each fraction by extrapolating the trendlines of each line and observing their intercept with the x axis.

At supra-optimal T, for accounting on the observed positive shift of $\Psi_b(g)$ as temperature increases, $\Psi_b(g)$ was modified according to Equation 5.4. To calculate the parameter k_T, $\Psi_b(g)$ was plotted against temperature for each seed fraction that germinated at 29°C (Figure 5.3), that is the 10th, 20th and 30th percentiles. The slope of the lines at supra-optimal T is the parameter k_T, which has here a value of 0.65.

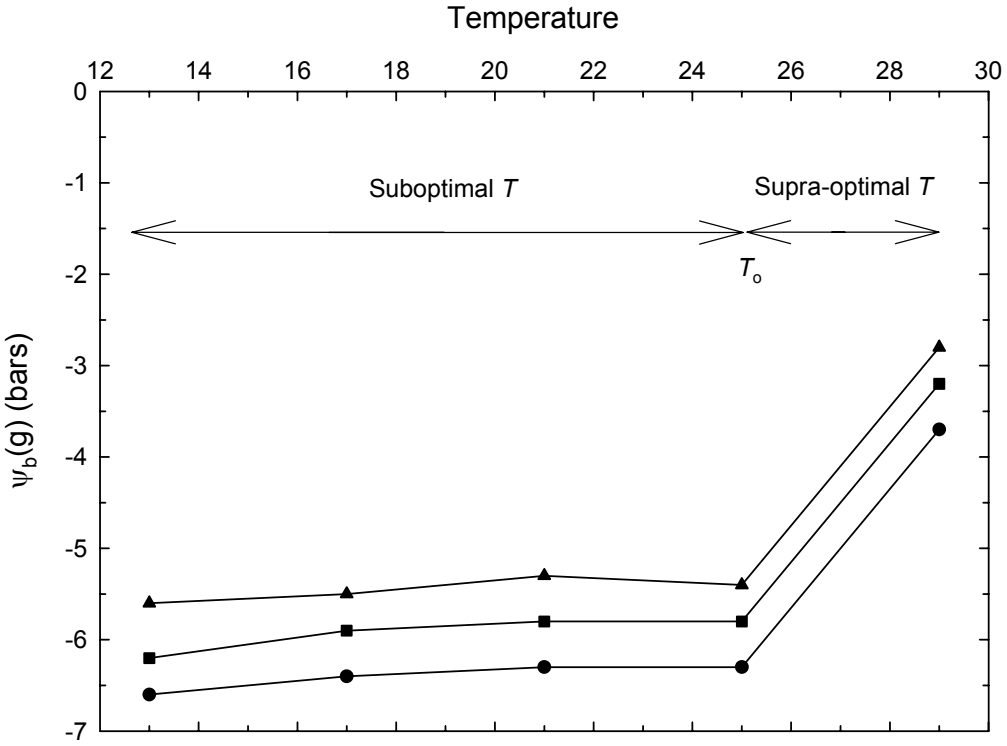


Figure 5.3: Base water potentials versus temperature for the 10th (●), 20th (■) and 30th (▲) percentiles. The direction of the slopes change from optimal temperature, and extrapolating the lines at supra-optimal T leads to an intercept of the x axis corresponding to ceiling temperatures for these fractions.

Actual values of $\Psi_b(g)_{T>To}$ inferred from extrapolation of the relation GR versus Ψ and their calculated values from Equation 5.4 have shown no difference, which confirms the relevance of the transformation proposed by Alvarado and Bradford (2002). At suboptimal temperatures, the lines representing $\Psi_b(g)$ demonstrate the constancy of the base water potential relative to seed fraction.

After calculating θ_H using Equation 5.2, the normal distribution of $\Psi_b(g)$ was linearised by a probit transformation. Probit(g) versus $\Psi_b(g)$ revealed at all temperatures a positive linear relationship (e.g.: Figure 5.4 shows data at 25°C).

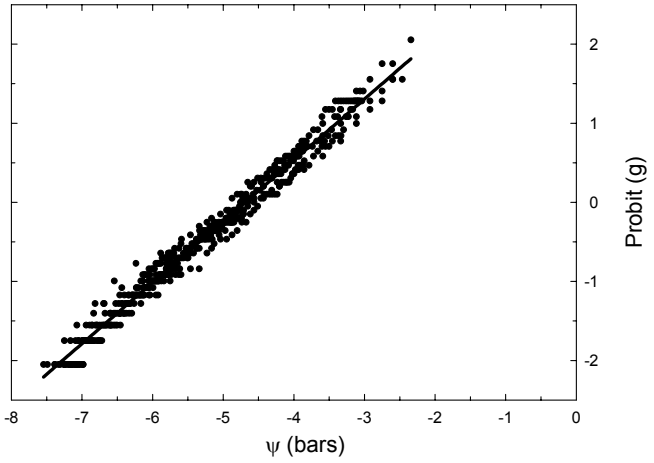


Figure 5.4: Actual data (dots) and trendline of the distribution of $\Psi_b(g)$ at 25°C linearised by probit transformation according to the hydrotime model. The regression line has a slope of 0.774.

This relationship enables the standard deviation of Ψ_b to be estimated as the inverse of the slope of this regression line. Mean Ψ_b ($\Psi_b(50)$) and its standard deviation are different for each suboptimal temperature. Results are presented in Table 5.1.

Table 5.1: Parameters for the hydrotime model describing the germination of *Buddleia davidii* **at any Ψ**. θ_H is the hydrotime constant, $\Psi_b(g)$ is the base water potential for the 50th percentile and σ (Ψ_b) is the standard deviation of Ψ_b.

A Sub-optimal T	T (°C)	θ_H (bars.h^{-1})	$\Psi_b(50)$ (Bars)	σ (Ψ_b) (Bars)			r^2
	13	1512	-4.70	1.33			0.98
	17	772	-4.79	1.33			0.99
	21	497	-4.67	1.31			0.98
	25	377	-4.70	1.29			0.98

B Supra-optimal T	T (°C)	θ_H (bars.h^{-1})	$\Psi_b(50)_{To}$ (Bars)	σ (Ψ_b) (Bars)	T_o (°C)	k_T (Bars °C^{-1})	
	29	377	-4.70	1.29	25	0.65	0.98

At supra-optimal temperature, that is 29ºC, the values for $\Psi_b(50)$ and $\sigma(\Psi_b)$ were kept the same than at optimum temperature. The positive shift of $\Psi_b(50)$ is accounted for by the transformation of $\Psi_b(g)$ using Equation 5.4.

The parameters from Table 5.1 were used in Equation 5.5 in order to plot together actual and predicted germination time courses (Figure 5.5).

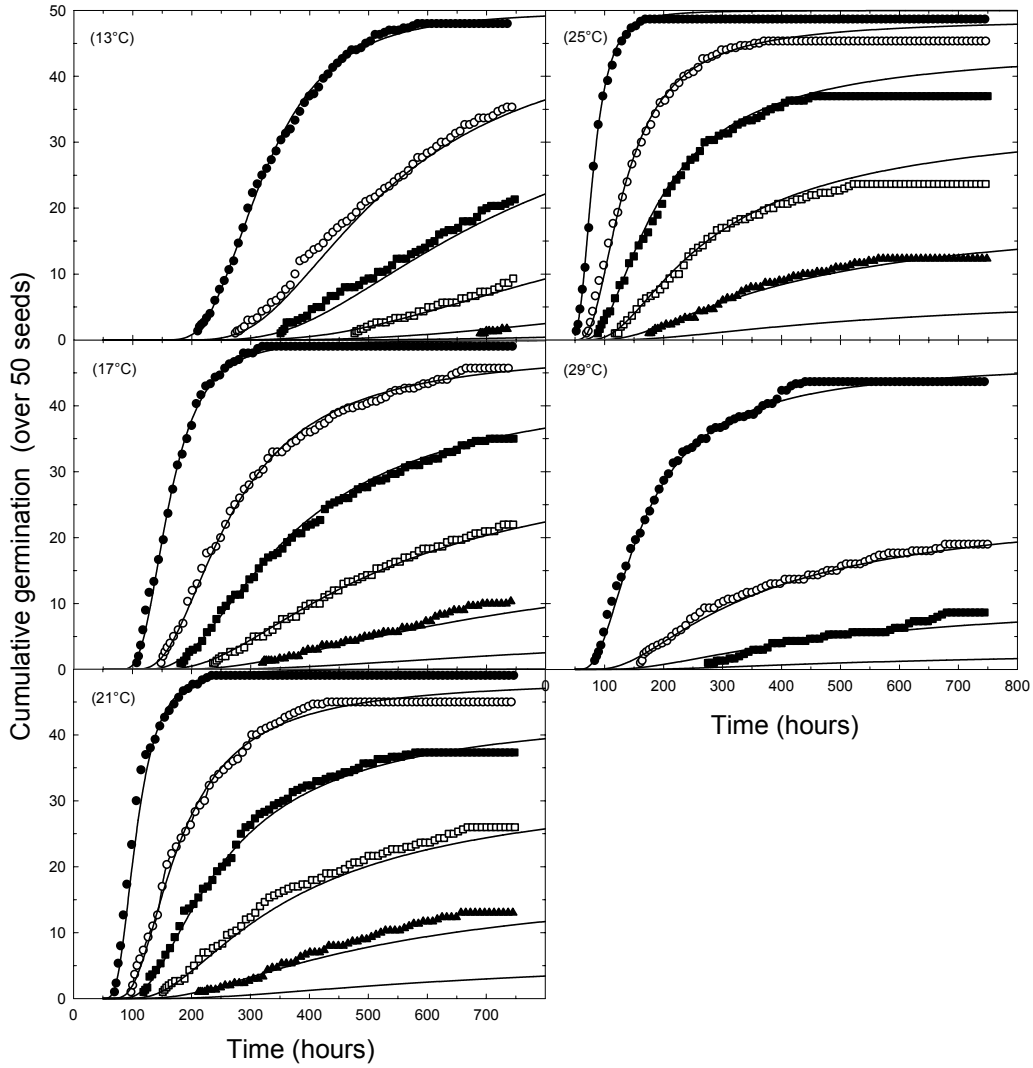


Figure 5.5: Germination time courses of predicted values from the hydrotime model (lines) compared with actual data (symbols) at sub-optimal temperatures (13 to 25°C) and supra-optimal temperature (29°C). Water potentials are 0 (●), -2 (○), -3 (■), -4 (□), and –5 (▲) bars.

Although the hydrotime model describes the pattern of germination at both sub-optimal and supra-optimal temperatures, it tends to overestimate once maximum germination is reached. At all temperatures the hydrotime model forecast germination at –6 bars while none was observed. This expected germination at –6 bars is confirmed by the extrapolation of $\Psi_b(g)$ which is lower (more negative) than –6 bars for the 10^{th} percentile at all sub-optimal temperatures (Figure 5.3), implying that germination should indeed occur at this water potential.

II. Hydrothermal time model:

The hydrothermal time constant θ_{HT} was estimated with Equation 5.7 at sub-optimal temperatures and Equation 5.8 at supra-optimal temperatures. Results are consistently similar throughout the temperature and water potential range at sub-optimal and supra-optimal temperatures. The distribution of $\Psi_b(g)$ was linearised by a probit transformation (Figure 5.7) and its standard deviation was calculated as the slope of the regression line. With these estimated values, the parameters θ_{HT}, $\Psi_b(g)$, $\sigma(\Psi_b)$, were estimated using the function proc nlin in the SAS software, with code written by Dr Mason. Results are shown in Table 5.2.

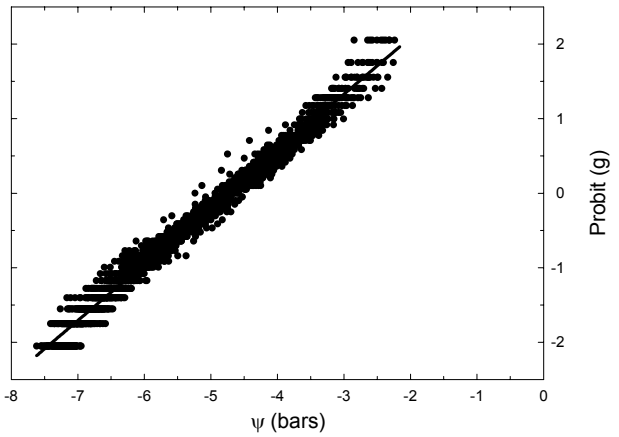


Figure 5.7: Actual data (dots) and trendline of the distribution of $\Psi_b(g)$ linearised by probit transformation at all sub-optimal and supra-optimal T and all Ψ according to the hydrothermal time model (Equation 5.8). The trendline has a slope of 0.760 and a r-square value of 0.984.

Table 5.2: Parameters used in the hydrothermal time model for the germination of *Buddleia davidii*. θ_{HT} is the hydrothermal constant, $\Psi_b(50)$ is the base water potential for the 50th percentile, $\sigma(\Psi_b)$ is the standard deviation of Ψ_b, T_o is the optimum temperature and k_T is a population constant.

T (°C)	θ_{HT} (Bars h)	$\Psi_b(50)$ (Bars)	$\sigma(\Psi_b)$ (Bars)	T_o (°C)	k_T (Bars °C^{-1})	r^2
13-29	6197.8	-4.7795	1.3229	25	0.65	0.98

The use of these parameters in Equation 5.7 allowed prediction of germination rate at any sub-optimal and supra-optimal T and any Ψ. Comparisons with actual data are shown in Figure 5.8.

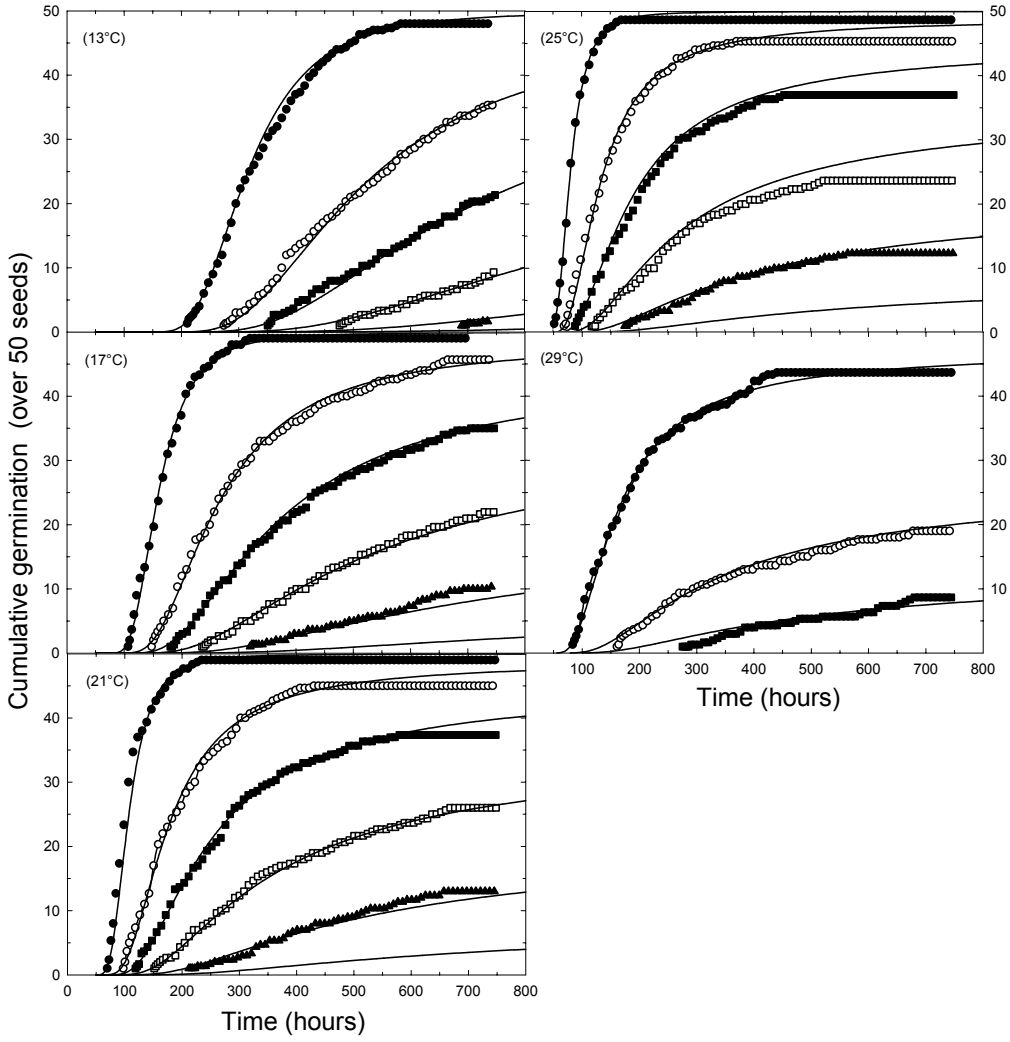


Figure 5.8: Normalized germination time courses of predicted values from hydrothermal time model (lines) compared with actual data (symbols) at sub-optimal and supra-optimal temperatures. Water potentials are 0 (●), -2 (○), -3 (■), -4 (□), and –5 (▲) bars.

The hydrothermal time model fitted almost perfectly to the actual germination time courses although it tended to overestimate the asymptotes. In some cases, mostly when temperatures were closer to either the base or ceiling temperatures, the model tended to overestimate time for germination at low ψ. As for the hydrotime model, in the sub-optimal range of temperatures, the hydrothermal time model predicted germination at -6 bars although none had been observed.

All germination time courses were transformed using Equation 5.10 in order to plot them on a normalised thermal time scale (Figure 5.9). The predicted time course in water had been calculated using the parameters for the hydrothermal time model (Table 5.2).

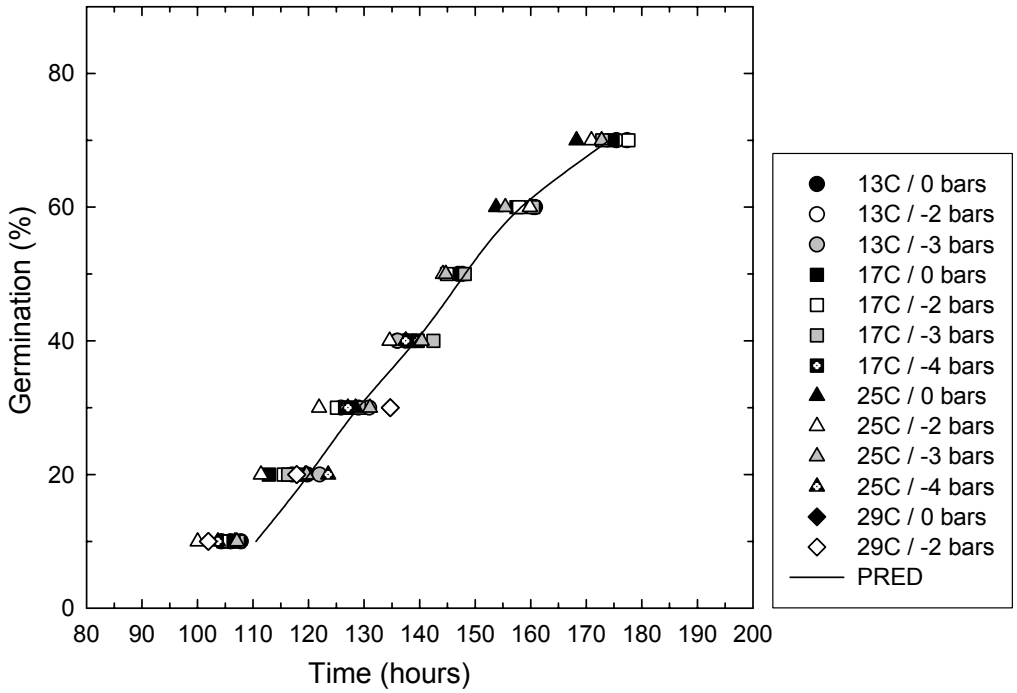


Figure 5.9: Germination time courses at all T and Ψ on a normalised thermal time scale (symbols) and the predicted germination time course normalised to a germination time course equivalent to that in water (line), calculated with the parameters shown in Table 5.2.

The normalisation of all germination time courses and their high coefficient of determination demonstrated the fit of the hydrothermal time model at any T and ψ. This also confirms that the model accounts well for the sensitivity of *Buddleia* seed population to both sub-optimal and supra-optimal temperatures and to water potentials.

Discussion

The hydrothermal time approach fitted the germination pattern observed in a laboratory, with much more accuracy than the hydrotime model. The discrepancy between the starting time of germination and predicted values at low water potentials can be linked to Kreabab and Murdoch (1999) study which stated an increase in T_b for seeds imbibed at low Ψ.

The fact that both the hydrotime and hydrothermal time models fitted germination at -6 bars while none have been observed during the experiments might be a result of (1) the fact that the accuracy of PEG 6000 solutions to provide a specific water potential has an uncertainty of ± 0.2 bars, or (2) the incubators showed variations in temperature of ± 1°C, which modify the water potential, although in this case all the

treatments should have been affected, or (3) a combination of both the previous variations.

The experiment was carried out during one month and a plateau in total germination was reached in most of the cases, however considering the Weibull distribution of the germination pattern of *Buddleia davidii*, it is very likely that a few late seeds might have germinated after one month, which might ultimately give reason to the predictions. However, as germination follows a Weibull pattern rather than a cumulative normal one, this may ultimately contribute to the overestimation of asymptotes.

In order to extend the predictions of this model to field conditions, one may want to model germination under varying temperature and/or water potentials. As specified by Bradford (2002), an accumulated hydrotime scale can be used under variable conditions, but either for only a specific seed fraction or by the incorporation into the model of the variation in $\Psi_b(g)$. Modifying the base water potential will adjust the fraction of germinable seeds, the rate of germination and the effect of T and Ψ on these (Bradford, 2002).

CHAPTER 6 - OVERALL DISCUSSION

The study described in Chapter 2 demonstrated that while location significantly influenced the germination pattern, the differences were minor at the scale of forest plantations. These results suggested that the germination model for *Buddleia davidii* might be applicable throughout New Zealand. Germination data from England showed the same sigmoid shape but the timing, rate and total germination were quite different. These differences could find their origin in ecotypic differentiation differentiation or perhaps in relative humidity during seed storage. However, considering that it is most likely that *Buddleia* species have a similar germination pattern around the world, *Buddleia* germination can still be predicted by a hydrothermal time model if previous germination tests are carried out to confirm or adjust the model parameters specific to a geographical area or to an ecotype which differentiated along the ages.

It was shown in Chapter 3 that if defoliation affected allometric relationships, it didn't modify in any way the germination of *Buddleia* in optimum conditions. However the impact on germination of the difference in seed size that appeared between defoliated and undefoliated plants cannot be clearly assessed because of the random selection of seeds. An interesting experiment to confirm the lack of effect of seed size on germination would be to divide seeds into lots of single weight classes and then germinate these lots separately. Knowing that in some cases defoliation affected seed size and seed size affects germination (Weis 1982, Dolan 1984, Hendrix 1984, Kalisz 1989), such an experiment would at least suppress the uncertainty of seed selection.

Key results of Chapter 4 were cardinal temperatures inferred for *Buddleia davidii*. In constant conditions, germination was achieved for temperatures between approximately 9 and 35°C. The rate of germination was fastest at 25°C, and from there appeared a decline in both the rate and total germination so that as temperature increases, fewer seeds germinate.

As demonstrated in Chapter 5, the rate of germination of *Buddleia davidii* seed was a function of hydrothermal time, as confirmed by the germination time courses and the models plotted on a similar normalised time scale and reduced to a single curve. In constant conditions, germination was predicted with much accuracy by Equation 5.9, however the latter equation needs to be checked for consistency through varying conditions experiments. The use of threshold models in varying conditions is an issue because when applied in varying conditions of temperatures and water potentials, Finch-Savage *et al*. (2000) observed that the time to germination of carrot in field conditions

was underestimated by the thermal time while it was overestimated by the hydrothermal time model except under very moist conditions. Bradford *et al*. (1993) showed that the hydrothermal time can be considered as a constant for specific seed samples and that it varies according to factors such as treatment and aging.

Regarding the temperature effects, Ellis and Barrett (1994) stated that rate of germination responds instantaneously to current temperatures. But the response to water potential variation seems to differ according to the species. While Rowse *et al*. (1999) noted that seeds of onion and carrot equilibrate immediately with the new water potential, Adams (1999) demonstrated that Callistris seeds retain the physiological changes induced by hydration, which suggests a hydration memory. The latter statement is confirmed by Vincent and Cavers (1978) and Dubrovsky (1996) who showed that hydrated-dehydrated seeds have the ability to retain physiological changes induced by the hydration phase. In addition, one weakness of the threshold models under their current expressions is that they do not take into account the fact that below the thresholds, a small advancement toward germination can still happen, even though it is too slow to be observed during experiments. Bradford (1995) stated that the addition of metabolic advancement below $\Psi_b(g)$ into the hydrothermal time concept might considerably improve predictions of germination time under field conditions. If *Buddleia* germination in constant conditions is now well predictable with the hydrothermal time model, a lot of work is required before we can apply these threshold models to seeds in variable field conditions.

CONCLUSION

This study highlighted the major importance of temperature and moisture and the insignificance of defoliation and location in regulating the germination of *Buddleia davidii*. Cardinal temperatures for *Buddleia davidii* had been defined as 8.9°C for the base temperature, 25°C for the optimum and from 30 to 35°C for the ceiling temperatures.

The three population threshold models demonstrated the sensitivity of *Buddleia* to heat and water sum above a threshold that allow it to go forth toward germination. The best fit of predictions against actual values was obtained with the hydrothermal time model, and its accuracy at any water potential and any sub- and supra-optimal temperature makes this model eligible to be used as a tool for describing and predicting *Buddleia* germination under constant conditions throughout New Zealand.

REFERENCES

Adams R. 1999. Germination of *Callistris* seeds in relation to temperature, water stress, priming, and hydration-dehydration cycles. *Journal of Arid Environments* 43: 437-448.

Aizen A.M. and Raffaele E. 1997. Flowering-shoot defoliation affects pollen grain size and postpollination pollen performance in *Alstroemeria aurea*. *Ecology* 79, No. 6, pp. 2133–2142.

Allen P.S., Meyer S.E., and Khan M.A. 2000. Hydrothermal time as a tool in comparative germination studies. In: M. Black, K.J. Bradford and J. Vazquez-Ramos (editors), *Seed Biology*: Advances and Applications, CAB International, Wallingford, Oxon, pp. 401-410.

Alvarado V. and Bradford K.J. 2002. A hydrothermal time model explains the cardinal temperatures for seed germination. *Plant, Cell and Environment* 25: 1061-1069.

Alm D.M., McGiffen Jr M.E. and Hesketh J.D. 1991. *Weed phenology*. In: Hodges T, ed. Predicting crop phenology. Boca Raton, FL: CRC Perss, 191-218.

Amen, R.D. 1963. The concept of seed dormancy. *American Scientist* 51: 409-424.

Apollo B., Orodho R.L. and Trlica M.J. 1998. Previous grazing or clipping affects seed of Indian ricegrass. *Journal of Range Management* 51: 37-41.

Bazzaz F.A., Chiariello N. R., Colley P.D. and Pitelka L. F. 1987. Allocating resources to reproduction and defence. *Bioscience* 37: 58–67.

Bell G., Lechowicz M.J. and Schoen D.J. 1991. The ecology and genetics of fitness in forest plants. III Environmental variance in natural populations of *Impatiens pallida*, *Journal of Ecology* 79: 697-713.

Bellingham P.J., Peltzer D.A. and Walker L.R. 2005. Contrasting impacts of a native and an invasive exotic shrub on flood-plain succession. *Journal of Vegetation Science* 16: 135-142

Bewley J.D. and Black M. 1978. Physiology and biochemistry of seeds in relation to germination. I. Development, Germination and Growth. Springer-Verlag, Berlin.

Biere A. 1991. Parental effects in *Lychnis flos-cuculi.* II: Selection on time of emergence and seedling performance in the field. *Journal of Evolutionary Biology* 3: 467–486.

Bierhuizen J.F. and Wagenvoort W.A. 1974. Some aspects of seed germination in vegetables. I. The determination and application of heat sums and minimum temperature for germination. *Scientia Horticulturae* 2: 213-219.

Binggeli P. 1998. An Overview of Invasive Woody Plants in the Tropics. School of Agricultural and Forest Sciences Publication Number 13, Unversity of Wales, Bangor.

Bonhomme R. 2000. Bases and limits to using 'degree.day.' units. *European Journal of Agronomy* 13:1-10.

Bradford K.J. 1990. A water relation analysis of seed germination rates. *Plant Physiology* 94: 840-849.

Bradford KJ. 1995. Water relations in seed germination. In: Kigel, J; Galili, G, eds. *Seed Development and Germination*. New York, USA: Marcel Dekker, 351–396.

Bradford K.J., Tarquis A.M., and Duran J.M. 1993. A population based-model describing the relationship between germination rates and seed deterioration. *Journal of Experimental Botany* 44: 1225-1234.

Bradford K.J. and Somasco O.A. 1994. Water relations of lettuce seed thermoinhibition. I. Priming and endosperm effects on base water potential. *Seed Science Research* 4: 1-10.

Bradford K.J. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science 50*: 248-260.

Brockerhoff E.G., Withers T. M., Kay M. and Faulds W. 1999. Impact of the defoliator *Cleopus japonicus* (Coleoptera: Curculionidae) on *Buddleja davidii* in the laboratory. Proc. 52nd N.Z. Plant Protection Conf. 113-118.

Brown R.F. 1987. Germination of *Aristida armata* under constant and alternating temperatures and its analysis with the cumulative Weibull distribution as a model. *Australian Journal of Botany* 35: 581-591.

Brown R.F. and Mayer D.G. 1988. Representing cumulative germination. 2. The use of the Weibull Function and other empirically derived curves. *Annals of Borany* 61: 127-138.

Brown J.S. and Venable D.L. 1986. Evolutionary ecology of seed bank annuals in temporally varying environments. *American Naturalist* 127: 31–47.

Chapin F.S. and Chapin M.C. 1981. Ecotypic differentiation of growth processes in *Carex aquatilis* along latitudinal and local gradients. *Oecologia* 76:158-159.

Choe H.S., Chu C., Koch G., Gorham J. and Mooney H.A. 1988. Seed weight and seed resources in relation to plant growth rate. Oecologia 76: 158–159.

Cohen D. 1966. Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology* 12: 119–129.

Covell S., Ellis R.H., Roberts E.H. and Summerfield R.J. 1986. The influence of temperature on seed germination rate in grain legumes. I. A comparison of chickpea, lentil, soybean, and cowpea at constant temperatures. *Journal of Experimental Botany* 37: 705-715.

Dahal P., Bradford K.J. and Jones R.A. 1990. Effects of priming and endosperm integrity on seed germination rates of tomato genotypes. I. Germination at sub-optimal temperatures. *Journal of Experimental Botany* 41: 1431-1439

Dahal P. and Bradford K.J. 1990. Effects of priming and endosperm integrity on seed germination rates of tomato genotypes. II. Germination at sub-optimal temperatures. *Journal of Experimental Botany* 41: 1441-1453

Dahal P. and Bradford K.J. 1994. Hydrothermal time analysis of tomato seed germination at sub-optimal temperatures and reduced water potential. *Seed Science Research* 4: 71-80.

Dias L.S. 2001. Describing phytotoxic effects on cumulative germination. *Journal of Chemical Ecology* 27: 411-418.

Dolan R. W. 1984. The effect of seed size and maternal source on individual size in population of *Ludwiqia leptocarpa*. *American Journal of Botany* 71: 1302-1307.

Doneen L.D. and MacGillivray J.H. 1943. Germination (emergence) of vegetable seeds as affected by different soil moisture conditions. *Plant Physiology* 18: 524-529.

Dubrovsky J.G. 1996. Seed hydration memory in Sonoran Desert cacti and its ecological implications. *American Journal of Botany* 68: 227-233.

Dumur D., Pilbeam C.J. and Craigon J. 1990. Use of the Weibull function to calculate cardinal temperatures in faba bean. *Journal of Experimental Botany* 41: 1423–1430.

Ellis R.H. and Barrett S. 1994. Alternating temperatures and rate of seed germination in Lentil. *Annals of Botany* 74: 519-524.

Ellis R.H., Covell S., Roberts E.H. and Summerfield R.J. 1986. The influence of temperature on seed germination rate in grain legumes. II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. *Journal of Experimental Botany* 37: 1503-1515.

Ellis R.H. and Butcher P.D. 1988. The effects of priming and 'natural' differences in quality amongst onion seed lots on the response of the rate of germination to temperature and the identification of the characteristics under genotypic control. *Journal of Experimental Botany* 39: 935-950.

Eriksson O. 1999 Seed size variation and its effect on germination and seedling performance in the clonal herb *Convallaria majalis*. Acta Oecologica-*International Journal of Ecology* 20: 61-66

Finch-Savage W.E. and Phelps K. 1993. Onion (Allium cepa L.) seedling emergence patterns can be explained by the influence of soil temperature and water potential on seed germination. *Journal of Experimental Botany* 50: 89-99.

Finch-Savage W.E., Steckel J.R.A. and Phelps K. 1998. Germination and post-germination growth to carrot seedling emergence: predictive threshold models and sources of variation between sowing occasions. *New Phytologist* 139: 505-516.

Finch-Savage W.E., Phelps K., Peach L. and Steckel J.R.A. 2000. Use of threshold germination under variable field conditions. Pages 489-497 nM. Black, K.J. Bradford and J.Vásquez-Ramos, eds. *Seed Biology*: Advances and Applications Wallingford, K, CABI Publishing.

Foster DR. 1986. On the adaptive value of large seeds for tropical moist forest trees: a review and synthesis. *Botanical Reviews* 53: 260–299.

Fyfield T.P. and Gregory P.J. 1989. Effects of temperature and water potential on germination, radicle elongation and emergence of Mungbean. *Journal of Experimental Botany* 40: 667-674.

Garcia-Huidobro J., Monteith J.L. and Squire G.R. 1982. Time, temperature and germination of pear millet (*Pennisetum thyphoides* S. and H.). I. Constant temperatures. *Journal of Experimental Botany* 33: 288-296.

Gerrit A.J. and Platenkamp G.A.J. 1991. Phenotypic plasticity and population differentiation in seeds and seedlings of the grass *Anthoxanthum odoratum*. *Oecologia* 88: 515-520.

Gillmann J.H., Dirr M.A. and Braman S.K. 1998. Effects of dolomitic lime on growth and nutrient uptake of *Buddleia davidii* `Royal Red` grown in pine bark. *Journal of Environmental Horticulture* 16: 111-113

Grant V. and Wilken D.H. 1988. Racial variation in *Ipomopsis tenuituba* (Poemoniaceae). *Botanical Gazette* 149: 443-449.

Gross K.L. 1984. Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. *Journal of Ecology* 72: 369–387.

Gross K.L. and Smith A.D. 1991. Seed mass and emergence time effects on performance of *Panicum dichotomiflorum* Michx. across environments. *Oecologia* 87: 270–278.

Grundy A.C., Phelps K., Reader R.J. and Burston S. 2000. Modelling the germination of *Stellaria media* using the concept of hydrothermal time. *New Phytologist* 148: 433-444.

Gummerson R.J. 1986. The effect of constant temperatures and osmotic potential on the germination of sugar beet. *Journal of Experimental Botany* 41: 1431-1439.

Hacker J.B. 1984. Genetic variation in seed dormancy in *Digitaria milanjiana* in relation to rainfall at the collection site. *Journal of Applied Ecology* 21: 947–959.

Hacker J.B. and Ratcliff D. 1989. Seed dormancy and factors controlling dormancy breakdown in buffel grass accessions from contrasting provenances. *Journal of Applied Ecology* 26: 201–212.

Harper J.L. 1977. The population biology of plants. London: Academic Press.

Harper J.L., Lovell P.H. and Moore K.G. 1970. The shape and sizes of seeds. *Annual Review of Ecology and Systematics* 1: 327–356.

Hegarty T.W. 1976. Effects of fertilizer on the seedling emergence of vegetable crops. *Journal of the Science of Food and Agriculture* 27: 962-968.

Hendrix S. 1984. Variation in seed weight and its effects on germination in *Pastinaca sativa* L. *American Journal of Botany* 71, 795-802.

Heslop-Harrison, J. 1964. Forty years of genecology. Advanced Botanical Research. 2: 159-247.

Hunter J.R. and Erikson A.E. 1952. Relation of seed germination to soil moisture tension. *Ibid* 44: 107-109.

Humphries R.N. and Guarino L. 1987. Soil nitrogen and the growth of birch and *Buddleia* in abandoned chalk quarries. *Reclamation and Revegetation Research 6:* 55-61.

Julien M.H. and Griffith M.W. 1998. Biological control of weeds: a World Catalogue of agents and their target weeds. CAB International, Wallingford, Oxon, UK. 223pp.

Kalisz S. 1989. Fitness consequences of mating system, seed weight, and emergence date in a winter annual, *Collinsia verna*. *Evolution* 43: 1263-1272.

Karssen C. 1982. The physiology and biochemistry of seed development, dormancy and germination. Elsevier-North Holland Biomedical Press. Amsterdam.

Koptur S., Smith C.L. and Lawton J.H. 1996. Effects of artificial defoliation on reproductive allocation in the common vetch, *Vicia sativa* (Fabaceae: Papilionoideae). *American Journal of Botany 83* (7): 886-889.

Kreabab E. and Murdoch A.J. 1999. Modelling the effects of water stress and temperature on germination rate of *Oribanche aegyptiaca* seeds. *Journal of Experimental Botany* 50: 655-664.

Labouriau L.G. 1970. On the physiology of seed germination in *Vicia graminea* Sm.-I. *Annals Academia Brasilia Ciencia* 42, 235-262.

Lalonde R.G. and Roitberg B.D. 1989. Resource limitation and offspring size and number trade-offs in *Cirsium arvense* (Asteraceae). *American Journal of Botany* 76: 1107–1113.

Larsen S.U., Bailly C., Côme D. and Corbineau F. 2004. Use of the hydrothermal time model to analyse interacting effects of water and temperature on germination of three grass species. *Seed Science Research* 14: 35-50.

Lee T. D. 1988. Patterns of fruit and seed production. Pages 179–202 *in* J. Lovett Doust and L. Lovett Doust, editors. Plant reproductive ecology: patterns and strategies. Oxford University Press, New York, New York, USA.

Levin D.A.; Kerster, H.W. 1968. Local gene dispersal in *Phlox pilosa*. *Evolution* 22: 130-139.

Lortie C.J. and Aarssen L.W. 1996. The specialization hypothesis for phenotypic plasticity in plants. *International Journal of Plant Science* 157: 484–487.

Macdonald S.E. and Chinnappa C.C. 1989. Population differentiation for phenotypic plasticity in the *Stellaria longipes* complex. *American Journal of Botany* 76: 1627–1637.

McFadyen R.E.C. 1998. Biological control of weeds. *Ann. Rev. Entomol*. 43: 369-393.

McGinley M.A. 1989. Within- and among-plant variation in seed mass and pappus size in *Tragopogon dubius*. *Canadian Journal of Botany* 67: 1298–1304.

McMillan C. 1965. Ecotypic differentiation within four North American prairie grasses. II. Behavioral variation within transplanted community fractions. *American journal of Botany* 52:55-65.

McWilliams E.L. 1966. Ecotypic differentiation within *Amaranthus retroflexus* L., *Amaranthus hybridus* L. and *Amaranthus powellii* Wats. Ph.D Thesis. Iowa State University, Ames, Iowa. 174 p.

McWilliams E.L., Landers R.Q. and Mahlstede J.P. 1967. Variation in seed weight and germination in populations *amaranthus retroflexus* L. *Ecology* 49: 290-296.

Mergen F. 1963. Ecotypic variation in *Pinus strobes* L. *Ecology* 44: 716-727.

Michel B.E. 1983. Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiology* 72: 66-70.

Miller A. 1984. The distribution and ecology of *Buddleja davidii* Franch in Britain, with particular reference to conditions supporting germination and the establishment of seedlings. CNAA. Oxford, Oxford Polytechnic: 262.

Mogie M., Latham J.R. and Warman E.A. 1990. Genotype-independent aspects of seed ecology in Taruxacum. *Oikos* 59: 175-182.

New, J.K. 1958. A population study of *Spergula arvensis*. *Annals of Botany* 22: 457-477.

Obeso J.R. 1993. Does defoliation affect reproductive output in herbaceous perennials and woody plants in different ways? *Functional Ecology* 7: 150–155.

Owen D.F. and Whiteway W.R. 1981. *Buddleia davidii* in Britain: history and development of an associated fauna. *Biological Conservation* 17: 149-155.

Platenkamp G.A.J. and Shaw R.G. 1993 Environmental and genetic maternal effects on seed characters in *Nemophila menziesii. Evolution* 47: 540–555.

Quesada M., Bollman K. and Stephenson A.G. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology* 76: 437–443.

Richardson B., Vanner A., Ray J., Davenhill N. and Coker G. 1996. Mechanisms of *Pinus radiata* growth suppression by some common forest weed species. *New Zealand Journal of Forestry Science* 26: 421-437.

Richardson B. and West G. 1993. Radiata growth trends following weed control. In: Proceedings, Paper presented at Weedworks '93, Forest Research Institute, Rotorua.

Roberts E.H. 1988. Temperature and seed germination. S.P. Long and F.I. Woodward, eds. Plants and Temperature. Cambridge. Society for Experimental Botany. Pages 109-132.

Roberts E. H. and Ellis R. H. 1989. Water and seed survival. *Annals of Botany* 63: 39-52.

Romo J.T. and Haferkamp M.R. 1987. *Forage kochia* germination response to temperature, water stress and specific ions. *Agronomy Journal* 79: 27-30.

Rowse H.R., McKee J.M.T and Higgs E.C. 1999. A model of the effects of water stress on seed advancement and germination. *New Phytologist* 143: 273-279.

Rowse H.R. and Ficnh-Savage W.E. 2003. Hydrothermal threshold models can describe the germination response of carrot(*Daucus carota)* and onion *(Allium cepa)* seed populations across both sub- and supra-optimal temperatures. *New Phystologist* 158: 101-108.

Schaal B.A. 1980. Reproductive capacity and seed size in *Lupinus texensis*. *American Journal of Botany* 67: 703-709

Simons A.M. and Johnston M.O. 2000. Variation in seed traits of *Lobelia inflata* (Campanulaceae): sources and fitness consequences. *American Journal of Botany* 87:124-132

Smale M.C. 1990a. Ecological role of *Buddleia* (*Buddleja davidii*) in streambeds in Te Urewera National Park. *New Zealand Journal of Forestry* 14: 1-6.

Smale M.C. 1990b. *Buddleia* - a growing weed problem in protected areas. *What's new in Forest Research* 185: 4pp.

Stanton M.L. 1984. Seed variation in wild radish: effect of seed size on components of seedling and adult fitness. *Ecology* 65: 1105–1112.

Taylor D.R., Aarssen L.W. 1988. An interpretation of phenotypic plasticity in *Agropyron repens* (Graminae). *American Journal of Botany* 75: 401–413.

Thompson P.A. 1973. Effects of fluctuating temperatures on germination. *Journal of Experimental Botany* 25, 164-175.

Venable D.L. and Lawlor L. 1980. Delayed germination and dispersal in desert annuals: escape in space and time. *Oecologia* 46: 272–282.

Vincent E.M. and Cavers P.B. 1978. The effects of wetting and drying on the subsequent germination of *Rumex crispus*. *Canadian Journal of Botany* 56: 2207-2217.

Volis S., Mendlinger S. and Ward D. 2002. Differentiation in populations of *Hordeum spontaneum* along a gradient of environmental productivity and predictability: life history and local adaptation. *Biological Journal of the Linnean Society* 77: 479–490.

Washitani I. and Saeki T. 1986. Germination responses of *Pinus densiflora* seeds to temperature, light and interrupted imbibition. *Journal of Experimental Botany* 37: 1376–1387.

Webb C.J., Sykes W.R. and Garnock-Jones P.J. 1988. Flora of New Zealand. Christchurch, Dept. of Scientific and Industrial Research.

Weis I. M. 1982. The effects of propagule size on germination and seedling growth in Mirabilis hirsute. Canadian Journal of Botany 60, 1868-1874.

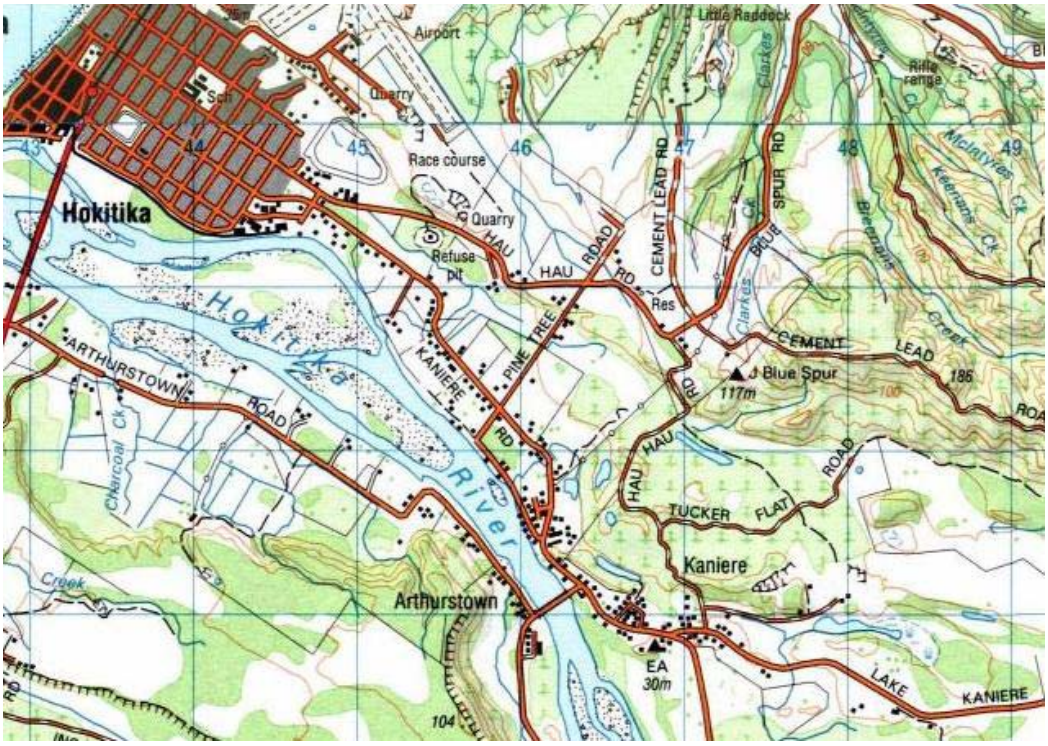
Wesson G. and Wareing P.F. 1968. The induction of light sensitivity in weed seeds by burial. Journal of Experimental Botany 20: 414-425.

Winn, A.A. 1988 Ecological and evolutionary consequences of seed size in Prunella vulgaris. Ecology 69: 1537–1544.

Wood H. and Degabriele R. 1985. Genetic variation and phenotypic plasticity in populations of Paterson's curse (Echium plantagineum L.) in South-eastern Australia. Australian Journal of Botany 33, 677-685.

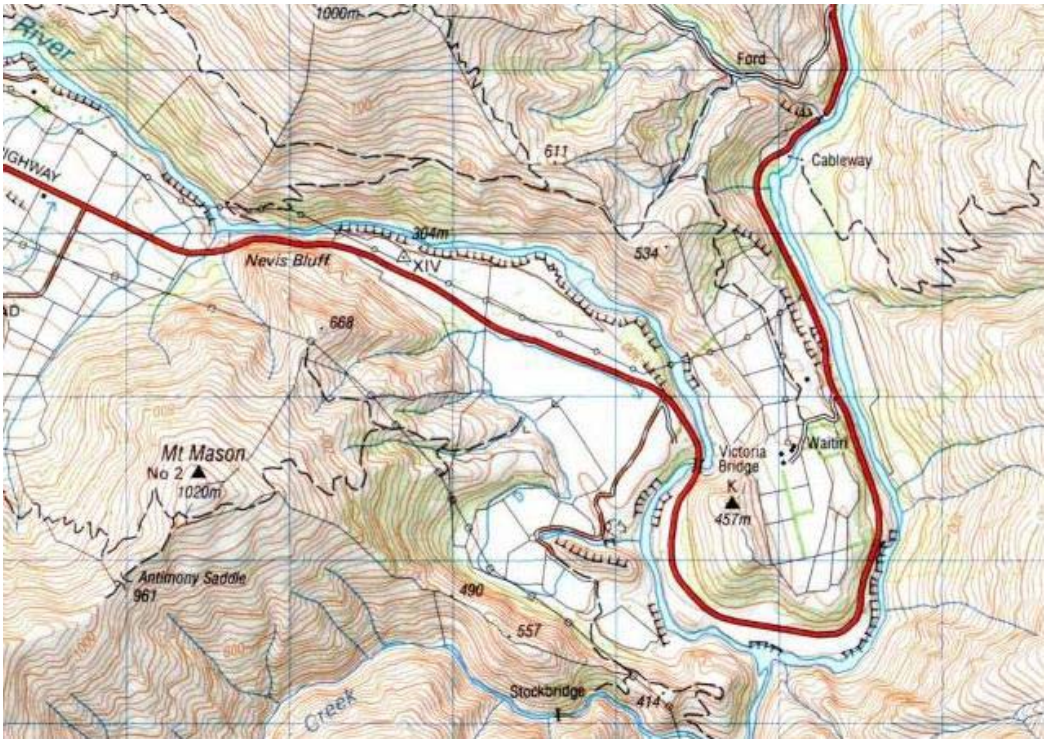
ANNEXE – SITE MAPS

- Hokitika: Kaniere



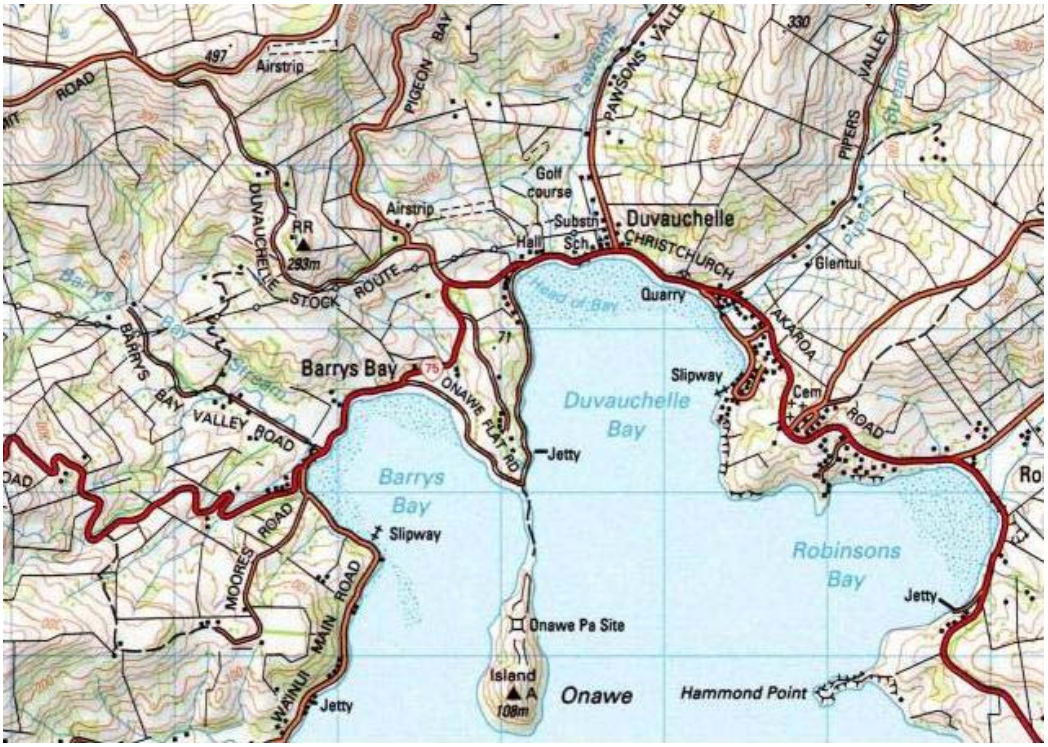
Coordinates: 42.74S / 171.01E

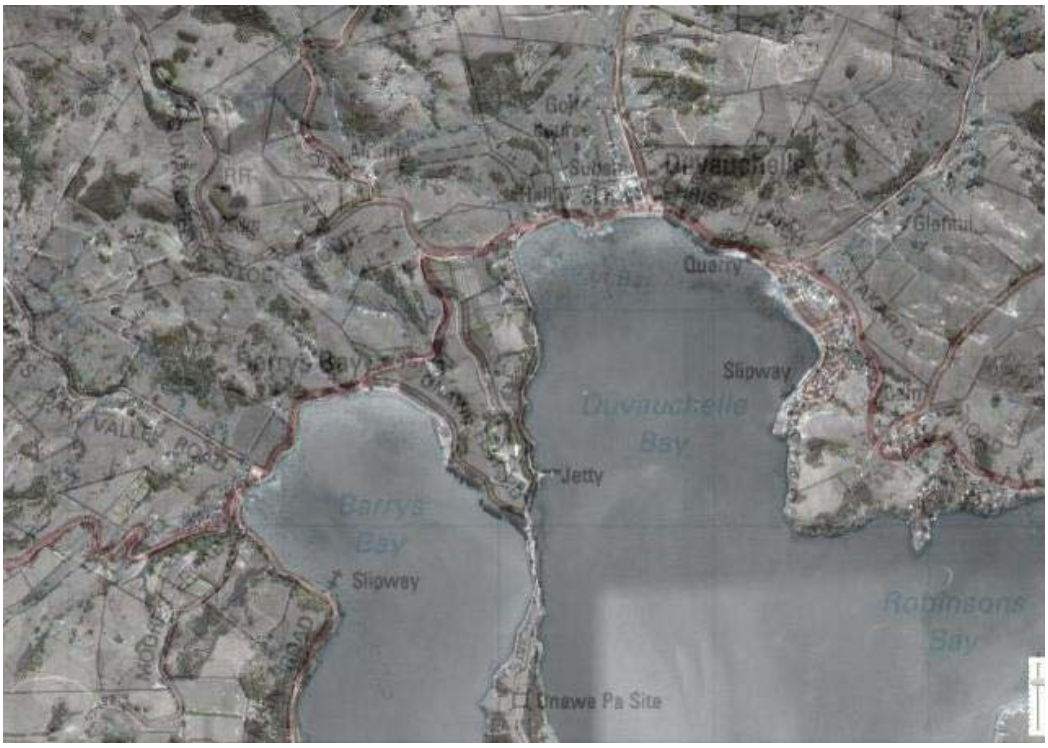
- Queenstown: Kawarau river



Coordinates: 45.04S / 169.04E

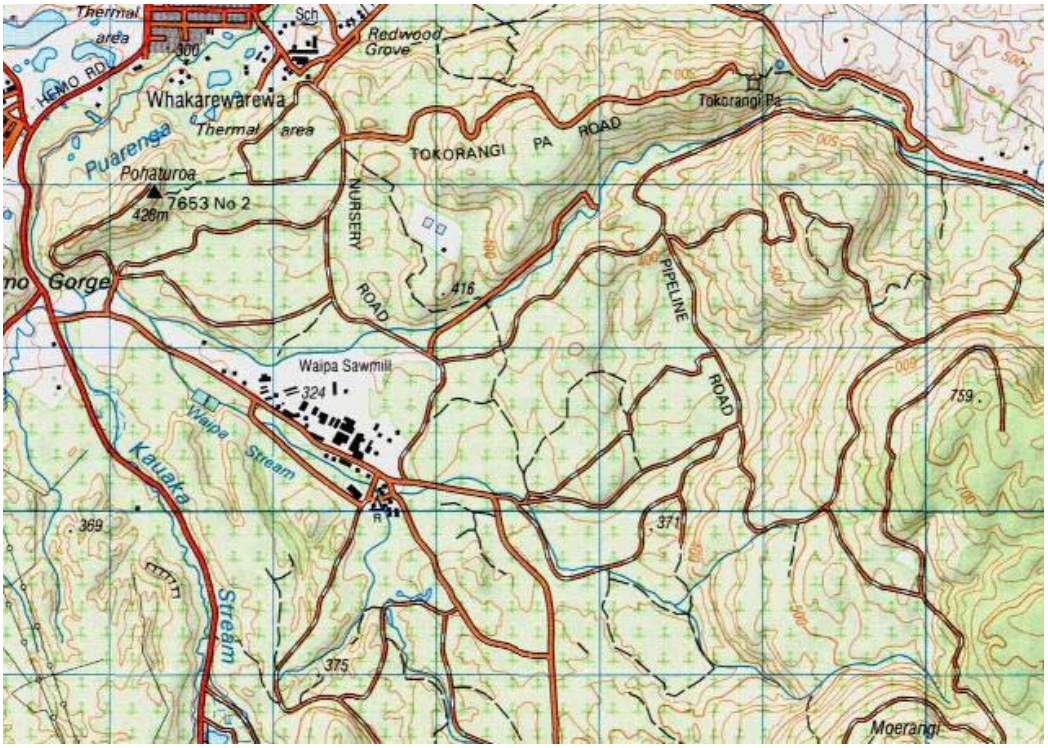
- Akaroa: Barrys Bay





Coordinates: 43.76S / 172.91E

- Rotorua:



Coordinates: 38.18S / 176.28E