The roles of temperature cues and resources in mast flowering

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Abstract

Masting is where individual plants within a species have highly variable, but synchronised, flowering within their population, a strategy which leads to increased fitness. Two important factors controlling the size of each year's flower crop in masting plants are temperature and resources. Under climate change, temperature cues plants respond to will be altered and may result in plants flowering regularly, which would remove the benefits of masting (e.g. predator satiation). Likewise, climate change can alter the resources available to plants for growth and flowering, potentially increasing their vulnerability to biotic and abiotic factors and also changing their flowering regime.

In this study, I set out to test the response of masting plants to temperature and resource cues to compare two previously hypothesised models for predicting the size and timing of masting events. 1) The temperature one year prior to flowering (T1) + resources (Monks *et al.*, 2016). 2) The temperature difference (DT) between one year prior and two years prior to flowering (T1 – T2 = DT)(Kelly *et al.*, 2013). I used two alpine snow tussocks (*Chionochloa pallens* and *C. macra*) and a mountain daisy (*Celmisia lyallii*) at Mt Hutt in Canterbury. Temperature cues were manipulated by transplanting plants to different altitudes on Mt Hutt and near sea level to University of Canterbury (UC). These manipulations allowed measurement of flowering responses to cooler and warmer temperatures, including extreme warming at UC. Resource cue experiments involved adding NPK fertiliser to naturally occurring tussocks at different altitudes on Mt Hutt and to potted transplants at UC. Plant responses (flowering and vegetative growth) were compared to control (unfertilised) plants at each site.

I constructed temperature series for Mt Hutt. The best estimates for gaps in on-site records were usually from NIWA VCSN data, though Darfield and Christchurch temperatures were also good. Temperature cues significantly explained the flowering response of plants in long observational datasets, with DT cues better than T1 (absolute) temperatures, supporting the DT model in these three species. In transplants moved to different temperatures, DT temperatures again gave significant predictors for *Chionochloa* but T1 better predicted for *Celmisia*, although the *Celmisia* dataset was small as none flowered at Mt Hutt. Fertiliser application produced no increase in flowering after one year for study species, but there was an increase in vegetative growth (new tillers or rosettes), suggesting that temperature cues are more important than resource cues for flowering in these species. Flowering did increase in the second year for fertiliser *C. pallens* plants which had been at UC for four years and fertiliser *C. lyallii* plants which had been at UC for 3 years, showing that resources do have an important, albeit secondary, role. With the temperature difference explaining flowering better in these species it implies that if global temperatures rise the variability of mast events should be maintained, and this should be true for other masting species where the DT model explains observed flowering. However, in the more extreme temperatures at UC some plants displayed abnormal flowering and growth responses suggesting climate change may have some quite negative consequences for masting species.

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Chapter 1. Introduction

Masting is a term used for the sporadic, but highly synchronised, reproductive effort of plants within and between species in populations, and can occur over large spatial scales (Kelly, 1994). During a high masting year, a large percentage of plants in a population will put a lot of effort into flowering and seed production. In contrast, in a low masting year, a small percentage of plants in a population will put minimal effort into reproduction, with a lot producing no flowers at all. The level of reproductive effort in masting species is not just high or low, however, and instead falls along a gradient from high to low including everything in between (Kelly, 1994). There is no set pattern for when high and low masting years occur, and it is possible for populations to produce few flowers for several years or to have two successive high masting years, although the latter is quite rare. Masting is a strategy utilised by plants worldwide, although it is especially prominent in New Zealand species (Webb and Kelly, 1993), and the synchrony of flowering at a particular location can occur between species from very different families (Kelly *et al.*, 2000; Schauber *et al.*, 2002; Kelly *et al.*, 2013).

1.1 Evolutionary factors behind masting as a strategy

For some time, scientists have been trying to understand the evolutionary (ultimate causes) and mechanistic (proximate causes) reasons behind masting in plants (Norton and Kelly, 1988; Kelly, 1994; Pearse *et al.*, 2014; Pearse *et al.*, 2016). Masting appears to be a costly strategy as there is more density-dependent mortality (Antonovics and Levin, 1980; Lambers *et al.*, 2002), and opportunities for reproduction (e.g. gaps where seedlings might establish) may be missed in low years. However, several compensating benefits have been identified from this reproductive strategy due to the economies of scale effect, such as pollen coupling, predator satiation (Kelly, 1994), and re-population after natural disasters (e.g. fire)(Pearse *et al.*, 2016). Firstly, the majority of masting plants are wind pollinated (Norton and Kelly, 1988) so the synchrony of flowering allows for greater pollination and cross-pollination within species at the same location (Kelly *et al.*, 2001). Even animal-pollinated plants might benefit during masting years as there are more flowers available to entice animal pollinators, such as insects or birds, to the area (Kelly, 1994). Secondly, species that suffer large losses to seed predators can reduce this loss because the predator's populations are kept low due to the small quantity of food available in low masting years (Kelly *et al.*, 2000). Subsequently, in masting years the low predator numbers are satiated by the large quantity of seeds and many more seeds survive, this is especially so if the seed predators are specialised to particular plant species (Kelly and Sullivan, 1997). Furthermore, it is suggested that one of the reasons for synchrony of

masting between different species is to help keep generalist seed predator numbers low so that seeds of each of the different species are more likely to survive during high masting years (Kelly *et al.*, 2000). Masting plants also tend to be long-lived (>100 years) so the costs of only reproducing intermittently are relatively low (Ims, 1990). Additionally, their seeds often last a long time in the seed bank (Rees *et al.*, 2002) so they can germinate several years after the initial seedfall, a handy factor after disturbance by fire.

1.2 Proximate factors controlling masting

The two main proximate mechanisms believed to control masting are climate and resources (Richardson *et al.*, 2005; Smaill *et al.*, 2011; Tanentzap *et al.*, 2012). Early studies identified a link between higher temperatures and the induction of flowering prior to masting events (Connor, 1966). Indeed, warmer than average temperatures appear to be responsible for inducing the mast flowering which takes place the next flowering season in multiple masting species (Mark, 1965c; Norton and Kelly, 1988; Schauber *et al.*, 2002; Övergaard *et al.*, 2007), albeit during specific times of the year for different species. Some researchers had further noticed a trend where a cooler and wetter year often occurred two years prior to large masting events (Norton and Kelly, 1988; Piovesan and Adams, 2001; Schauber *et al.*, 2002; Smaill *et al.*, 2011). For example, Piovesan and Adams (2001) found that a lack of water during a hot drought period one year prior to flowering correlated well with masting in North American and European *Fagus* (beech) species, but even more so if they had also experienced a cooler, wetter summer two years prior to flowering. Kelly *et al.* (2013) proposed that the temperature difference (ΔT or DT) between two years prior (T_{-2}) and one year prior (T_{-1}) to a flowering event determines how big the flower or seed crop will be (i.e. $T_{-1} – T_{-2}$ = DT which predicts population flowering effort). Therefore, the larger the DT the larger the flowering event will be. There is some debate about masting being solely controlled by a temperature cue, however, and Monks *et al.* (2016) suggest that the effect of T_{-2} in the DT model is just a proxy for flowering in year T_{-1}, which will be low if T_{-2} was cold, so that free resources in T_{-1} are higher. Hence Monks *et al.* (2016) say the mechanism is driven by last summer's temperatures (T_{-1}), and free resources that same summer.

Mark (1968) mentions a personal communication with C. J. Burrows where Burrows suggested that flowering in *Chionochloa* after high temperatures may be an indirect effect of an increase in available nutrients, particularly nitrogen and phosphorus, due to greater temperature-induced mineralisation of humus. Burrows also informed Mark (1968) of his observation that in light to very light flowering years *Chionochloa* flowering could be confined to areas such as along the edges of scree or erosion patches. Factors leading to increased flowering in low-masting years on

edges of plant patches were investigated in *Chionochloa* at Mt Hutt by Hay *et al.* (2008) who found significantly greater amounts of nitrogen in the soil in the upper 2 m of up-slope edges, and a similar (but non-significant) trend in phosphorus, compared to both the interior and down-slope edge of patches. This suggests that greater quantities of these two nutrients are important for higher levels of flowering. Additionally, Körner (1989) found that globally, high alpine plants invariably had higher nitrogen levels within their leaves than conspecifics at lower altitudes, suggesting high altitude plants are not limited in nitrogen accumulation. These higher nitrogen concentrations may be the result of high altitude plants maintaining smaller stature, despite having nitrogen levels allowing greater growth (Körner, 1989), due to smaller sizes being more advantageous in regard to other alpine environmental factors, such as temperature, snow, and wind. Körner (1989) advised that finding the reason for this nitrogen-accumulation increase with altitude would require fertiliser experiments monitoring the response of growth and development in plants.

Resource budget models were put forward as possible mechanistic explanations for the pattern of flowering in masting plants (Isagi *et al.*, 1997). Although early empirical studies looked at the role of carbon stores on masting (Richardson *et al.*, 2005; Crone *et al.*, 2009), carbon was later shown to be an unlikely resource-limiting factor (Crone and Rapp, 2014) as it is not depleted during seed production (Hoch *et al.*, 2013) or plays a minor role in seed production compared to nitrogen (Abe *et al.*, 2016) in some studied species. Resource budget models were reframed to concentrate on more likely resource-limiting nutrients, nitrogen and phosphorous (Callahan *et al.*, 2008; Ichie and Nakagawa, 2013; Crone and Rapp, 2014). Empirical studies have found these two nutrients to play an important role in flowering (Hay *et al.*, 2008; Han *et al.*, 2014; Miyazaki *et al.*, 2014).

A link between the use, and depletion, of seasonally stored nitrogen for leaf production and flowering has been identified in *Fagus crenata,* where a predetermined nitrogenous effort goes into leaf production each year but greater nitrogen depletions occur after a masting year (Han *et al.*, 2008). Therefore, stored nitrogen availability appears to be an important factor inhibiting *F. crenata* from flowering again the year after heavy masting as nitrogen is required in great quantity to produce reproductive tissue. Han *et al.* (2014) later found that the nitrogen levels in mature *F. crenata* trees are significantly reduced the year following a large masting event. The trees then required a year to restore these internal nitrogen levels before they could flower again, with older stands of trees being replenished faster than younger stands and able to flower again sooner (Han *et al.*, 2014).

Experiments analysing the effects of nitrogen-fertiliser addition on various masting species have generally resulted in an increase in flowering. Smaill *et al.* (2011) added nitrogen-fertiliser to *Nothofagus solandri* (mountain beech), which increased seedfall compared to unfertilised trees in

the years where there was a temperature cue to initiate flowering. In a genetic study, Miyazaki *et al.* (2014) found that nitrogen-fertiliser addition significantly upregulated the expression of flowering genes in *F. crenata*. Furthermore, the fertilised trees all flowered whereas none of the controls did, and when Miyazaki *et al.* (2014) repeated the exact same fertiliser application on the same trees for a second year running the same outcome was achieved, giving support to the resource budget model and establishing the importance of nitrogen in the regulation of mast flowering. Bogdziewicz *et al.* (2017a) studied the long-term effects (25+ years) of nitrogen-fertiliser application at low (50 kg/ha/year) and high (150 kg/ha/year) rates on *Quercus rubra* (red oak). Over a three-year period, acorn production significantly increased in fertilised trees, more in the high application rate than the low rate, with high-rate trees significantly greater than controls in all three years (Bogdziewicz *et al.*, 2017a). A negative effect of this increased acorn production with high nitrogen levels was the removal of predator satiation benefits (Bogdziewicz *et al.*, 2017a).

Mast-flowering can occur without corresponding mast-seeding, as seen in the dioecious *Juniperus thurifera*, showing that uncoupling of these two events is able to take place. Two possible reasons for this are resource-limitation and extreme weather events. If the female plants do not have the resources to produce high seed-set after receiving a temperature initiation cue then abortions may occur as flower production is far less resource-demanding than seed production (Montesinos *et al.*, 2012). Likewise, if severe weather events take place after fertilisation of ovules seed production may be vetoed (Bogdziewicz *et al.*, 2018) . The destructive effect of severe weather events may be a further way some masting species can synchronise as their resource stores will fluctuate similarly if seed production has been halted to a large degree throughout an entire population (Bogdziewicz *et al.*, 2018).

Satake and Bjørnstad (2008) altered the resource budget model to account for variation in the productivity (resource availability) of the sites that masting plants of the same species may occupy and which result in differences in masting frequencies. The study species used to test this altered model was *Sorbus aucuparia* (Rowan), which generally masts every two years in west-southern Norway but every three years in east-southern Norway. Satake and Bjørnstad (2008) showed that variation in masting intervals can vary within a species depending on the availability of resources at specific sites.

Whether climate or resources are the main contributing mechanism resulting in the amount of flowering effort in masting plant populations from year to year is yet to be unequivocally determined. Temperature has been shown to explain a large proportion of flowering in several masting plants (Kelly *et al.*, 2008; Kelly *et al.*, 2013), but the exact role of climate and resources, and

their interaction, is still unclear. The actual mechanism is likely to be a combination of the two factors (Pearse *et al.*, 2016), with the amount of impact each factor has varying between species.

1.3 Study species

Chionochloa is a genus of long-lived (>100 years), native New Zealand alpine tussock grasses that has been widely studied as a model masting group due to its highly variable masting behaviour (Kelly *et al.*, 2000), easily counted inflorescences, and its small size allowing for experimental transplantations. *Chionochloa pallens* and *C. macra* are two species which are found widely on Mount Hutt, in the Canterbury region of the eastern foothills of the Southern Alps of New Zealand, where both species are found between 800 m to 1650 m, with *C. macra* dominating above 1200 m and *C. pallens* below this elevation (Kelly *et al.*, 2008). Long-term datasets have been collated on some *Chionochloa* populations at Mt Hutt recording individual plant flowering, sizes and the temperatures at different sites.

Celmisia lyallii is an endemic New Zealand alpine daisy found between 800-1700 m throughout New Zealand (Fenner *et al.*, 2001), and is also a masting species (Campbell, 1981; Kelly *et al.*, 2013). On Mt Hutt, *C. lyallii* is found amongst *Chionochloa* populations but only above approx. 1300 m. Very few previous masting studies have included *C. lyallii*. Of note regarding flowering in *C. lyallii,* a lot of apical buds in *Celmisia* species can be initiated, and visible by microscope in autumn, yet very few complete development, with *C. lyallii* having a high floral bud abortion rate (Mark, 1970). This seems to occur often in *C. lyallii* as data from Mt Hutt have been shown to include more zero flowering years compared to *C. pallens* (Kelly *et al.*, 2013), a fact that may signal the use of environmental vetoes in this species (Bogdziewicz *et al.*, 2017b).

Chionochloa species have been identified as being highly synchronised in their flowering efforts throughout a range of altitudes due to individuals adjusting to the local altitudinal temperature (Mark, 1965a), which invariably changes to nearly the same degree across all altitudes but with up to 8˚C difference in absolute mean temperatures between the highest and lowest altitudes (Mark, 1965b). Mark (1965c) found that the sporadic nature of flowering in *C. rigida* (and later *C. macra* also (see below)) is predominantly environmentally controlled. He later stated that the magnitude of the flowering effort is controlled in part by the period of high temperatures and in part by the availability of resources Mark (1968).

Plants of the same species but at different altitudes can respond in different ways. In situ, *Chionochloa* generally have a winter dormancy period from May-August, where no vegetative growth occurs (Mark, 1969). The length of the vegetative growing season is dictated to a large degree by snow cover. *Chionochloa* at lower altitudes, with little to no snow, can start growing again when spring starts in September, whereas those at higher altitudes may be delayed by up to two

months due to snow cover. Mark (1969) found that *C. rigida* plants transplanted to Dunedin, near sea level, from 910 m and 1220 m all grew continually throughout the year, with no dormancy period. In contrast, plants brought down from 1590 m still adhered to a two to three month dormancy period despite the winter in Dunedin being warmer than their home location. The sections of *C. rigida* from the highest site at 1590 m showed distinctly different patterns in growth and reproduction compared to sections from lower sites when relocated near sea level, and Mark (1965a) described these as a separate "ecotype". However, these ecotypically differentiated shorter-leaved snow tussocks were later described as a different species and renamed *C. macra* (Zotov, 1970). Mark (1969) suggested this difference in growing pattern was likely controlled by photoperiod rather than temperature.

The effect of temperature and altitudinal changes on *Chionochloa rigida* have previously been studied by Mark (1965a). Large plants were taken from four different altitudes (*C. rigida* at 870 m, 910 m, 1220 m, and *C. macra* at 1590 m), split into five sections, and a section of each reciprocally transplanted to one of the four original sites and one to Dunedin. Mark (1965a) found that increasing the temperature increased the flowering along a gradient, with the plants at the lowest altitude flowering the most including even in non-masting years, while a decrease in temperature by being moved upslope inhibited flowering. Connor (1966) also found that *C. rigida* grown from seed near sea level flowered every year compared to plants in their natural range. In the Mark (1965a) experiment, over the three years of post-transplantation observations the *Chionochloa* left at their original sites flowered only in the 1962-63 season, which was noted as a highly synchronised masting year (Mark, 1965a).

In November 1965, Mark (1968) also transplanted *C. flavescens* and *C. crassiucula* segments from Mt Brewster, near Haast, among the three source sites (1265 m, 1430 m and 1680 m) and to a fourth site in Dunedin. The 1966-67 summer was a reasonable flowering year and those plants moved uphill produced few to no inflorescences while plants moved downhill produced more inflorescences than those from their original sites (Mark, 1968), the same effect as seen in *C. rigida* (Mark, 1965a).

Fourteen years after Mark (1965a) reciprocally transplanted *C. rigida* sections, Greer (1979) dug up some of these plants, sectioned them, and re-reciprocally transplanted these sections among some of the same sites to follow their growth and flowering again. Leaf length and growth rate showed distinct genetic control after 14 years away from original sites, but flowering showed plasticity, with transplanted plants responding similarly to resident plants in terms of flowering (Greer, 1979). This suggests that with time the flowering of translocated plants fell into synch with local temperatures.

Datasets from masting species at Mt Hutt have previously been used (with datasets from other locations) to support the DT model (Kelly *et al.*, 2013). If the DT model can be shown to accurately predict the size of future masting events in other species too it could be very beneficial to conservation efforts in New Zealand, and worldwide, in attempting to reduce the impacts of introduced predators on local wildlife following masting years.

1.4 Thesis outline and objectives

The objective of my study was to attempt to determine the relative importance of temperature and resources in controlling flowering in masting species, especially in *Chionochloa* and *Celmisia*. Temperature effects were studied in plants in their natural environment on Mt Hutt as well as by transplanting plants to higher or lower altitudes to decrease or increase their local temperature, respectively. To study the effects of resources on plants, fertiliser was applied to some, at different altitudes, and these plants were monitored alongside unfertilised controls for a treatment effect. I also investigated other effects of increased nutrients in plants, such as vegetative growth. Additionally, I tested how well the DT model predicted the size of the flower crop in a particular year in masting species.

This research used long standing temperature and flowering datasets from Mt Hutt as well as recently collected data from manipulation experiments. I also utilised temperature data from NIWA's National Climate Database and VCSN virtual climate station database to fill in any missing temperature data and to determine the best way to estimate any missing temperatures. To meet my objectives, I used these data to answer the following specific questions:

Temperature effects on *Chionochloa* and *Celmisia* (Chapter 2)

1) What is the best way to estimate missing temperature data for a site?
2) Using long-term observational flowering datasets, does the DT model explain the flowering response better than the T1 model (the absolute temperature one year before flowering)?
3) How does a change in temperature, due to transplantation to higher or lower altitudes, affect flowering in these masting species?
4) Does the DT model explain the flowering in transplanted plants better than the T1 model?

Fertiliser effects on *Chionochloa* (Chapter 3)

1) Does the addition of nutrients, via fertiliser, increase vegetative growth of masting plants by,
    a. increasing the number of tillers and live basal area of plants?
    b. increasing daughter tiller production?
    c. increasing the survival of adult tillers?

2) Does the addition of nutrients increase the flowering response of *Chionochloa*,
    a. one year after fertiliser application?
    b. two years after fertiliser application?

Fertiliser effects on *Celmisia lyallii* (Chapter 4)

1) Does the addition of nutrients, via fertiliser, increase vegetative growth by increasing the number of daughter rosettes produced,
    a. one year after fertiliser application?
    b. Two years after fertiliser application?
2) Does the addition of nutrients increase the number of *C. lyallii* rosettes within patches at Mt Hutt?
3) Does the addition of nutrients increase the flowering response of *C. lyallii*,
    a. one year after fertiliser application?
    b. two years after fertiliser application?

Answering these questions will help to, firstly, understand how reproduction in masting plants may respond to rising global temperatures; secondly, define the main controlling mechanism behind flowering in masting plants; and thirdly, support the use of the easy-to-use DT model to predict when high masting years will occur which will be of benefit to conservation efforts in New Zealand and worldwide.

Chapter 2. Temperature effects on *Chionochloa* and *Celmisia*

2.1. Objectives

Temperature is known to play an important role in the flowering of masting plants. Often, an above average temperature during the floral induction period precedes mast flowering (Schauber *et al.*, 2002; Övergaard *et al.*, 2007). But, to what degree does temperature control the frequency of masting events? The DT model was put forward as a seemingly reliable way to predict the timing and size of flowering events in masting plants (Kelly *et al.*, 2013). The direction (negative or positive) and size of the difference in temperatures from two years before flowering and one year before flowering explains the observed flowering events to a high degree in some masting species it has been studied in (Pearse *et al.*, 2014; Kon and Saito, 2015; Davi *et al.*, 2016). The prediction of flowering levels by DT implies that temperature plays an important role in controlling the size of each flower crop. As it is important to be able to predict large masting events due to the downstream negative effects seedfall quantity can have on native wildlife (Elliott and Kemp, 2016), the DT model can be a very useful conservation tool (Holland *et al.*, 2015).

In order to assess the reliability of the DT model, long-term flowering data are required as short-term temperature quirks may distort flowering patterns over a short period and not give an accurate depiction of the actual flowering responses at a given site. Long-term flowering data are available for my study species, *Chionochola pallens, C. macra* and *Celmisia lyallii*, from Mt Hutt. However, the floral data has been collected for longer than on-site weather stations have been in place. Also, after the installation of on-site weather stations at Mt Hutt, there were periods when these stations were not recording due to faults or damage. Therefore, to measure the observed flowering against the DT temperatures it was necessary to estimate the temperatures for the missing periods. A previous study used estimates created from the NIWA-maintained Christchurch Botanic Gardens weather station temperatures (National Institute of Water and Atmospheric Research) to predict missing Mt Hutt temperatures (Kelly *et al.*, 2008). But, as Christchurch is a low-lying coastal city and Mt Hutt is an alpine environment, how accurate are these predictions likely to be?

One of my aims was to test whether there are better ways to estimate missing temperatures, especially for alpine environments. This involved utilising two similar, but different, temperature resources from NIWA. The first was to use NIWA weather stations closer to the location

of interest, in this case Mt Hutt. The second was to use the NIWA Virtual Climate Station Network (VCSN) predictions which give New Zealand-wide temperature predictions at a 5 km resolution.

Another aim of this study was to further investigate the accuracy of the DT model in explaining flowering events in masting species. This was done in two ways. Firstly, by comparing the complete on-site temperature records with the long-term flowering of all three study masting species at Mt Hutt. Secondly, by transplanting plants of two alpine masting species from Mt Hutt, *C. pallens* and *C. lyallii*, to higher and lower altitudes to decrease and increase their temperatures, respectively, and follow their flowering response. All tests were run using the T1 (absolute mean temperature one year prior to flowering) and DT models to identify which explained the flowering response better. It was predicted that one year after transplantation, decreasing the temperature would result in less flowering than plants at the home site, and that increasing the temperature would result in more flowering than home site plants. For plants moved to warmer locations, after two summers at a new site, the flowering response of plants were predicted to follow the temperature differences of their new site in accordance with the DT model. They were not expected to flower much in the second year after transplantation due to expending extra resources in flowering heavier after their first year. Those moved to cooler sites, after two years, were expected to display similar flowering to other plants at their new site. Additionally, it was predicted that the scale of change in the flowering of transplants would be better explained by the DT model than the T1 model. To summarise my aims, my key questions were:

1) What is the best way to estimate missing temperature data?
2) Using long-term observational flowering datasets, does the DT model explain the flowering response better than the T1 model (the absolute temperature one year before flowering)?
3) How does a change in temperature, due to transplantation to higher or lower altitudes, affect flowering in these masting species?
4) Does the DT model explain the flowering in transplanted plants better than the T1 model?

2.2. Methods

Study sites and experimental methodology

Most of the fieldwork for this thesis research was conducted at Mount Hutt, with some also occurring at the University of Canterbury glasshouses (UC, 15 m above sea level). Five study sites were used on Mt Hutt which all contain naturally occurring populations of *Chionochloa pallens* and *C. macra*, at 1620 m, 1540 m, 1520 m, 1350 m, and 1070 m above sea level (see Fig. 2.1), while

Celmisia lyallii are found naturally at all except 1070 m. A temporary site at 450 m was used to hold *C. pallens* plants for a four-month summer period in 2016/17.

Long-term flowering observations have been recorded for *Chionochloa* at Mt Hutt since 1986, and on *C. lyallii* since 1996. In 1986, three 20 m monitoring lines were set up at 1070 m, which ran horizontally across a hillside with a 10 m gap between each line. Every *C. pallens* and *C. macra* touching each of these lines was mapped in 1990, and their sizes (basal area (BA) in dm^2) were recorded in 1990, 2006, summer 2016/17, and summer 2017/18. Throughout this thesis these monitoring lines, combined, are referred to as the 1070 m control plot. Flower counts have been made on all plants since the establishment of these monitoring lines, and in the following monitoring zones since their respective establishments. At 1070 m most of the plants were *C. pallens*.

In 1992, two 20 m monitoring lines were set up at 1540 m on Mt Hutt, with a 5 m gap between the two lines. All *C. pallens* and *C. macra* plants touching these lines were mapped, and their sizes were recorded in 1992 and 2004. At 1540 m most of the plants were *C. macra*. *Celmisia lyallii* plants above these lines were mapped and flowering was monitored from 1997 onwards.

In 1996, a 20 x 2 m plot was set up at 1620 m, on a slope above the ski field carpark. All *C. pallens* and *C. macra* plants were mapped, with *C. pallens* being more common, and sizes of each were recorded in 1996, 2004, and 2016. *Celmisia lyallii* within this plot were also monitored and annual inflorescence counts began.

In 2006, a second 20 x 2 m control plot was set up at 1520 m on Mt Hutt. All *C. pallens* and *C. macra* plants, and *C. lyallii* patches in the plot were mapped, with rosette patch flower counts made every year since, and sizes of plants recorded in 2006, summer 2016/17, and summer 2017/18. The 1520 m plot had 56 *C. pallens* and 44 *C. macra*.

Some *Chionochloa pallens* and *C. lyallii* plants from populations at 1620 m, 1520 m, 1350 m, and 1070 m on Mt Hutt had previously been experimentally manipulated by being transplanted to new sites. Transplanted plants were dug up and moved either to a higher site, to reduce the temperature and hypothetically lower the amount of flowering in the following flowering season, or to a lower site, to increase the temperature and hypothetically increase the amount of flowering the next season. Details of these transplanted plants are summarised in Table 2.1, with further details below.

In November 2014, 12 *C. pallens* plants were dug up at 1620 m on Mt Hutt and transplanted down to UC. Larger plants were split into two or four segments, so a total of 20 individual plants or plant segments were placed into unfertilised potting mix in 70 L garden bags (No formal record was made at the time of the type of potting mix both *Chionochloa* and *Celmisia* transplants in 2014 and 2015 were put into. I collected and processed soil samples *a posteriori* to determine this, and the

results are written here. See Appendix A for soil analysis details.). In December 2014, 31 *C. pallens* plants were dug up at 1070 m on Mt Hutt; 11 of these plants were moved higher up the mountain to 1520 m and larger plants were split into two or three segments so that a total of 20 individual plants or plant segments were transplanted; 20 plants were replaced in the same holes to be "dug controls" to see if the handling and any severing of roots affected the health of the plants.

In November 2015, 10 *C. pallens* plants were dug up at 1070 m and halved. Half of each plant was placed back into the same hole to act as dug controls, and the other half was transplanted to UC and placed into 70 L garden bags with fertilised potting mix. Also, in December 2015, 70 small patches of *C. lyallii* were dug up at 1350 m; 20 patches (50 rosettes) were transplanted higher up the mountain to 1520m; 10 patches (27 rosettes) were put back in the same holes to act as dug controls; 20 patches (57 rosettes) were transplanted lower down the mountain to 1070 m; 20 patches (56 rosettes) were transplanted to UC and potted with fertilised potting mix; while another 10 individual rosettes at 1350 m were marked as undug controls

In November 2016, 10 *C. pallens* plants were dug up at 1070 m and halved. Half of each plant was moved lower down the mountain to 450 m, where each was placed into a dug hole lined with weed mat to stop the roots from spreading and obtaining extra nutrients from the site. The other half of each plant was moved to UC and placed in unfertilised potting mix in 70 L garden bags. After four months at warmer temperatures, in March 2017, both plant halves were again dug up, reunited and returned to their original holes at 1070 m. This was to give the plants a short period of elevated summer temperatures followed by normal winter temperatures.

In January 2018, 10 *C. pallens* plants were dug up at 1070 m and halved. Half of each plant was transplanted higher up the mountain to 1520 m, the other half was transplanted down to UC and placed into unfertilised potting mix in 70 L garden bags. Patches of *C. lyallii* were removed from the holes dug for the *C. pallens* plants at 1520 m and transplanted down to UC. These were placed into pots with unfertilised soil (24 pots containing 31 rosettes).

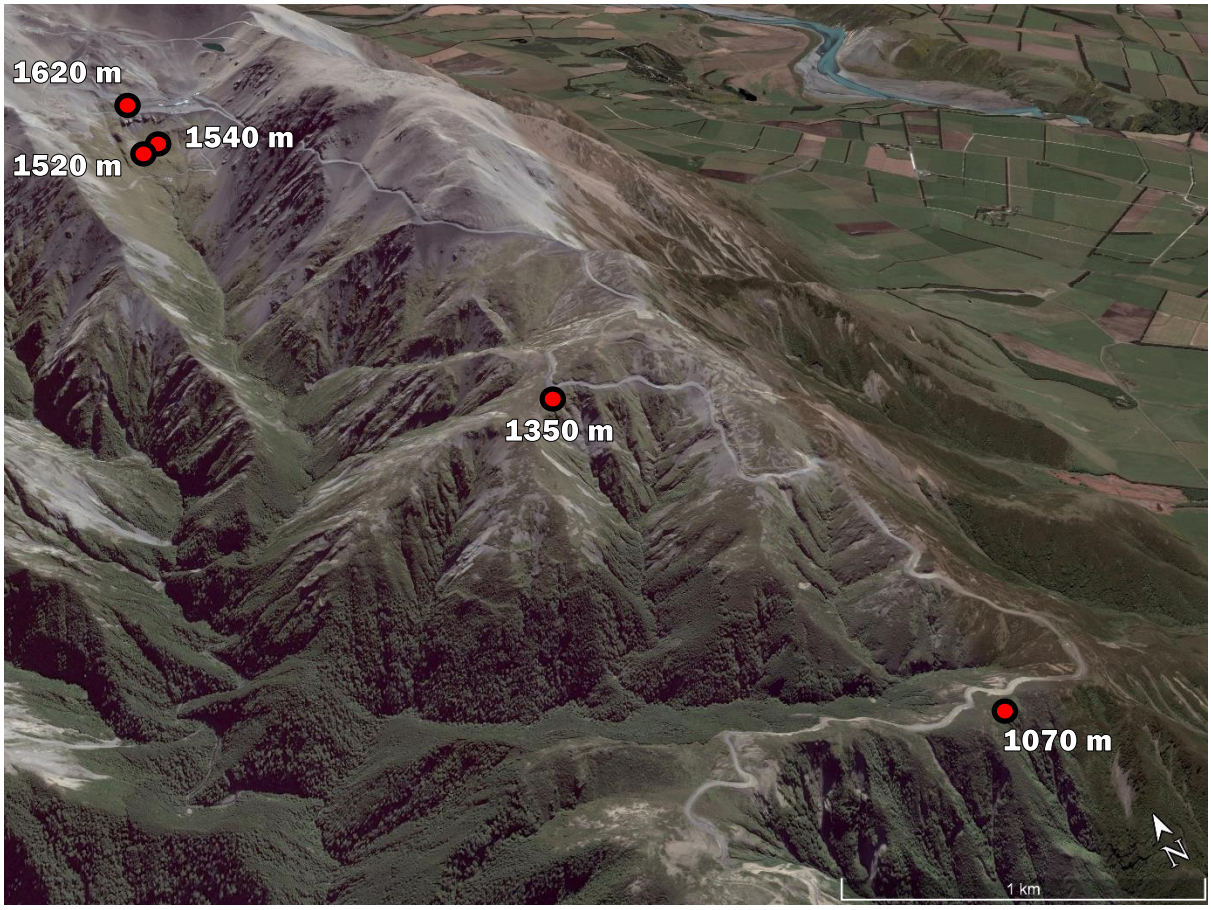


Figure 2.1. Mt Hutt and the ski field access road, showing the five study sites used at Mt Hutt throughout this thesis research. Both *Chionochloa* species were naturally found at all five sites. *Celmisia lyallii* were found naturally at all sites except 1070 m. The ski field base station is to the right of the 1620 m site. Image modified from Google Earth Pro.

Table 2.1. *Chionochloa pallens* and *Celmisia lyallii* plants transplanted from locations on Mt Hutt to higher or lower altitudes to manipulate the flowering responses with decreased or increased temperatures, respectively. A * denotes plants that were moved for one four-month summer period then returned to their home site at 1070 m. University of Canterbury (UC) is 15 m above sea level.

Species	Cohort year	Group name	n plants	Home site	Trans site	Trans date
C. pallens	**2014**	14Warm	20	1620 m	UC	16/12/2014
		14Cool	20	1070 m	1520 m	9/01/2015
		14Dug	20	1070 m	1070 m	9/01/2015
	2015	15Warm	10	1070 m	UC	25/11/2015
		15Dug	10	1070 m	1070 m	25/11/2015
	2016	16Warm	10	1070 m	450 m*	24/11/2016
		16Hot	10	1070 m	UC*	24/11/2016
	2017	17Cool	10	1070 m	1520 m	9/01/2018
		17Hot	10	1070 m	UC	9/01/2018
C. lyallii	**2015**	15Warm	56	1350 m	UC	23/12/2015
		15Warmish	57	1350 m	1070 m	23/12/2015
		15Cool	50	1350 m	1520 m	15/01/2016
		15Dug	27	1350 m	1350 m	15/01/2016
	2017	17Warm	31	1520 m	UC	9/01/2018

Estimating missing temperatures, and analysing long-term flowering datasets to test DT vs. T1

The transplants moved in 2014, 2015 and 2016 were performed before I started this MSc, but I was involved in the 2017 transplanting, and have monitored flowering and tiller growth on all treatments since late 2017 at UC and Mt Hutt.

On-site daily mean, maximum and minimum temperatures have been recorded from weather stations on Mt Hutt at 1070 m since 1995, and at 450 and 1520 m since January 2005 (Kelly *et al.*, 2008), and those for UC are taken from the Christchurch Botanic Gardens from The National Climate Database (www.cliflo.niwa.co.nz) maintained by NIWA. Missing temperature records consist of large periods of time before on-site weather stations were established and smaller periods after stations were in place.

As observational flowering data has been collected from Mt Hutt since 1986, before the placement of on-site weather stations, it was necessary to predict the temperatures for the early years, but hard to know how accurate these predictions are. Previous studies from Mt Hutt have used estimates made from temperatures at the Christchurch Botanic Gardens from The National Climate Database (CliFlo) as these temperatures were highly correlated with those on Mt Hutt but 6.0˚C warmer (Kelly *et al.*, 2008). I aimed to find out if there were any closer stations which may give a better estimate of these missing temperatures and to also test the NIWA VCSN predicted temperatures.

Faults and damage to on-site weather stations have left periods of days, months, or years where a weather station may not have been recording. To fill these gaps predictions are often made from off-site weather station recordings. This is done by working out a regression equation between the local site of interest and the predicting site over a large period where both have overlapping daily temperature data. The resulting equation is then used to fill in the missing temperatures for the local site. A problem with this technique is that the local sites temperature pattern now follows that of the predicting site which may not necessarily be an accurate depiction of what occurred at the local site at that given time. But, are some predicting stations better to use than others? Does it matter how close or far they are from the local station, or whether the altitudes are similar? For some of the missing temperatures on Mt Hutt since 2005 another on-site station has been able to be used to predict the missing temperatures. For example, the 1520 m has been predicted from the 1070 m site at times, as the weather at these two sites are highly correlated (Kelly *et al.*, 2008) with 1070 m being 2.0˚C warmer.

I located several CliFlo weather stations that were closer to Mt Hutt including Snowdon, Darfield, and Coleridge, and obtained their temperature recordings over the years of interest (1984 – now), as well as those for the Christchurch Botanic Gardens. Some closer stations started later or

ended sooner than the times of interest or had large periods of data missing so were not able to be used. The closest station that had complete temperatures for the period I needed was Darfield, and this was the station that was used in my analyses.

The VCSN predicts climate variables throughout the whole of New Zealand using observational climate data from around 150 locations (Tait and Macara, 2014). For this study I was just interested in the daily maximum and minimum temperatures. Grid points at a 5 km spatial scale have been plotted throughout New Zealand and temperature predictions made for each point. These predictions are based on a smoothed elevation grid, meaning the VCSN temperatures are predicted over the coarser 5 km elevation data at the same 5 km spatial resolution, not at the exact location the grid point falls on (pers. comm. A. Tait). The VCSN data used were the improved 'Norton' data which have been shown to better predict maximum (Mason *et al.*, 2017) and minimum temperatures, especially at higher altitudes (Tait and Macara, 2014). The Norton data include different lapse rates for maximum and minimum temperature as ˚C/km for each of the three-month seasonal periods (Norton, 1985; Tait and Macara, 2014). Norton (1985) calculated these lapse rates by creating regression equations that incorporated data on latitude, altitude and distance from the nearest coast from 301 temperature stations throughout New Zealand. For this study, the two closest points to our Mt Hutt weather station sites, which also had similar elevation differences to our weather stations, were chosen to obtain temperature predictions from the VCSN (Fig. 2.2). Grid point #15657 has a smoothed elevation of 1873 m and was used to predict estimates for the upper Mt Hutt weather station at 1520 m, while grid point #15658 has a smoothed elevation of 1025 m and was used for estimates for the 1070 m weather station. The VCSN data used in this study were provided by Andrew Tait, NIWA's principal climate scientist.

In a study looking at multiple sites along the east coast of New Zealand, from Christchurch to Wairarapa, Mason *et al.* (2017) found that on-site maximum and minimum temps correlated better with VCSN than using nearby NIWA weather stations, although all were high (R^2s: VCSN Norton maximum 0.98, minimum 0.97, NIWA station maximum 0.96, minimum 0.91). Adding the Norton equations (for elevation, latitude, and distance to the nearest coast) reduced the bias in the temperature estimates, especially for maximum temperatures (Mason *et al.*, 2017). This study aimed to identify the correlative ability of nearby climate stations and VCSN predictions to on-site Mt Hutt measurements also.

Testing the relationship between temperatures at the two Mt Hutt on-site weather stations (1070 m and 1520 m) and each of the four temperature estimate data sources (Christchurch Botanic Gardens, Darfield, VCSN lower grid point, and VCSN higher grid point) was done using regression analyses in Microsoft Excel. Temperatures for these regression analyses included only January and

February mean daily temperatures (see below for reasoning) and began when each on-site weather station recording began, i.e. 1996 for 1070 m and 2005 for 1520 m. These regression analyses gave an R^2 for each pairing and determined which of the temperature estimate data source temperatures were most aligned with the on-site temperatures at each altitude.

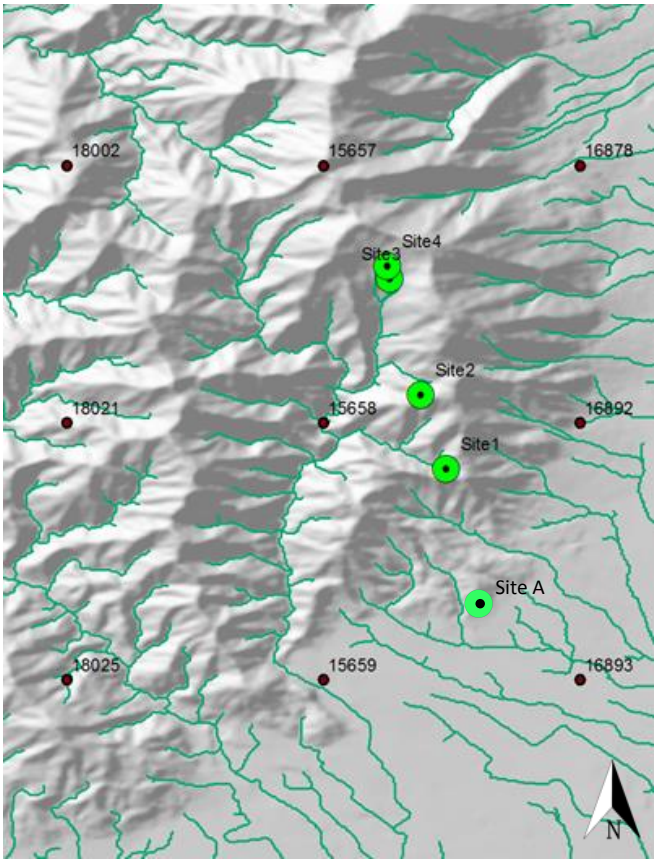


Figure 2.2. Map of the Mt Hutt area showing the VCSN grid points at their 5 km spatial scale (small, red and black dots) and five of the sites of interest on Mt Hutt (large, green dots). On-site weather stations are found at 450 m (site A), 1070 m (site 1), and 1520 m (site 3). Plants used for this study were found at four of the sites (1, 2 (1350 m), 3, and 4 (1620 m)), while site A was used to temporarily house *Chionochloa* transplants for the 2016/17 summer. The grid points used for this study were #15657 (1873 m smoothed elevation) and #15658 (1025 m smoothed elevation). Map courtesy of A. Tait, NIWA.

For tests using the long-term flowering data, I used the temperature data recorded on site when available and estimated any missing daily temperatures using the other on-site station (i.e. 1070 m for missing 1520 m temperatures, and vice versa). However, where no on-site recordings were available (mainly 1984-1994), to test which off-site missing estimates were best to fill the gaps I used three different datasets: one estimated these gaps from the Christchurch Botanic Gardens, one from Darfield, and one using the VCSN (VCSN-estimates; which was further split into the lower site, #15657 for 1070 m, and the higher site, #15658 for 1520 m). I also included a dataset which was entirely VCSN data (VCSN-only) to test how well the VCSN predicted temperatures compared to ones with on-site temperatures included.

My analyses used the mean daily temperatures for the January and February period each year as these have been shown to be the important induction months, as the day length is in excess of 14 hours (Mark, 1968) and where temperatures best explain the flowering response the following year (Kelly *et al.*, 2000; Kelly *et al.*, 2008; Kelly *et al.*, 2013). I further separated the data into the

individual month periods and worked out the regression equations using January daily means for the three on-site and off-site datasets (Botanic Gardens, Darfield, and VCSN) for 1070 m from 1996 – 2002 and for 1520 m from 2005-08 and 2010-12. These seven-year periods had unbroken temperature recordings for January and February for all off- and on-site weather stations. I calculated regression equations the same way for every February for the same periods also. I used these individual month regression equations to predict the missing daily January and February temperatures on Mt Hutt at the lower and higher sites. The datasets including the predicted daily means were then averaged per month per year. Each January and February monthly mean for each year was then averaged again and used in the statistical analyses. This same process was used for obtaining the VCSN-only dataset, with mean January-February temperatures calculated for every year. The final statistical analysis dataset contained both T1 (the temperature one year before flowering) and DT (the difference in temperatures two years before flowering or T1 – T2) for each of the four sets of temperature estimate data.

Observational flowering data began at 1070 m in 1986 with *C. pallens*, with other sites and species being added in later years (see Table 2.3 in Results). Flowering data for *Chionochloa* were measured as mean inflorescences per plant from within plots or along transect lines, and *C. lyallii* were measured as mean inflorescences per area from within plots. Regression analyses were run testing flowering data (as log (x + smallest non-zero number) due to lots of zeroes for some years) for each species at each site with all temperature estimates for each respective site over the years available. The R^2 was obtained for each regression to identify which of the four sets of temperature estimates gave the best fit to each set of flowering data at each site. A relative fit was then calculated for each species at each site, where the highest R^2 was assigned 100%, and the equation: R^2 / highest R^2 x 100 was used to calculate each other R^2 in relation to the highest. The average for each of *C. pallens, C. macra* and *C. lyallii* at each site was then calculated to find the best overall estimation data source for each category of plants at Mt Hutt.

Analysing transplant data

Temperatures for the entire length of the transplant experiment on Mt Hutt were taken from on-site weather stations at 1070m and 1520 m. As no weather station was sited at 1350 m, I used temperatures from 1070 m and 1520 m to estimate those for 1350 m. The 1070 m site is 280 m lower, and 1520 m 170 m higher, than 1350 m, so a respective 40:60 temperature weighting was used between the two sites to calculate temperatures for 1350 m. I then used these estimated T1s to calculate the DT for 1350 m. For the temperatures at the 1620 m site, I used those from the 1520 m weather station, directly, as the temperature would not vary much over the 100 m difference in elevation. Temperatures for UC were from the Christchurch Botanic Gardens, as above.

In *C. pallens*, all transplanted plants and dug control plants had total tiller counts and total inflorescences per plant recorded every year starting from the year they were first dug up. Total tiller counts were not made on any undug or unmanipulated plants at 1070 m or 1620 m, but long-term observation control plot plants had been measured for their basal area (BA) in dm^2. The transplants had also had their BAs measured so annual inflorescences per dm^2 were used to test whether digging up the plants significantly altered their flowering responses in later years. This test was performed using a multi-factor anova with year (as factor), group, and whether plants were dug or controls as the predictor variables.

Initially, to test whether the T1 or DT model explained the resulting flowering responses of *C. pallens* plants moved to different temperatures, I created a dataset with separate rows for each flowering year for each group. The flowering tillers and non-flowering tillers per plant were totalled for all observed plants within each row category (i.e. all tiller data totalled across the relevant plants, to avoid having a nested data structure) with their respective T1 and DT temperatures. Quasibinomial glms were run due to overdispersion in the data. The proportion of tillers per plant that produced inflorescences was used as the response variable. Each of T1s and DTs were analysed in an additive model which included all the *C. pallens* groups mentioned in table 2.1 as a 'group' factor (the interactions of T1 x group (p-value = 0.860) and DT x group (p-value = 0.282) were non-significant so were removed). However, as there were very few observed flowering years for some groups (minimum of two flowering years) this was not the best way to test these data, although the DT model was shown to better explain the flowering ($F_{17,25}$ = 15.475, p = 0.001) than the T1 model ($F_{17,25}$ = 5.214, p = 0.036). The results were, however, used to create graphs (Appendix C) for each of the T1 and DT models plotting each observed flowering response at the specific model temperature and then using the coefficients to overlay trendlines for each respective group. The trendlines were back-transformed fitted means predicted for each 0.2˚C interval for the total range of temperatures observed in this study for each of the T1 and DT models. The data were displayed as a smooth line instead of individual data points.

To better test the relationship between the resultant *C. pallens* flowering and the T1 and DT models, Generalised linear mixed models (GLMMs) were used with the raw dataset containing each individual plant flowering response. These GLMMs included the random effects for each individual plant within each group, and each group itself, using the code: +(1|group/plant_ID). The response variable was still the proportion of flowering tillers per plant, while the fixed effect was either T1 or DT. The model was run using was a binomial GLMM. The coefficients from each of the T1 and DT models were back-transformed and used to create an overall trendline for the flowering responses

of all plants under each model using the same technique as mentioned above. These trendlines were overlaid on the same plotted group per year data point observations as above.

To further evaluate the temperature effects on the flowering of *C. pallens* transplants I made graphs for each of the cohort years in Table 2.1. These graphs showed the mean percentage of flowering tillers in plants for each cohort group across all the years since each cohort was transplanted. The flowering responses of the 14Dug and 15Dug control plants were also shown on each graph to better compare increases and decreases in flowering with changes in temperature due to transplantation. Identifying differences and similarities in flowering responses was easier to see when separated out into cohorts. As the 15Warm plants were placed into fertilised potting mix at UC, compared to all other UC transplants being placed into unfertilised potting mix, these graphs enabled a more thorough evaluation of the effect fertilised potting mix had too.

For *Celmisia lyallii,* no flowering occurred in any of the study plants at any altitude on Mt Hutt so tests could not be run to see if digging up plants affected their flowering response. This had been planned to be tested in 10 plants at 1350 m that were dug up and replaced in the same holes and with 10 tagged but undug plants. However, the fact that plants transplanted to UC did flower well shows that the flowering response was unlikely affected by digging. Looking at the flowering response of *C. lyallii* over a range of elevations and temperatures was not possible with the data collected as only those plants moved to UC flowered at all. However, the responses of the two groups of plants moved to UC, 15Warm and 17Warm, was assessed by graphing the number of inflorescences per rosette for *C. lyallii* across each year since they were transplanted. Note that half of the 15Warm *C. lyallii* plants (Table 2.1) had additional fertiliser added for the fertiliser experiment (Chapter 4), so the number used for this analysis was halved from 56 to 28 rosettes.

2.3. Results

Estimating missing temperatures, and analysing long-term flowering datasets to test DT vs. T1

The R^2s from the regression analyses looking at the relationship between each temperature estimate data source (i.e. Christchurch Botanic Gardens, Darfield, VCSN low, or VCSN high) and the temperatures from on-site weather stations at 1070 m and 1520 m on Mt Hutt are shown in Table 2.2. VCSN predictions were closer to those measured at on-site weather stations than the CliFlo temperatures. Both the lower and higher VCSN predictions gave very good relationships to both the 1070 m and 1520 m on-site temperatures, with the lower VCSN slightly better for 1070 m and the higher VCSN slightly better for 1520 m. The similar results for both VCSN predictions for both on-site weather stations come from the two VCSN predictions being very highly related (Table 2.2), as expected from the nature of the VCSN predictions and the close proximity of the two VCSN grid

points. Out of the two CliFlo weather stations, the temperatures at the closer site, Darfield, were more closely related to temperatures at both on-site weather stations than Christchurch Botanic Gardens. This suggests that using a station closer to the location of interest will likely result in estimates that better resemble on-site temperatures than one further away and in a different landscape type. However, the relationship between temperatures for the two on-site weather stations was the best overall, showing missing temperatures at one site are still best predicted from the other site.

The four temperature estimate data sources were used to create temperature predictions for missing daily temperature periods from both the 1070 m and 1520 m on-site stations. These missing on-site temperatures were filled with predicted temperatures from Christchurch Botanic Gardens, Darfield, and VCSN (VCSN-estimates), with the lower VCSN grid point predictions used for 1070 m and the higher VCSN grid point predictions used for 1520 m and above. A fourth missing temperature estimate consisted entirely of VCSN predictions (VCSN-only).

Long-term flowering data from Mt Hutt tested against each of the four missing temperature data estimates (Christchurch Botanic Gardens, Darfield, VCSN-estimates, VCSN-only) showed that, with one exception, all DTs gave better fits than the T1s (e.g. Fig. 2.3 A and B). Regression graphs and R^2s for all tested models can be seen in Appendix B. The exception was for *C. pallens* at 1520 m where the T1 R^2s for the on-site weather station (0.65, no missing temps so no estimates required) and VSCN-only (0.74) were higher than the DT R^2s on-site (0.63) and VCSN-only (0.70). Besides this one exception where T1s were better than DTs, the rest of the T1s were considerably lower than the DTs, and all but three of the T1 R^2s fell under 0.5, with the lowest being 0.11. Because the DTs were clearly giving a better overall explanation of the flowering data the rest of the analyses were performed using only the DT model.

I used the DT R^2s to create a relative fit comparing each of the missing temperature estimate sources to the flowering for each species at each site (Table 2.3). These relative fits showed that for both *Chionochloa* species most estimates resulted in a good relationship, whereas, unexpectedly, *C. lyallii* flowering was best explained by the VCSN-only and not any dataset containing actual on-site temperature recordings (Table 2.3, Fig. 2.3 E and F). For *Chionochloa,* the best site averages came from estimating the missing temperatures with the VCSN at both sites, but especially the lower site.

Table 2.2. Actual R^2s for the relationship between each temperature estimate source and the on-site weather stations at 1070 m and 1520 m on Mt Hutt, with additional testing of the relationship between the two VCSN prediction sources. Temperatures were mean daily temperatures for January and February each year since the establishment of on-site weather stations (1996 for 1070 m and 2005 for 1520 m) through to 2018, excluding any days without on-site temperature recordings.

Weather	**Actual R^2s**				
station site	**Botanic Gardens**	**Darfield**	**VCSN low**	**VCSN high**	**1520 m**
1070 m	0.577	0.763	0.863	0.854	0.871
1520 m	0.515	0.619	0.815	0.822	
VCSN low				0.997	

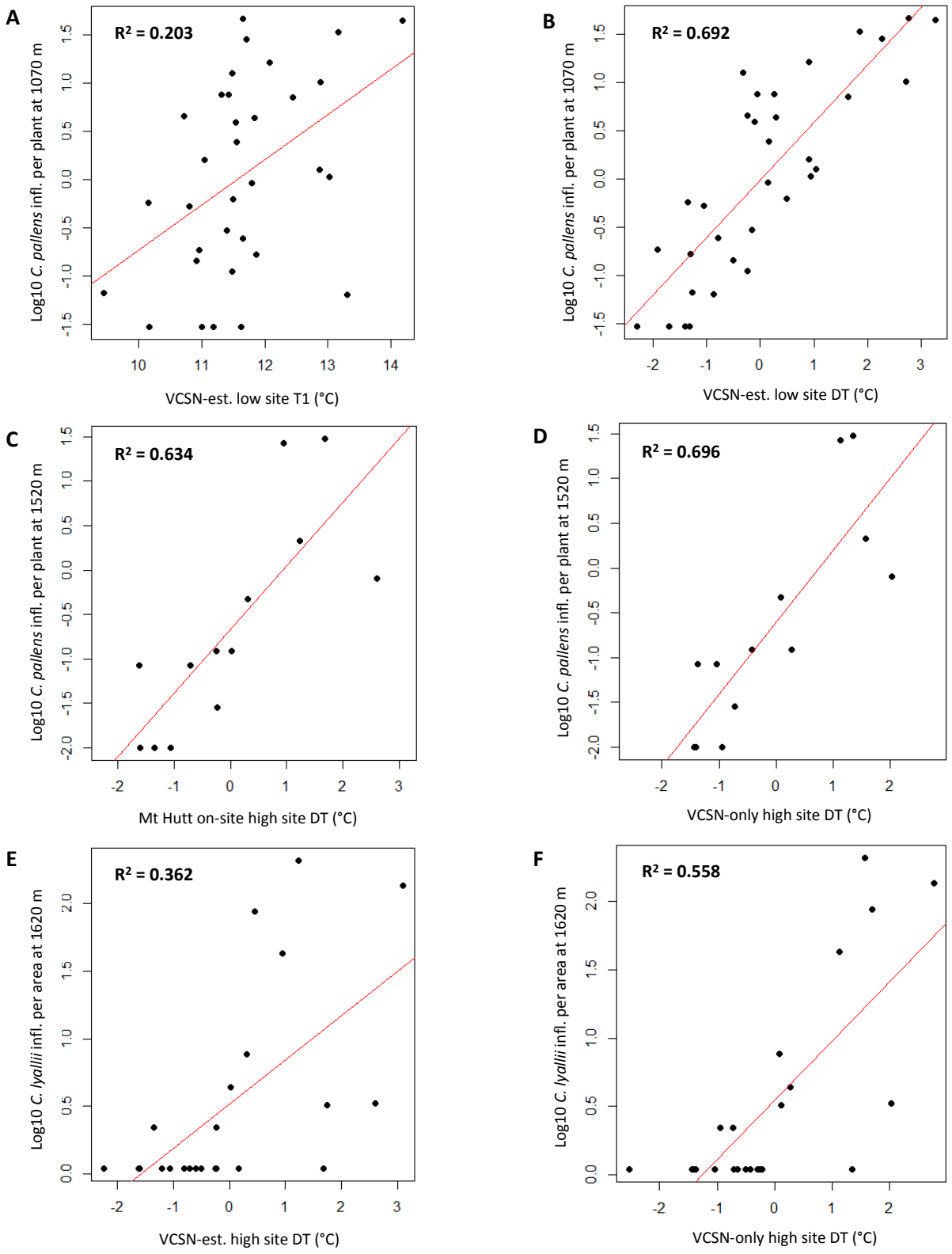


Figure 2.3. DTs consistently explained flowering better than T1s, as seen with *C. pallens* inflorescences per plant at 1070 m on Mt Hutt when filling missing temperatures with VCSN estimates: **A** = T1 (absolute temperature for January-February the year before), **B** = DT (January-February T_{-1} minus January-February T_{-2}). Oddly, in datasets that only contained the on-site temperatures (**C**) the VCSN-only temperatures explained flowering slightly better (**D**), shown for *C. pallens* inflorescences per plant at 1520 m. *Celmisia lyallii* flowering had low R^2s with all three on-site temperatures + missing estimates, e.g. with VCSN-estimates (**E**), with the best fits all from VCSN-only temperatures (**F**), as seen with DTs for inflorescences per area at 1620 m.

Table 2.3. Data used to identify the best missing temperature estimates to use for Mt Hutt, showing each site and the year flowering observations began for *C. pallens, C. macra* and *C. lyallii.* The R^2 results (a) for each individual regression were used to calculate relative fit percentages (b) and show which estimate was the best for each species at each site and the overall best for *C. lyallii* and *Chionochloa* at lower and higher altitudes. The best site averages are shown in bold. For datasets starting in 2006, all temperatures were from on-site weather stations so no gaps needed to be filled. Only DT results shown as these resulted in better fits than T1 (see results text).

(a) Actual R^2s

				Actual R^2 - DT				
Species	**Site**	**Year started**	**N years**	**All on-site**	**Bot. Gardens**	**Darfield**	**VCSN est.**	**VCSN-only**
C. pallens	1070 m	1986	32		0.669	0.575	0.692	0.616
C. macra	1070 m	1990	28		0.430	0.358	0.443	0.419
C. pallens	1520 m	2006	12	0.634				0.696
C. macra	1540 m	1992	16		0.627	0.648	0.619	0.479
C. pallens	1620 m	1996	22		0.644	0.630	0.669	0.514
C. lyallii	1520 m	2006	12	0.261				0.365
C. lyallii	1540 m	1997	21		0.467	0.476	0.339	0.554
C. lyallii	1620 m	1996	22		0.345	0.337	0.362	0.558

(b) Relative fits as a percentage

				Relative fit % - DT				
Species	**Site**	**Year started**	**N years**	**All on-site**	**Bot. Gardens**	**Darfield**	**VCSN est.**	**VCSN-only**
C. pallens	1070 m	1986	32		96.7	83.0	100	89.0
C. macra	1070 m	1990	28		97.1	80.8	100	94.5
Site average					96.9	81.9	**100**	91.7
C. pallens	1520 m	2006	12	91.1				100
C. macra	1540 m	1992	16		96.7	100	95.4	73.8
C. pallens	1620 m	1996	22		96.3	94.2	100	76.8
Site average					94.7	95.1	**95.5**	83.5
C. lyallii	1520 m	2006	12	71.4				100
C. lyallii	1540 m	1997	21		84.3	85.9	61.2	100
C. lyallii	1620 m	1996	22		61.8	60.5	64.8	100
Site average					72.5	72.6	65.8	**100**

Analysing transplant data for Chionochloa pallens

My first analysis tested whether the act of digging *C. pallens* plants up at 1070 m on Mt Hutt for transplantation significantly affected the flowering responses of these plants in future flowering seasons. The “dug control” plants were dug up then immediately replaced in their original holes and

their future flowering responses tested against that of control plants which had no manipulation. As the control plants had not had total tiller counts measured I used the number of inflorescences per dm^2 as the response variable for each group of plants. A significant effect of year was found (Table 2.4), with flowering varying across years for all groups and being especially higher in 2016 (Fig. 2.4). Importantly, no significant difference was found between the flowering responses of individual groups within the same flowering year or due to having been dug up compared to undug controls (Table 2.4). This meant that for the following analysis I could justifiably add the 14Dug and 15Dug plants as representatives of the flowering effort of untransplanted plants across years and compare them to the flowering responses of transplants due to temperature change at other sites. The transplant plants and dug controls all had total tiller counts collected, a measurement which gives a more accurate depiction of flowering effort compared to the inflorescences per dm^2 used in this section.

Table 2.4. The effects of year, group (14Dug, 15Dug, 1070 m Control, 1620 m Control), and if plants had been dug on inflorescences per dm^2 in *C. pallens* at 1070 m and 1620 m on Mt Hutt. This was testing whether digging up plants significantly affected their future flowering (*n* = 20 for 14Dug, 10 for 15Dug, 82 for 1070 m Control, and 76 for 1620 m Control). Significant effects indicated in bold.

(a) Analysis of variance table. Evaluated using type III sums of squares when entering each term into the model last.

Response: Log10 (inflorescences/dm2 + 0.01)					
	Df	**Sum Sq**	**Mean Sq**	***F* value**	***P* value**
as.factor (year)	3	495.6	165.21	603.459	**< 0.001**
group	2	0.9	0.44	1.623	0.198
dug	1	0.0	0.00	0.003	0.953
residuals	735	201.2	0.27		

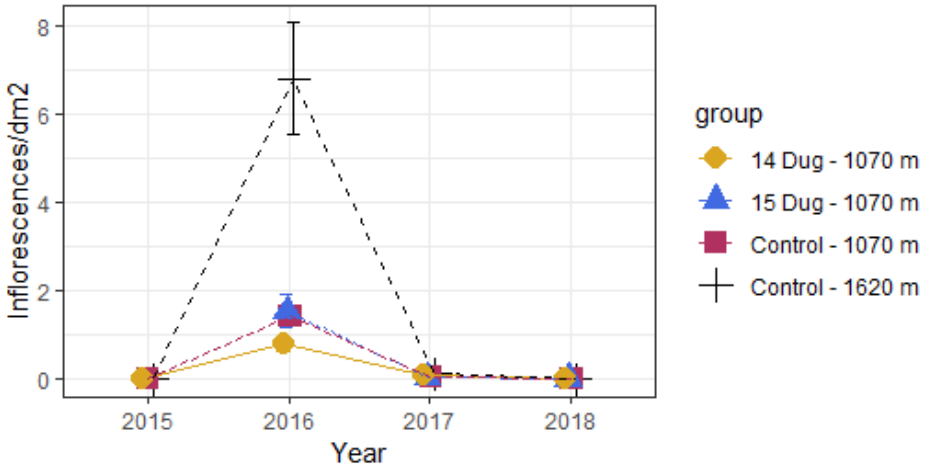


Figure 2.4. The variation in flowering (inflorescences per dm^2) across four consecutive flowering years in *C. pallens* control plants at 1070 m and 1620 m on Mt Hutt, as well as the corresponding flowering of plants dug up and replaced in their original holes in 2014 and 2015 at 1070 m. No significant difference was found between the flowering responses of each group or for whether plants were dug up or not, but there was a significant difference in flowering effort for all groups across years. Error bars show SEM.

My second analysis combined the flowering responses of all transplanted *C. pallens* plants and the dug controls across all years starting from each transplant group's first flowering episode in the flowering season they were manipulated. Note that flowering in the year of manipulation should be controlled by (unmanipulated) temperatures in the previous two summers. I analysed the relationship between all transplant plants and their respective T1 model temperatures and DT model temperature differences. Significant relationships were found between both models and the flowering responses of the transplanted plants (Table 2.5). However, the DT model explained this relationship better than the T1 model, as determined by the higher *z* score for the DT model (Table 2.5). The greater explanation of the respective flowering responses of transplant groups using the DT model can be visualised in Fig. 2.5. Here, the coefficients for each model have been back-transformed and used to predict a trendline through the plotted data points including each flowering year for each of the transplants. This was done for both the T1 and DT models. The spread of the data points around the DT trendline is clearly less than those around the T1 trendline (Fig. 2.5), especially for high T1 and DT values.

Table 2.5. The relationship between the two temperature models and the proportion of flowering tillers for all transplanted *C. pallens* plants, including dug controls, combined across all years. Significant effects in bold.

(a) T1 model (January-February absolute mean temperature 1 year before flowering),

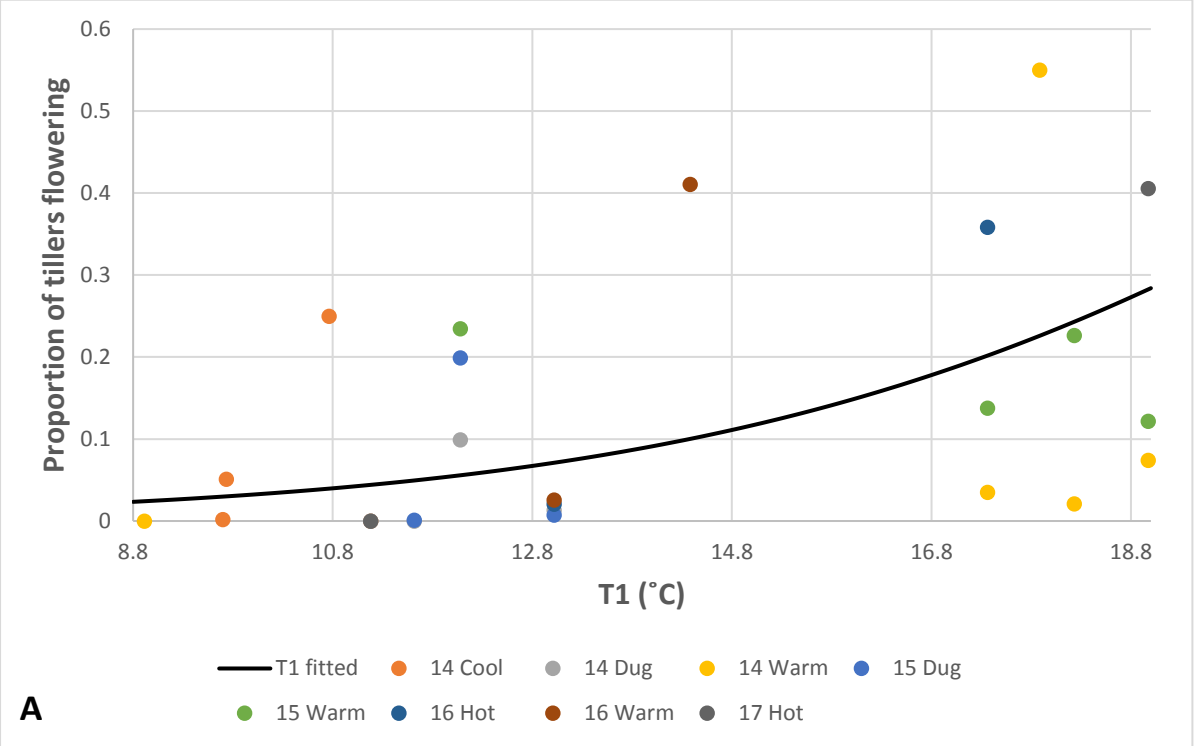
Random effects	**Variance**	**Std. Dev.**
plant ID:group	0.477	0.690
group	0.386	0.621

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-6.154	0.285	-21.61	< 0.001
T1	0.275	0.011	24.36	**< 0.001**

(b) DT model (difference in mean January-February temperatures over the two years prior to flowering, i.e. temperature 1 year before – temperature 2 years before)

Random effects	**Variance**	**Std. Dev.**
plant ID:group	0.556	0.746
group	0.331	0.576

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-2.988	0.219	-13.62	< 0.001
DT	0.378	0.008	49.73	**< 0.001**



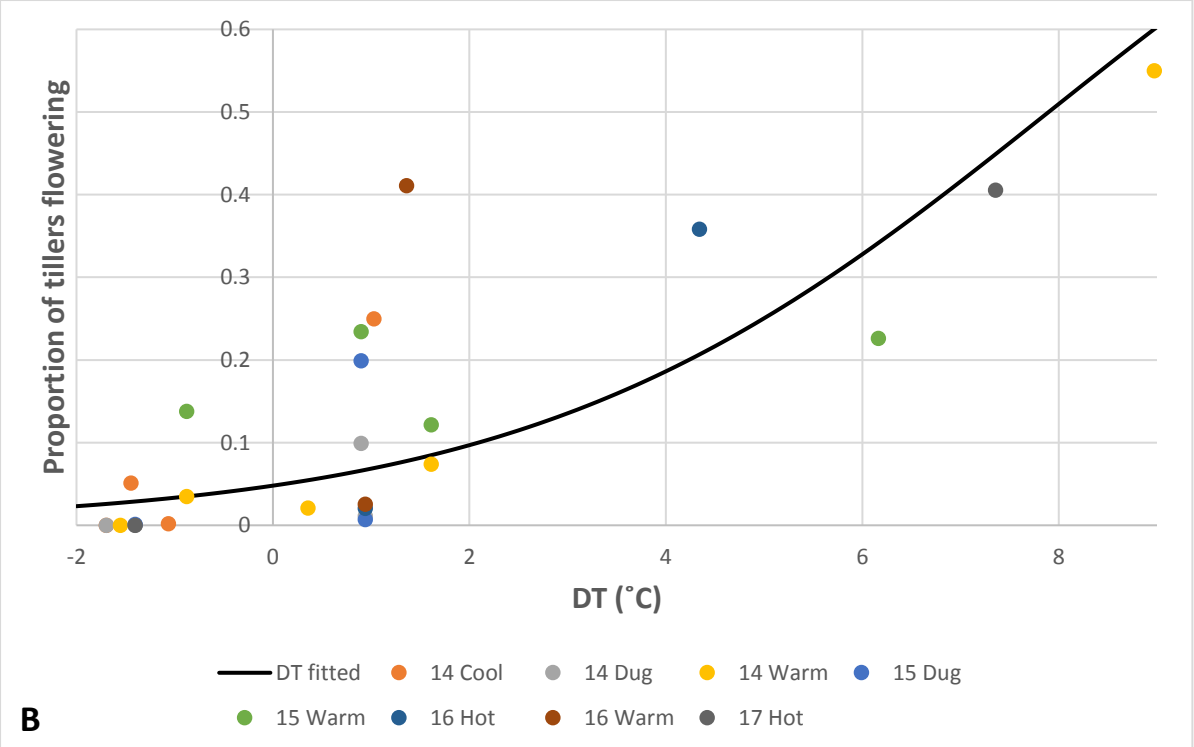


Figure 2.5. The mean proportion of tillers flowering per plant for each group of transplants across all years since they were first moved predicted from either T1 or DT temperature cues. Overlaid are the back-transformed fitted mean trend lines for all points combined for each temperature model. **A** = T1 (absolute temperature 1 year prior to flowering), **B** = DT (difference in temperatures over the two years prior to flowering, i.e. temperature 1 year prior – temperature 2 years prior). Each point is an average across all plants in a group for one year.

Of further interest regarding transplanted *C. pallens* plants were the flowering responses of each individual group of transplanted plants manipulated in the same year, and how these flowering responses varied across the years since they were first transplanted. Figure 2.6 shows these responses as the mean percentage of tillers flowering per plant across years separated out into cohorts for the year each was dug up and transplanted. There are five main things to note from these graphs of flowering responses. **1**) In plants moved to warmer temperatures (warm and hot groups), one year after they were transplanted, all showed large increases in flowering percentage compared to the dug controls. This was the predicted response. **2**) The 14Warm plants flowered to a higher percentage in their first year after transplantation (55% of tillers flowered) than any other plants moved to UC. **3**) In plants moved to cooler temperatures (14Cool, Fig. 2.6A), one year after transplantation, they expectedly decreased flowering compared to controls. However, unexpectedly, in their second year after transplantation they had a considerable increase in flowering compared to the control plants. **4**) Although the 16Warm plants experienced a 1.4˚C DT increase, while 16Hot plants experienced a 4.4˚C DT increase, both groups of plants flowered similarly one year later (Fig. 2.6C) instead of 16Hot flowering more than 16Warm, as was expected under both the T1 and DT models. **5**) The 14Warm plants had very low flowering in their second and third years after transplantation, which was expected after heavy flowering one year after transplantation. This is a key difference between the DT and T1 models. Under the T1 model, flowering should be heavy every year at low altitude, whereas under the DT model, by the second year at low altitude plants should flower similarly to plants that had been permanently at that site. Yet, the 15Warm plants still flowered heavily in both their second and third years after transplantation without any low flowering period to recover. The implications of these five main points will be discussed below.

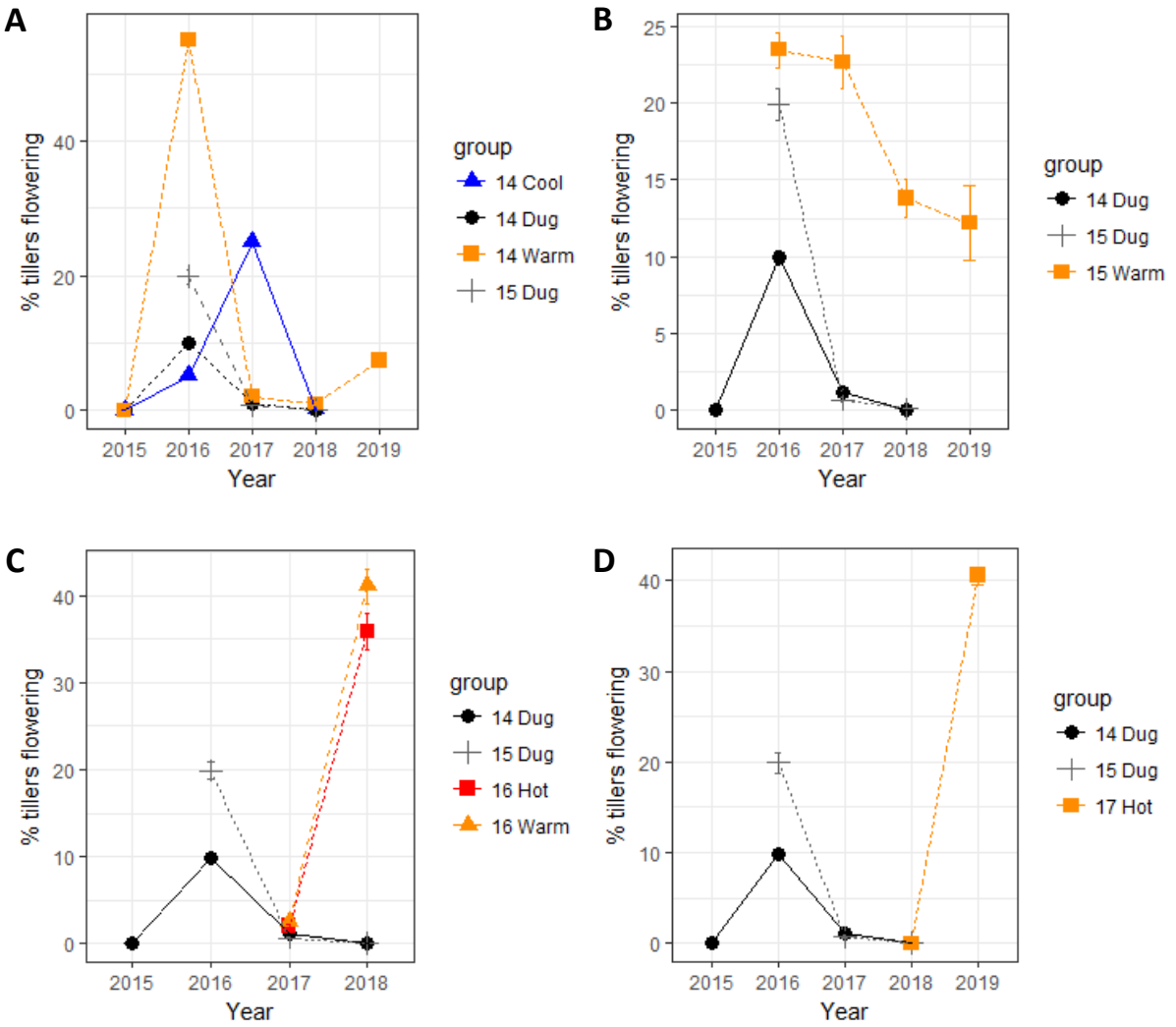


Figure 2.6. Raw mean percentage of tillers per plant that flowered for transplants and dug controls, separated out into **A**, 2014/15 transplants, **B**, 2015/16 transplants, **C**, 2016/17 transplants, and **D**, 2017/18 transplants. All transplants were originally from 1070 m on Mt Hutt except for the 14Warm plants which came from 1620 m. Dug plants remained at 1070 m, square symbols were all transplanted to UC, 14 Cool plants were transplanted to 1520 m, 16Hot and 16Warm plants were moved to UC and 450 m, respectively, for the 2016/17 summer then moved back to 1070 m.

Analysing transplant data for Celmisia lyallii

No flowering occurred in any of the observed *C. lyallii* plants on Mt Hutt over the whole period since the first plants were transplanted in summer 2015/16. Therefore, for several groups of plants (15Dug, 15Undug, 15Cool, and 15Warmish) the flowering response across all years was entirely zeroes. In contrast, the plants transplanted to UC flowered well every year since they were moved (Fig. 2.7). Since there were only four instances of flowering from only two of the six groups of plants, statistical analysis became difficult. However, the flowering responses of the *C. lyallii* transplants were significantly explained by both the T1 and DT models (Table 2.6), but more by the T1 model, as

determined by the higher *z* score. This is the opposite to what was found in *C. pallens* transplants and in the long-term flowering for *C. lyallii, C. pallens* and *C. macra*.

We can see, just looking at Fig. 2.7, that the increase in temperature for *C. lyallii* transplanted to UC clearly increased the flowering response of these plants compared to any on Mt Hutt. It was predicted that heavy flowering would be seen one year after transplantation, as was in fact seen in both 15Warm and 17Warm plants. However, under the DT model this heavy flowering was expected to drop in the second year after transplantation due to used resources. This effect was not seen in the 15Warm plants, which surprisingly further increased in flowering two years after transplantation and then flowering still remained high in the third year after transplantation (Fig. 2.7). This continuous high flowering at low altitudes is more consistent with the T1 model, a fact which was supported by the T1 model fitting the transplant flowering data better than DT (Table 2.6), albeit with very few flowering episodes to run the test on.

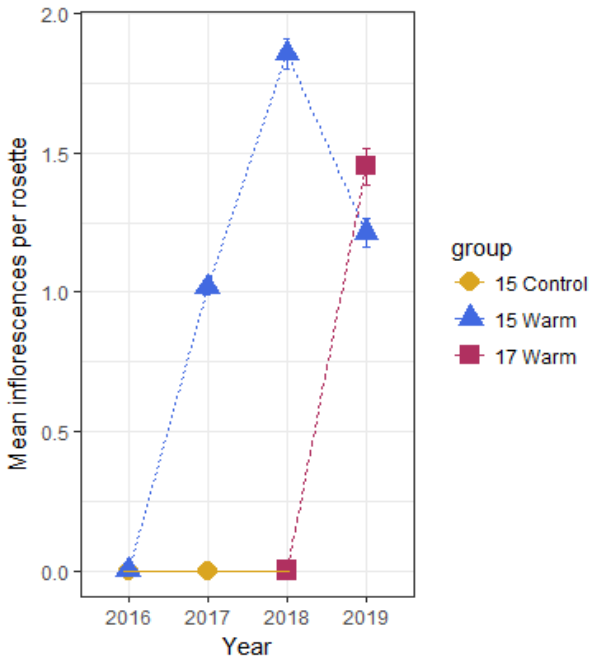


Figure 2.7. Flowering responses of *Celmisia lyallii* transplanted to UC (15Warm (*n* = 28), 17Warm (*n* = 31)), due to the increase in temperature at their new site, and of control plants (*n* = 20) at the original sites at 1350 m and 1520 m on Mt Hutt. Flowering is shown as the mean number of inflorescences per rosette and for the years since transplantation that data was currently available for.

Table 2.6. The relationship between the two temperature models and the number of inflorescences per rosette for all *C. lyallii* transplants, including dug and undug controls, combined across all years. Significant effects in bold.

(a) T1 model (January-February absolute mean temperature 1 year before flowering),

Random effects	**Variance**	**Std. Dev.**
plant ID:group	1.039	1.019
group	0.000	0.000

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-9.915	1.024	-9.680	< 0.001
T1	0.520	0.056	9.231	**< 0.001**

(b) DT model (difference in mean January-February temperatures over the two years prior to flowering, i.e. temperature 1 year before – temperature 2 years before)

Random effects	**Variance**	**Std. Dev.**
plant ID:group	1.239	1.113
group	21.849	4.674

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-6.648	4.978	-1.336	0.182
DT	0.107	0.020	5.245	**< 0.001**

2.4. Discussion

Estimating missing temperatures and T1 vs DT using long-term flowering data

All four temperature estimate sources produced high correlations with on-site temperatures, with both the CliFlo stations and the lower and higher VCSN prediction grid points giving good fits. Overall, the VCSN grid point predictions gave the best fits, with the lower point better for the lower site and the higher point better for the higher site. This result supports the Mason *et al.* (2017) findings that VCSN temperature predictions were a slight improvement on individual nearby weather station temperatures at predicting on-site recorded temperatures. However, I found the improvement of the relationship to on-site temperatures using VCSN predictions compared to CliFlo temperatures to be notably greater than those of Mason *et al.* (2017). This may be due to Mason *et al.* (2017) using coastal forest sites compared to the alpine environment used in this research. For the prediction of missing temperatures using off-site weather stations from CliFlo, using a station closer to the location of interest showed a better fit to on-site temperatures than a site further away. Whether this was due to the Darfield site being inland, compared to the Christchurch Botanic Gardens being coastal, would need further investigation. Other inland sites from further away would need to be tested to see if this was the case.

When the estimate sources were tested using the long-term flowering from Mt Hutt some interesting patterns emerged. For *C. pallens*, *C. macra* and *C.lyallii* the DTs all gave notably better fits than T1s, except for the 1520 m *C. pallens* where T1 was slightly better than DT. This may be due to the 1520 m *C. pallens* flowering data being the shortest dataset as flowering records only began in 2006. Kelly *et al.* (2013) found DTs better explained seedfall than T1s for both *C. pallens* (T1 R^2 = 0.145, DT R^2 = 0.484) and *C. lyallii* (T1 R^2 = 0.248, DT R^2 = 0.418) for Mt Hutt sites combined during the January-March periods. For the two *Chionochloa* species, the VCSN-estimated missing temperature predictions gave consistently better fits for the flowering than the NIWA weather stations, although the NIWA stations were often only slightly lesser fits than VCSN. Both these findings are similar to that of Mason *et al.* (2017). At 1070 m, the estimates from the Christchurch Botanic Gardens produced a better fit than those from Darfield for both *Chionochloa* species. This shows that using a weather station closer to the site of interest does not necessarily explain the flowering better than one further away, even though the temperatures from the closer site better fitted those on-site. Therefore, the estimates used in the Kelly *et al.* (2008) and Kelly *et al.* (2013) papers, based on estimates where necessary from Christchurch, were applicable. In a study where temperature data loggers were placed on individual *Quercus lobata* trees, a Californian masting species, it was found that fine-scale differences in flowering and seeding between trees was highly correlated to the microclimate individual trees experienced (Koenig *et al.*, 2015), with less inter-tree temperature variability resulting in more synchronous flowering leading to higher pollen availability and fertilisation success. The Koenig *et al.* (2015) results show that more accurate temperature data allows for finer-scale understandings of the processes and mechanisms occurring in masting species. However, as Körner and Hiltbrunner (2017) point out "Whatever the focus, the temperature that matters, is that actually imposed on the processes/organs of interest and at a temporal resolution that matches with the biological reactions." Therefore, my results show that using broader-scale on-site temperatures (or being able to accurately predict these temperatures) provides very useful data for determining the effects of temperature on masting, with far less work involved.

At the higher sites on Mt Hutt (1520-1620 m), the VCSN-only temperatures on average gave worse fits in explaining observed flowering than any of the sources using on-site temperatures with estimated missing temperatures. This may be related to the known discrepancy between VCSN temperature predictions at higher altitudes compared to lower altitudes, with higher altitude temperatures often predicted as warmer than they actually are (Tait and Macara, 2014). The warm bias at higher altitudes was found to be mostly during summer months and was improved by using the Norton equations (Tait and Macara, 2014). As my study used only summer temperatures my results may be displaying the remaining warm bias. However, the effect was small as the average R^2

for the three high altitude flowering correlations using VCSN-only was 0.56 compared to 0.64 for each of the other three estimate sources, which is still a reasonable correlation with which to make predictions from. The last, and particularly interesting, finding was that the flowering in *C. lyallii* was better explained by the VCSN-only estimates and any using the on-site temperatures were markedly lesser fits. In contrast, for *C. pallens* the best fits came from using on-site temperatures, although the best estimated missing temperatures were achieved using the VCSN estimates for both lower and higher altitudes.

The long-term flowering of *C. pallens*, *C. macra* and *C.lyallii* were best explained by the DT model than the T1 model (with one minor exception, see above). However, *Chionochloa* and *Celmisia* appear to be responding to different facets of the DT temperature cue. Both *C. pallens* and *C. macra* flowering responses were better explained by the on-site temperatures, and therefore the more accurate measure of what the plants actually experienced, with any missing daily temperatures filled in by another source (VCSN gave the best fit), than the VCSN-only temperatures. The VCSN-only temperatures still explained *C. pallens* and *C. macra* flowering well but the fit dropped off as the altitude increased, which may be a result of remnant warm-bias of temperatures at higher altitudes (Tait and Macara, 2014). *Celmisia* flowering, on the other hand, was better explained by the VCSN-only temperatures than any of the temperatures using on-site records. As all the *C. lyallii* long-term flowering data were from the higher altitude sites, therefore using the higher VCSN grid point temperatures for predictions, perhaps something in this warm-bias is better explaining *C. lyallii* flowering than actual on-site temperatures. It is possible that *C. lyallii* require extra warm temperatures to flower, which may help explain why *C. lyallii* have been observed having more frequent zero-flowering years at Mt Hutt than *Chionochloa* species, yet heavy flowering following large DTs (Kelly *et al.*, 2013).

Effect of transplantation on flowering and T1 vs DT

Crone and Rapp (2014) say there have been almost no successful manipulative tests of temperature cues in masting species. They failed to note the Mark (1965a) and Mark (1968) transplant experiments in *Chionochloa* where Mark successfully showed that increasing temperatures (moving to lower altitudes) increased flowering and decreasing temperatures (moving to higher altitudes) decreased flowering compared to flowering at the source sites. My own results also show similar flowering responses to those found in the Mark (1965a) and Mark (1968) transplant experiments.

The flowering responses of all plants used in the *C. pallens* transplant experiment were significantly explained by both the T1 and DT models. Yet, as was expected, the flowering was better explained by the DT model. One year after transplantation to a lower and warmer location, either permanently or just for the summer period, *C. pallens* plants flowered much more heavily than those

at their home sites, as predicted. Mark (1965a) also found that *C. rigida* transplants moved to lower altitude sites all flowered heavily the year after they were transplanted. For the 14Warm plants, the summer after they were transplanted they flowered to a higher percentage than any other *C. pallens* plants moved to UC (Fig. 2.6). This may be because the 14Warm plants were transplanted from 1620 m instead of 1070 m like the other transplants. As Fig. 2.4 shows, the control plants from 1620 m also flowered more heavily than any others in that same year, summer 2015/16 (a mast year). Heavier flowering by higher altitude plants in mast years has been noted before (Kelly *et al.*, 2008) and may explain the high percentage of flowering in the 14Warm transplants. Furthermore, the current summer (2018/19) is predicted to be a mast year, and the percentage of flowering in the 14Warm plants did increase in accordance with the UC temperatures, with a DT of 1.61°C. However, the percentage of flowering in the 14Warm plants was lower than that of the 14Dug plants in the 2015/16 summer where the DT was 0.89°C. This may be due to the plants being in unfertilised potting mix in self-contained bags where no additional nutrients are available than what breaks down from the potting mix or decaying plant matter. Yet, the current flowering in 14Warm plants was still better explained by the overall DT trendline than the T1 trendline.

For the *C. pallens* plants moved to UC and 450 m on Mt Hutt for a single summer period of less than 4 months (16Hot and 16Warm, respectively), the predicted increase in extra flowering with the greater temperature increase at UC (16Hot) than at 450 m (16Warm) was not seen. Instead, both sets of plants flowered similarly, about 40% of tillers flowering, a level which was also seen in the 17Hot plants moved to UC one year later. This suggests the possibility of flowering being saturated once the temperature cue reaches a certain point. For plants from 1070 m, their maximum increase in flowering effort due to increases in temperature was approximately 40%, compared to the 1620 m plants which got up 55% of tillers flowering. This would mean that once the plants have nearly all capable tillers flowering, further increases in temperature cannot increase flowering, and the DT model would be better fitted by a sigmoid curve than the exponential curve shown in Fig. 2.5B. Interestingly, a sigmoid curve would fit visually onto Fig. 2.5B.

Studies on *Chionochloa* in their natural environment between 900-1050 m in Takahe Valley in the Murchison Mountains, Fiordland, showed that *C. pallens* had an average of 11% of tillers flowering during years where at least one of the 10 monitored plants flowered (Tanentzap *et al.*, 2012); and in years where more than half of the monitored plants flowered there was also an average of 11% of tillers flowering across five *Chionochloa* species combined (Tanentzap *et al.*, 2014). These numbers are similar to my findings for *C. pallens* at 1070 m on Mt Hutt, as in 2015/16, a mast year, the 14Dug plants had an average of 10% of tillers flowering and the 15Dug plants had an average of 20% of tillers flowering.

The *C. pallens* plants moved to higher and cooler sites flowered the next year less than those from their 1070 m home site, as predicted, even though this was the 2015/16 heavy flowering mast year. However, unexpectedly, the 14Cool plants flowered more heavily in their second summer at the cooler site than any of the 2014 cohorts did that same year, and heavier than the 1070 m plants had flowered in the previous mast year. No explanation has been found for this yet. However, it is in contrast to the Mark (1965a) finding that plants moved to higher altitudes did not flower over the next three years, leading him to suggest that flowering in cold-shifted *Chionochloa* "is most unlikely". Greer (1979) found that some plants from the Mark (1965a) study that had spent 15 years at a lower location to their home site, when moved back to their original site flowered as others from the same site did, showing a possible genetic relationship to temperatures at their home site and that plants moved from warmer to cooler climates can indeed flower.

The 15Warm *C. pallens* plants responded differently to all other transplants. They flowered to a similar degree as the 15Dug controls in the year they were moved, which was the 2015/16 mast year. Flowering in that year was determined by temperature before moving, so this was as expected. One year after transplantation they did flower heavier than all others, as expected, but to a lesser degree than other transplants moved to UC. This was likely due to the heavier flowering they had done in the previous year reducing the supply of tillers competent to flower in 2016/17. Against predictions, in their second and third years after transplantation, the 15Warm *C. pallens* plants continued to flower well instead of having their flowering level drop down to very low levels like the 14Warm plants did. This difference may be due to a treatment difference: during transplanting the 15Warm plants were placed into slow-release fertilised potting mix at UC, whereas the 14Warm plants went into unfertilised potting mix. The extra nutrients and resources may have enabled the 15Warm plants to produce more tillers (see Chapter 3) and continue flowering more than expected for consecutive years. A similar effect was seen in *C. rigida* plants transplanted to Dunedin by Mark (1965a) where plants from various mountain altitudes flowered well for three successive years near sea-level. The Mark (1965a) plants were placed into the ground in a garden where soil was likely quite fertile and would have provided continual resources for the plants compared to plants in this study being placed into discrete garden bags with limited resources (especially the unfertilised 14Warm plants which had their bags filled with potting mix which is largely bark and sand, rather than soil). Therefore, the 15Warm plants, which had slow-release fertiliser, probably responded more similarly to a plant placed into the ground than to how the resource-limited 14Warm plants responded across years. The 15Warm plants possibly had an easier life than even plants in the field as they would not have had any root competition from other plants. It will be interesting to see the flowering responses of the 2016 and 2017 transplants, as the 2016 plants were returned to their

original holes in the ground at 1070 m and the 2017 plants were put into unfertilised potting mix in bags at UC. The 2016 transplants are unlikely to produce a lot of inflorescences in 2018/19 as they flowered heavily the previous year with very few new tillers seen by me in a check of these plants on 15/01/2018, leaving few competent tillers remaining to flower this coming flowering season. However, a high percentage of these remaining tillers may still flower if this is a heavy masting season as predicted. The 17Warm transplants are expected to have low flowering in the 2019/20 season, after their second year at UC.

Celmisia lyallii displayed a different pattern to flowering on Mt Hutt than *Chionochloa* for long-term flowering data. In low flowering years for *Chionochloa* on Mt Hutt, monitored *C. lyallii* produced no inflorescences. The Kelly *et al.* (2013) paper showed this same effect where there was either virtually no flowering or high flowering in *C. lyallii* compared to other masting species and years of zero flowering were very frequent. This lack of flowering in Mt Hutt *C. lyallii* meant there was very little flowering data available to run statistical tests on.

The *C. lyallii* transplanted to UC flowered every summer after being moved (none flowered in the year they were moved, the same response as *C. lyallii* plants on Mt Hutt for that year). Statistical tests run on the *C. lyallii* UC transplant flowering data showed that the observed flowering in *C. lyallii* transplants was better explained by the T1 model than DT, a direct contrast to that seen for *C. pallens* transplants and to long-term observational data for *C. lyallii, C. pallens,* and *C. macra*. This inconsistency may be due to the *C. lyallii* transplant dataset being very small, with only four flowering episodes to run statistical tests on, all of which were from UC as no flowering occurred on Mt Hutt in any year since the beginning of the transplant experiment. Perhaps the 2018/19 flowering season will provide flowering data from Mt Hutt for *C. lyallii* that will allow more comprehensive statistical tests to be run. The response of the 17Warm *C. lyallii* in 2019/20 will further add to this dataset. Both 2015 and 2017 *C. lyallii* cohorts brought down to UC flowered heavily one year after they were transplanted, as expected. However, the 15Warm plants did not decrease in flowering in their second summer after transplantation, as predicted by the DT model, but in fact increased their flowering even more. This was followed by a third consecutive year of quite heavy flowering in *C. lyallii* after transplantation to UC. These unexpected results may be due to these plants also being placed into slow-release fertilised potting mix when brought down to UC, as extra resources would have been available for them to recover quickly and flower again. It will be interesting to see what the 17Warm plants do in their second year after transplantation as they were placed in unfertilised potting mix so may show a different response to the 15Warm transplants.

In conclusion, where long-term flowering datasets were available, the DT model better explained the flowering in all three study species, *C. pallens, C. macra*, and *C. lyallii*, than the T1 model. For *C. pallens* the DT model was shown to be a reliable predictor of both long-term flowering and of short-term responses to manipulated temperature changes. The lack of flowering in *C. lyallii* at different elevations on Mt Hutt shows that *C. lyallii* has a different sensitivity to temperature in regard to flowering than does *C. pallens*. The 15Warmish *C. lyallii* transplanted from 1350 m to 1070 m on Mt Hutt had a small positive DT (1.88˚C) one year after they were transplanted but this was not enough to provoke flowering the following year. The 15Warm *C. lyallii*, transplanted to UC, had a 7.10˚C DT that resulted in heavy flowering. This lack of flowering in *C. lyallii* with small but positive DTs and the frequent zero years in observational data seem to show that only an infrequent very large T1 or DT is sufficient to provoke flowering in *C. lyallii*. The current 2018/19 season is expected to be a heavy masting year but the DT for 1070 m, and the 15Warmish *C. lyallii,* is only 1.69˚C so it is possible we may again see a zero flowering year for this species. However, the T1 for January-February at 1070 m was 13.31˚C, the highest it has been for 19 years, so this may be enough to initiate flowering in *C. lyallii*, but this T1 is not much more than the 13.02˚C the 15Warmish plants experienced the year after they were moved and did not flower. Hopefully this question will be resolved when the 2018/19 flowering observations are recorded.

Whether plants were put into fertilised or unfertilised potting mix after transplantation apparently made a large difference in the flowering responses in later years, showing that resources do play an important role in the regularity of flowering in both these species when moved to higher temperatures. Looking at the effect substantial increases in temperature could have on these plants is a very important and timely exercise and is easiest done by moving plants down near sea-level. However, both *C. pallens* and *C. lyallii* are well below their natural elevation range when moved to UC and 450 m, as well as to 1070 m for *C. lyallii*. The results seen in the UC transplants may not be an accurate depiction of what would occur at different elevations within the natural range of each species in situ on Mt Hutt as the unknown effects of dealing with an elevation they are not adapted to comes into play also. Moving plants within their respective higher and lower altitude range sites (e.g. *C. pallens* transplanted from 1620 m to 1070 m and vice versa) would enable us to verify if the responses seen at UC are like what would occur in situ, albeit with smaller manipulatable DTs. The flowering responses of transplants that were moved within Mt Hutt ranges in the coming 2018/19 flowering season will help to clarify this. If the flowering responses of transplanted plants at higher altitudes during a mast year in the Mark (1965a) study are anything to go by we should see a moderate to heavy amount of flowering on Mt Hutt in summer 2018/19. The impact of these findings in relation to climate cues and masting globally will be discussed in the final chapter

(Chapter 5). For now, we have successfully run further temperature manipulation experiments on masting species, similar to the successful experiments by Mark (1965a) and Mark (1968) which Crone and Rapp (2014) failed to mention. Our temperature manipulation experiments showed direct effects on flowering in *Chionochloa* and *Celmisia* due to altered temperatures.

Chapter 3. Fertiliser effects on *Chionochloa*

3.1 Objectives

Resources are an important factor for reproduction in all plants, and they can determine when and how much plants will flower in a given year (Bazzaz *et al.*, 1987). Masting plants, by definition, have infrequent flowering to start with, although the main factors controlling this flowering pattern are yet undefined. Heavy masting years use up a lot of stored resources in plants and it has been suggested that the time it takes for plants to regain enough resources to flower again is one factor which controls the sporadic flowering pattern in masting plants (Isagi *et al.*, 1997; Han *et al.*, 2014). Resources have been shown to have this effect in some masting plants in natural populations (Han *et al.*, 2008; Satake and Bjørnstad, 2008; Han *et al.*, 2014) and under experimental conditions (Miyazaki *et al.*, 2014).

This study used two New Zealand alpine masting species, *Chionochloa macra* and *C. pallens* (snow tussocks), to look at the importance and control of resources on mast flowering. This was not something that had been tested in either of these species before, and as they are herbaceous monocots, instead of the dicot trees that have previously been studied in this regard, it posed a further interesting dimension. The method used to study the impact of resources on flowering in *C. macra* and *C. pallens* was the addition of complete fertiliser to plots at Mt Hutt and individual bagged plants at UC, which had previously been transplanted from Mt Hutt. The study was done over multiple altitudes, with flowering responses monitored over consecutive years. In addition to flowering responses, plant size and vegetative growth was also measured to identify any changes due to fertiliser application. I used these variables to test the following key questions:

1) Does the addition of nutrients, via fertiliser, increase vegetative growth of masting plants by,
    a. increasing the number of tillers and live basal area of plants?
    b. increasing daughter tiller production?
    c. increasing the survival of adult tillers?
2) Does the addition of nutrients increase the flowering response of *Chionochloa*,
    a. one year after fertiliser application?
    b. two years after fertiliser application?

The overall aim was to determine how big an effect resources have on the frequency and quantity of flowering in masting species. This was achieved by testing whether additional resources, via fertiliser

application, directly affected flowering, especially one year after application, to show a resource-based control of flowering response in these plants.

3.2 Methods

Chionochloa plants from populations at 1520 m and 1070 m, and *Celmisia lyallii* plants from 1520 m and 1350 m (fertiliser effects on *C. lyallii* are discussed in Chapter 4), on Mt Hutt had been experimentally manipulated by having resources (fertiliser) added (see Chapter 2 methods for Mt Hutt locations). Fertiliser experiments were also set up on plants previously brought back to University of Canterbury (UC, 15 m above sea level).

At UC, 10 of 20 *Chionochloa* plants and 10 of 20 *Celmisia* pots had fertiliser added. These experiments were set up initially for the collection of leaf tissue samples for a concurrent epigenetic study investigating the switching on or off of gene expression in these species in relation to tillers flowering or remaining vegetative. Additionally, it was necessary to collect data on the size of the flowering episodes and sizes of individual plants used for these epigenetic experiments, data which was used for this study also.

On Mt Hutt, fertiliser plots were set up next to existing long-term monitoring at 1070 m and 1520 m, these long-term monitored plants were used as controls for each site. Another plot was set up at 1350 m which consisted of a fertiliser and control pairing.

In November 2016, at the 1520 m site, a 20 x 2 m plot was set up 10 m to the west of, slightly below, and running parallel to, the control plot that was established in 2006 (see Chapter 2 methods). This newer plot had fertiliser added to it (including a 1 m buffer around all sides) twice during the 2016/17 summer period. All fertiliser applications were made once in December 2016 and again in March 2017 (Table 3.1). All *Chionochloa* plants (*C. pallens* and *C. macra* were both found in all fertiliser experiment plots) and *C. lyallii* patches in the plot were mapped, and sizes of plants and rosette patch counts, respectively, were recorded, as per the control plot.

At 1070 m, in December 2016, a 2 x 20 m plot was set up 5 m below, and running horizontal to, the bottom control line set up at this site in 1990. The *Chionochloa* plants in this plot were mapped and had sizes measured. Fertiliser was added twice during the 2016/17 summer period, including a 1 m buffer around all sides.

At 1350 m, a 20 x 2 m plot was set up in December 2016, where the top 11 x 2 m was unfertilised and the lower 9 x 2 m (plus a 1 m buffer on both sides and at the bottom) was fertilised twice during the 2016/17 summer period. The smaller size of the fertiliser ‘half’ was due to two control DNA plants being inadvertently sampled and tagged below the 10 m mark prior to any fertiliser addition. Therefore, the final fertiliser and control sizes within this plot required a reshuffle

before fertiliser addition to account for this. *Chionochloa* plants and *C. lyallii* patches were mapped, and the number of rosettes per patch was recorded.

At UC, fertiliser was added twice during the 2016/17 summer to 10 of the 20 *C. pallens* bagged plants that were brought down from Mt Hutt in December 2014. Additionally, half of the pots the *C. lyallii* plants were placed in, that were brought down from Mt Hutt in December 2015, had fertiliser added to them at the same times.

Fertiliser was applied at a rate of 200 kg/ha of calcium ammonium nitrate ($CaNH_4NO_3$) and 500 kg/ha of superphosphate. This rate was based on that used by Platt *et al.* (2004). For plants in bags or pots (at UC) fertilizer amount was based on the surface area of the bag, while for field plots on Mt Hutt, as noted above, we included a 1 m buffer zone around the edges of the marked plots to cover any roots from plants within the plot that were growing outside the plot. See Table 3.1 for the areas which fertiliser was applied to at each site and application dates. Fertiliser was applied by hand to all sites. Original fertiliser applications were carried out at all sites in December 2016, with a second maintenance application made in March 2017. I helped apply the fertiliser in the 2016/17 season before starting this MSc, and recorded the flowering and tiller responses from late 2017 onwards.

Table 3.1. Fertiliser application table showing the size of the areas where fertiliser was placed at a rate of 20 g per m^2 of calcium ammonium nitrate ($CaNH_4NO_3$) and 50 g per m^2 of superphosphate to all plants (*Chionochloa* and *Celmisia*) and plots, and the date these applications took place.

Site	Size (including buffer)	Total area	1st application	2nd application
UC *Chionochloa pallens* bags	0.145 m^2 x 10 bags	1.45 m^2	22/12/2016	30/3/2017
1070 m fertiliser plot (*Chionochloa* and *Celmisia spectabilis*)	22 x 4 m	88 m^2	13/12/2016	15/3/2017
1520 m fertiliser plot (*Chionochloa* and *Celmisia lyallii*)	22 x 4 m	88 m^2	2/12/2016	15/3/2017
UC *Celmisia lyallii* pots	0.04 m^2 x 10 pots	0.4 m^2	22/12/2016	30/3/2017
1350 m fertiliser plot (*Celmisia lyallii*)	10 x 4 m	48 m^2	2/12/2016	15/3/2017

Pre-fertiliser data (plant size, tiller counts, and flowering data) were collected in the same season as the fertiliser was applied (2016/17), but before any fertiliser effects would have had time to occur (Table 3.2). Plants were followed for 1-2 years post-fertilizer, and comparison was made to the relevant non-fertilizer plants which were recorded as part of other experiments (Table 3.2).

The control plants for the RNA-sampled plants (which had leaves removed for RNA sampling) were not within the control plots but established along a line running parallel with the fertiliser plots at both 1070 and 1520 m to avoid any manipulation taking place in the long-term control plots.

These control lines were set up 2 m uphill and 5 m uphill of the fertiliser plots at 1070 m and 1520 m, respectively, so they would not receive any leached fertiliser. The analyses below looking at tiller survival and daughter tiller production had their control data collected from these control lines, whereas the fertiliser data came from within the fertiliser plot at 1070 and 1520 m. There was also a plot set up at 1350 m on Mt Hutt that was used primarily for the study of *Celmisia lyallii* but which also contained *Chionochloa* plants. These *Chionochloa* plants within the 1350 m plot also had their basal area and percentage of live tillers collected post-fertiliser but because similar data were not collected pre-fertiliser they could not be used in these analyses.

Table 3.2. Data recorded for both *Chionochloa* species showing the number of plants at each site under each treatment and the data gathered before the commencement of the fertiliser experiment (2016/17), one year after the fertiliser experiment (2017/18), and at UC only, two years after fertiliser (2018). Included are some plants that are part of other studies (see Chapter 2) but whose data were used in some of the current analyses. *Live BA* is the live tussock basal area (dm^2); *Tillers* signifies whole plant tiller counts; *Daughters* signifies the tagging of individual tillers on RNA-sampled plants which allowed counts of daughter tiller production; *Infl.* signifies counts of inflorescences produced per plant. The control 'plot' at 1070 m consists of three 20 m lines which have been monitored since 1990.

					2016/17 summer season			2017/18 summer season				December 2018		
Species	**Site**	**Group label**	**Treatment**	**No. plants**	**Live BA**	**Tillers**	**Infl.**	**Live BA**	**Tillers**	**Daughters**	**Infl.**	**Live BA**	**Tillers**	**Infl.**
Chionochloa pallens	1520 m	Fertiliser plot	Fertilised	69	✓		✓	✓			✓			
		-includes RNA-sampled plants	Fertilised	10	✓		✓	✓	✓	✓	✓			
		Control plot	Unfertilised	55	✓		✓	✓			✓			
		Control line	Unfertilised	10			✓	✓	✓	✓	✓			
		14 Cool	Unfertilised	20	✓	✓	✓	✓	✓		✓			
	1070 m	Fertiliser plot	Fertilised	76	✓		✓	✓			✓			
		-includes RNA-sampled plants	Fertilised	10	✓		✓	✓	✓	✓	✓			
		Control plot (lines x 3)	Unfertilised	82	✓		✓	✓			✓			
		Control line	Unfertilised	10			✓	✓	✓	✓	✓			
		14 Dug	Unfertilised	20	✓	✓	✓	✓	✓		✓			
		15 Dug	Unfertilised	10			✓	✓	✓		✓			
		16 Hot	Unfertilised	10	✓	✓	✓	✓	✓		✓			
		16 Warm	Unfertilised	10	✓	✓	✓	✓	✓		✓			
		17 Cool	Unfertilised	10				✓	✓		✓			
		17 Hot	Unfertilised	10				✓	✓		✓			
	UC	Fertiliser plants	Fertilised	10		✓	✓		✓	✓	✓	✓	✓	✓
		Control plants	Unfertilised	10		✓	✓		✓	✓	✓	✓	✓	✓
Chionochloa macra	1520 m	Fertiliser plot	Fertilised	58	✓		✓	✓			✓			
		Control plot	Unfertilised	44	✓		✓	✓			✓			
	1070 m	Fertiliser plot	Fertilised	1	✓		✓	✓			✓			
		Control plot (lines x 3)	Unfertilised	26	✓		✓	✓			✓			

Whole-plant tiller counts consisted of counting the number of fully-grown adult, or parent, tillers and the number of younger daughter tillers. Daughter tillers are tillers which have recently been formed as offshoots from a parent tiller. I included as a daughter tiller any tiller whose longest leaf was less than half the length of the leaves on adult tillers; these were also usually very narrow in diameter compared to fully-grown tillers. Daughter tillers may be too small or young to flower (Connor, 1966). Connor (1966) states that plants raised from seed do not flower for the first time until after four to five years, and says this suggests "floral initiation is not controlled only by summer temperatures but also by the age of the tillers." He dissected flowering tillers but found no indication of the absolute age of the tillers from this, yet he said tillers are not ready for floral induction in their first year and possibly not in their second year either. However, in glasshouse-grown plants raised from seed at UC, flowering has sometimes been observed in tillers in their first year (Dave Kelly, pers. comm.). Both Connor (1966) and Mark (1965c) state that flowering tillers "invariably" produce one or two axillary tillers before the emergence of the panicle (flowering leads to the eventual death of the parent tiller). I had some fertiliser tillers which produced 3 and 4 daughter tillers. Here I assume that full-sized tillers (my "adult" tillers) could be capable of flowering. Usefully, where individual tillers were marked with coloured adhesive tape for RNA sampling, daughter tillers emerged within the same tape markers (a similar technique to that used by Mark (1965b)), so we have definite counts of daughter tiller production for RNA-sampled tillers as well as each parent tiller's subsequent survival. Total tiller counts included both the adult and daughter tillers, and these are what were used for most of the analyses. Due to the total tiller count data not meeting the assumptions of equal variances and linearity I performed log (ln) transformations on these data (of x + 1 to allow for zeroes) for all analyses, after which the assumptions were met. Log (ln) transformations were used throughout this chapter.

As a caveat, due to our RNA-sampled tillers periodically having leaf sections removed for gene analysis, it is worth mentioning that Mark (1965b) stated that clipping the leaves of even a few tillers of a whole *Chionochloa* plant can have detrimental effects on the on the normal growth pattern of the whole plant. However, the clipping treatment Mark (1965b) used involved cutting the outer leaf low down, at the top of the sheath. For our sampling each leaf was cut higher up, if possible, and often the cut leaf continued to elongate after clipping. Most of our RNA-sampled tillers only died after flowering and no deterioration was noted due to leaf clipping, therefore I have assumed that any impact of leaf clipping on tiller survival or daughter tiller production was negligible.

Chionochloa basal area (BA) was measured using a dbh (diameter at breast height) tape just above ground level. Also recorded was the (visually estimated) percentage of that area which carried

live tillers (% live). The BAs of individual *Chionochloa* plants generally do not change much from year to year as new 'daughter' tillers are produced from within current 'parent' tillers, meaning plants only increase slowly in diameter like trees (Mark, 1969). However, the proportion of live tillers within a plant can change rapidly from year to year depending on the conditions, with often heavy dieback in the year after heavy flowering. Hence my calculated size of plants (liveBA) included both the basal area and the percentage of the given basal area that was live, using the equation:

$$\text{liveBA } (dm^2) = ((\pi \times \text{diameter}^2) / 4 \times \% \text{ live} / 100) / 100 \text{ where diameter is in cm}$$

The conversion to BA in dm^2 follows Kelly *et al.* (2008), and is purely to have flowering counts per unit area which have fewer leading zeros after the decimal point.

To calculate the variation in growth rates between control and fertilised *Chionochloa* plants for the 2016/17 to 2017/18 period, a liveBA ratio was created using the liveBAs collected pre-fertiliser and post-fertiliser using the equation:

$$\text{liveBa ratio} = \text{post-fertiliser liveBA} / \text{pre-fertiliser liveBA}$$

For analysis I always used (ln) log of this ratio.

All analyses were run using the statistical programme R (version 3.4.2, 2017). *Chionochloa* total tiller counts, liveBA (dm^2), liveBA ratios, and pre-fertiliser inflorescences per plant were included as numerical variables and all were natural log-transformed (ln) to account for the right-skewed nature of these data. Species was a categorical factor with two levels, *C. macra* and *C. pallens*. Individual *Chionochloa* tiller survival was binomial (dead or alive), and daughter tillers were counts per parent tiller (ranging from 0-4) so followed the poisson distribution. *Chionochloa* pre-fertiliser and post-fertiliser flowering data were counts of inflorescences per plant. Treatment was a categorical factor containing two levels: control or fertilised; while site was a categorical factor with up to four levels: UC, 1070 m, 1350 m, and 1520 m, depending on what was being tested and the data collected.

Chionochloa macra and *C. pallens* size and growth rates were analysed using multi-factor ANOVAs to test for any differences in growth between the two species. Both of these species are found growing together on Mt Hutt in the plots at 1070 m, 1350 m, and 1520 m. We know from previous work that the two species are highly synchronised in their flowering effort (Kelly *et al.*, 2000). I wanted to find out whether the two species responded similarly to the treatments, in which case I could combine both for analysis. Models were run with the response variable being log liveBA ratio and the predictors being species, treatment (fertilised or control), site (1070 m, or 1520 m), and all possible interactions.

Tiller counts are the most accurate measure of the plant size for relating to inflorescence counts, but tiller counts were not available for all plants. Additionally, counting tillers is very time-consuming so having a faster technique for gathering data on the size variable, such as liveBAs, would be very useful. Multiple regressions were used to analyse the total tiller counts and liveBAs collected post-fertiliser to test whether they were closely related and whether liveBAs were a good representation of the size of the plants where tiller counts were not available. These analyses were performed using all plants which had both total tiller counts and liveBAs collected during the 2017/18 flowering season (Table 3.2). Models were run including the effect of treatment on the relationship between tiller counts and liveBA.

Survival data for individual *C. pallens* tillers were analysed using binomial GLMMs (generalised linear mixed models) with the *lme4* package in R. The individual plant IDs (there were three tillers on each of the 10 plants per treatment per site) were used as a random effect to control for the nested structure of these data. The fixed effects were treatment, site (UC, 1070 m, or 1520 m), and treatment x site. Daughter tiller production was analysed using poisson GLMMs, again with plant ID as a random effect and the same treatment x site fixed effects.

The effects of fertiliser addition on flowering in *Chionochloa* at Mt Hutt were analysed using quasipoisson GLMs (generalised linear models) to correct for overdispersion. Firstly, I tested whether any flowering differences existed in between pairs of plots, prior to the addition of fertiliser to one of each pair, due to effects of (future) treatment. This was to ensure there was no pre-existing significant difference between treatment and control plots. The response variable was counts of inflorescences per plant within the plots. I further included additive effects of log pre-fertiliser liveBA and site in the model as these two factors were found to influence flowering in this study. Formal testing for the effect of fertiliser on flowering one year after application was not possible, due to an extremely low flowering year (five inflorescences in total among all monitored plots on Mt Hutt) that resulted in a dataset almost completely of zeroes and which would not run in the statistical analyses.

The 20 *C. pallens* plants previously transplanted to UC in summer 2014/15 were also used to test the effects of fertiliser on flowering. Half the plants had fertilizer added in 2016, after the plants had had two years to acclimate to the higher temperatures at low altitude. These plants produced more flowers than those on the mountain, so tests were able to be run on flowering both before the application of fertiliser and afterwards. As these plants flower earlier than those at Mt Hutt (June-October vs December-January), due to warmer temperatures and lack of snow cover, the flowering response two years after the application of fertiliser (the 2018/19 season) was able to be counted in December 2018 and included in this thesis. The UC plants had all had tiller counts made on them

every year since being transplanted from Mt Hutt, therefore I was able to use the number of flowering tillers per plant as the response variable. I ran a binomial GLMM using the individual plant IDs for each plant as a random effect. The fixed effects were treatment and year (as in flowering year, i.e. 2017 for the 2016/17 flowering season, 2018 for 2017/18, etc.). This test was run to ensure there was no pre-treatment difference in the flowering response of the two groups of plants, one of which later had fertiliser added, and to test for any increase in flowering one and two years after the application of fertiliser to half of the plants.

3.3 Results

Effect of fertiliser on vegetative growth in Chionochloa pallens *and* C. macra

I start this section analysing the growth rate of *Chionochloa* plants, measured as log LiveBA ratio, over the period 2016/17 to 2017/18 (the year fertiliser was added and one year afterwards). First, I found that there was no difference in growth rates between the two species of *Chionochloa* found in the plots used for this analysis, nor any species x treatment interaction (Table 3.3). Since the two species did not differ in their growth rates or responses to fertiliser they were combined for subsequent analyses using whole plot data.

Fertiliser addition significantly increased the overall growth rate in plants one year after it was added at both sites, and interestingly, there was a significant interaction effect of treatment x site on growth (Table 3.3). The interaction effect was due to a large difference in growth rate at the two sites for unfertilised plants, whereas for fertilised plants the growth rate at the two sites was almost the same. This means that although unfertilised plants grew at different rates between the two sites, with 1070 m plants growing at a significantly higher rate than 1520 m plants (back-transformed fitted final/initial size ratio = 1.080 and 0.836, respectively), the addition of fertiliser led to a significant increase in growth rate at both sites, but especially at 1520 m where plants ended up growing at a similar rate to the 1070 m plants (back-transformed size ratio = 1.510 and 1.449, respectively), as can be seen in Fig. 3.1. The higher liveBA ratios seen after the addition of fertiliser was not due to large increases in BA in these plants but due to live tissue filling more of the same BA (i.e. an increase in live %). One year after treatment, fertilised plants had more leaves as well as thicker, brighter green leaves compared to the control plants.

Table 3.3. The effects of species, treatment, and site on the log liveBA ratios for *Chionochloa macra* and *C. pallens* over the period 2016/17 to 2017/18. Significant effects in bold.

(a) Analysis of variance table

Response: log (liveBA ratio)					
	Df	**Sum Sq**	**Mean Sq**	***F* value**	***P* value**
species	1	0.01	0.01	0.03	0.860
site	1	0.25	0.25	1.20	0.275
treatment	1	21.06	21.06	101.36	**< 0.001**
treatment x species	1	0.01	0.01	0.07	0.796
treatment x site	1	2.15	2.15	10.36	**0.001**
residuals	400	83.10	0.21		

(b) Coefficients from the model

Predictor	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	0.143	0.068	2.100	0.036
species pallens	-0.087	0.069	-1.262	0.208
site 1520 m	-0.274	0.065	-4.202	**< 0.001**
treatment fertiliser	0.213	0.117	1.817	0.070
treatment fertiliser x species pallens	0.102	0.106	0.958	0.339
treatment fertiliser x site 1520 m	0.321	0.100	3.219	**0.001**

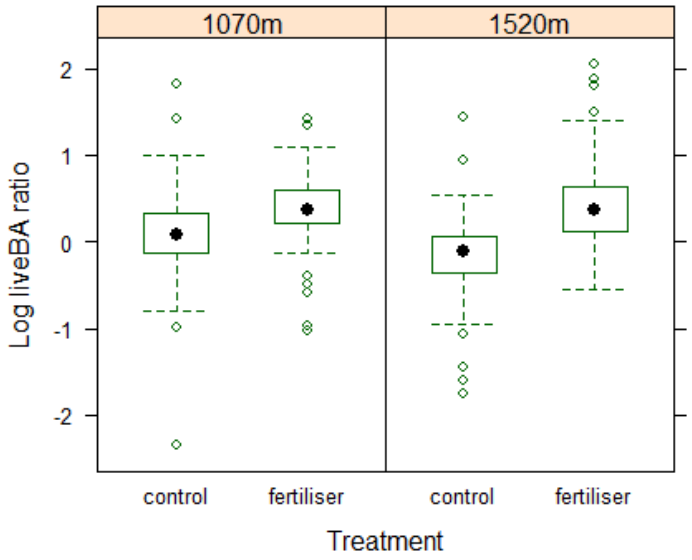


Figure 3.1. Effects of treatment and site on the growth rate (log liveBA ratio) of *Chionochloa* plants for the period 2016/17 to 2017/18, one year after the addition of fertiliser. Solid black dots show the median, green boxes the interquartile range (from 25-75% of the data), vertical dashed error bars the total range minus the outliers, and green circles the outliers (points more than 1.5 times above or below the interquartile range).

Next, I analysed whole plant tiller counts and their relationship to the liveBA measurement one year post-fertiliser treatment. Since tiller counts were only carried out on *C. pallens* plants, these analyses include only one species. There was no significant treatment x log liveBA interaction on the log total tillers (Table 3.4), so the interaction was removed from the model. A highly significant relationship was found between log liveBA and log total tiller counts in *C. pallens* plants at both 1070 m and 1520 m on Mt Hutt (R2 = 0.864, Table 3.4). Therefore, the liveBA measurement was a good predictor of tiller counts and thus is a useful measure of size and variation in size in *C. pallens*.

Treatment had a significant effect on log total tillers (Table 3.4). For the same liveBA the fertilised *C. pallens* plants showed a greater number of tillers, they produced 1.71 times more tillers per unit area than unfertilised plants (Fig. 3.2, Table 3.4c). This is likely due to an increased rate of daughter tiller production within the same plant basal area in the fertilised plants compared to the unfertilised plants (see results below), and shows that the liveBA measurement underestimates the fertiliser effect on vegetative growth.

Table 3.4. The effects of log (liveBA) and treatment on the total number of tillers for *Chionochloa pallens* at both 1070 m and 1520 m in the 2017/18 season, one year after the addition of fertiliser to some groups of plants. Significant effects indicated in bold.

(a) Including interaction term

Response: log (total tillers)					
	Df	**Sum Sq**	**Mean Sq**	***F* value**	***P* value**
log (liveBA)	1	82.42	82.42	758.680	**< 0.001**
treatment	1	3.37	3.37	30.976	**< 0.001**
log (liveBA) x treatment	1	0.08	0.08	0.714	0.4
residuals	124	13.47	0.11		

(b) Excluding interaction term. Evaluated using type III sums of squares when entering each term into the model last.

Response: log (total tillers)					
	Df	**Sum Sq**	**Mean Sq**	***F* value**	***P* value**
log (liveBA)	1	48.02	48.02	443.0	**< 0.001**
treatment	1	3.37	3.37	31.05	**< 0.001**
residuals	125	13.55	0.11		

(c) Coefficients from the model

Predictor	**Estimate**	**Std. Error**	***t* value**	***P* value**
(Intercept)	4.343	0.115	37.824	< 0.001
log (liveBA)	0.578	0.027	21.048	**< 0.001**
treatment unfert	-0.539	0.097	-5.572	**< 0.001**

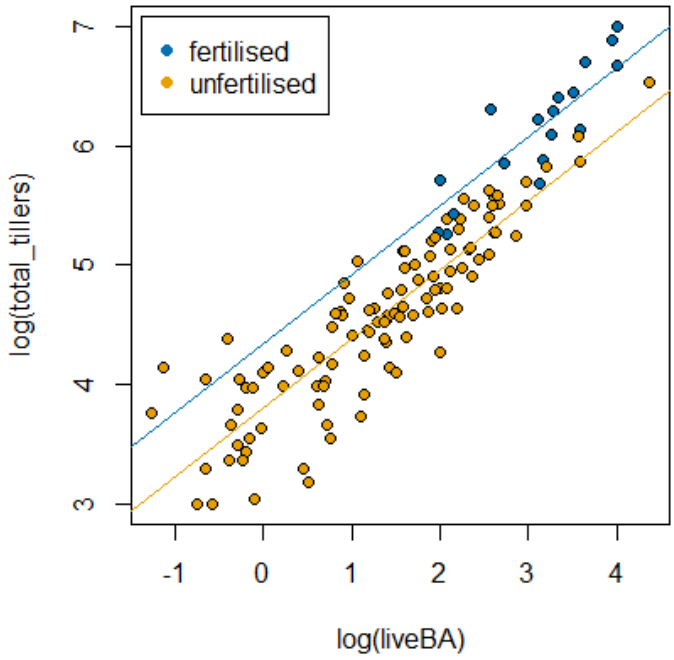


Figure 3.2. The effect of fertiliser on the relationship between liveBA and total tiller counts in *Chionochloa pallens* plants one year after fertiliser treatment, with trend lines for each treatment. Plants are from both 1070 m and 1520 m combined, with fertilised plants, *n* = 20, and unfertilised plants, *n* = 110. As the interaction term was non-significant it was omitted from the final model (i.e. the lines are parallel).

The next analyses focused on the individual tagged tillers within *C. pallens* plants at UC, 1070 m, and 1520 m. The fates (survived or died) of tillers within *C. pallens* plants showed no significant treatment x site interaction (Table 3.5a), so this interaction was removed. Tiller survival for both fertiliser and control plants showed no significant effect of fertiliser treatment (Table 3.5b; back-transformed fitted mean survival rate per treatment: control = 91%, fertilised = 86%), so the addition of fertiliser had no effect on the survival rate of individual tillers within plants. However, the tillers under both treatments at 1520 m survived to a significantly greater degree than those at UC and 1070 m (Table 3.5b; back-transformed fitted mean survival rate per site: UC = 81%, 1070 m = 89%, 1520 m = 98%) showing a higher tiller survival rate at the highest elevation.

For the production of daughter tillers, there was also no treatment x site interaction (Table 3.6a), so the interaction was removed from the model. However, the addition of fertiliser did significantly increase the number of daughter tillers produced by plants at all sites (Table 3.6b). When looked at in more detail, compared to 1070 m, there was a significant decrease in daughter tiller production at UC and a significant increase at 1520 m (Table 3.6b, Fig. 3.3). This shows that daughter tiller production significantly increased with elevation across sites, but that this effect was

significantly intensified by the addition of fertiliser. Therefore, these results imply that the significant increase in growth rate (liveBA ratio) seen above for fertilised plants compared to unfertilised plants was due to increased production of daughter tillers in the fertilised plants. Furthermore, the increase in growth rate seen in fertilised plants at 1520 m that led to them growing at the same rate as fertilised 1070 m plants was facilitated by greater survival and production of tillers at the higher elevation.

Table 3.5. GLMM analysis output showing tiller survival rate from 3 tillers x 10 *C. pallens* plants per treatment (fertilised/unfertilised) at each of three sites (UC, 1070 m, and 1520 m) with significant effects indicated in bold. Binomial error distribution used.

(a) Including interaction term

Random effects	**Variance**	**Std. Dev.**
Plant ID	1.492	1.221

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	1.579	0.691	2.286	0.022
treatment fertiliser	1.601	1.084	1.476	0.140
site 1520 m	2.487	1.182	2.104	**0.035**
site UC	0.182	0.908	0.201	0.841
treatment fertiliser x site 1520 m	-1.729	1.737	-0.995	0.320
treatment fertiliser x site UC	-1.780	1.414	-1.259	0.208

(b) Excluding interaction term

Random effects	**Variance**	**Std. Dev.**
Plant ID	1.629	1.276

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	2.041	0.666	3.064	0.022
treatment fertiliser	0.484	0.671	0.721	0.471
site 1520 m	1.842	0.859	2.145	**0.032**
site UC	-0.576	0.694	-0.831	0.406

Table 3.6. GLMM analysis output using daughter tiller production from 3 parent tillers x 10 *C. pallens* plants per treatment (fertilised/unfertilised) at each of three sites (UC, 1070 m, and 1520 m). Significant effects in bold. Poisson error distribution used.

(a) Including interaction term

Random effects	**Variance**	**Std. Dev.**
Plant id	0	0

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-1.204	0.333	-3.612	< 0.001
treatment fertiliser	1.204	0.380	3.168	**0.002**
site 1520 m	-0.251	0.504	-0.499	0.618
site UC	-1.504	0.782	-1.924	0.054
treatment fertiliser x site 1520 m	0.893	0.552	1.618	0.106
treatment fertiliser x site UC	0.742	0.846	0.877	0.381

(b) Excluding interaction term

Random effects	**Variance**	**Std. Dev.**
Plant id	0	0

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-1.626	0.270	-6.028	< 0.001
treatment fertiliser	1.725	0.256	6.741	**< 0.001**
site 1520 m	0.495	0.203	2.438	**0.015**
site UC	-0.891	0.297	-3.001	**0.003**

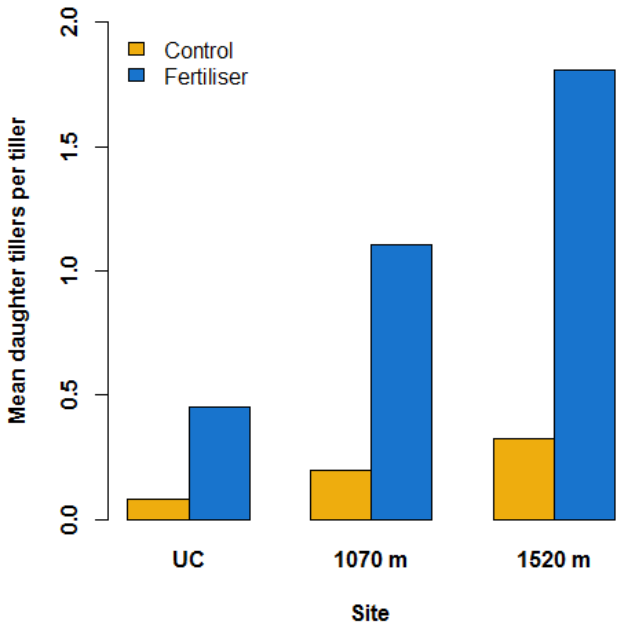


Figure 3.3. The effect of fertiliser and the variation in daughter tiller production between sites for *C. pallens* plants one year after the addition of fertiliser, shown as back-transformed fitted mean number of daughter tillers produced per parent tiller.

Effect of fertiliser on flowering in Chionochloa pallens and C. macra

I measured the effect of fertiliser on flowering, in field plots at Mt Hutt at two altitudes (1070 m and 1520 m), and in potted transplants at UC. At Mt Hutt, observations occurred on a background of relatively heavy flowering in 2015/16 (one year before fertiliser was applied), low flowering in 2016/17 (the year fertiliser was applied), and very low background flowering the following year. At UC, plants flowered at least to some extent in every season.

Prior to the addition of fertiliser at Mt Hutt, in the 2016/17 summer season, out of a total of 411 *Chionochloa* plants 100 of these plants produced 218 inflorescences among the four plots, two plots at each of 1070 m and 1520 m. The mean inflorescences per plant are given in Table 3.7 below and show that flowering was of low relevance as it was a low flowering year. The effect of "treatment" (in this case the two plots, one of which would later get fertiliser added) was non-significant on the pre-fertiliser flowering response in *Chionochloa* (Table 3.8). There was a significant effect of plant size (liveBA pre-fertiliser) on the pre-fertiliser flowering response (Table 3.8), where plants with larger liveBAs produced more inflorescences (Fig. 3.4), as expected since larger plants contain more tillers so can produce a greater number of inflorescences. This effect was indeed seen through significantly more flowering in plants at 1070 m than at 1520 m (Table 3.8), and although not significant, the average size of plants at 1070 m was greater than those at 1520 m (Fig. 3.5). Additionally, we already know that the growth rate of control plot plants at 1070 m was significantly greater than 1520 m plants (Table 3.3). Given the low flowering that occurred the year fertiliser was applied, biologically, the potential for flowering the following year should not have been compromised for any of these plants. Therefore, interpretation of flowering results one year after the fertiliser addition experiment would not have been confounded by the flowering response in 2016/17.

The season following the addition of fertiliser, 2017/18, was an extremely low flowering year (Table 3.7b). Only five inflorescences were produced, one on one plant and four on another, out of the total 411 plants monitored for this study. Both flowering plants were from the 1070 m fertiliser plot. Due to such low flowering numbers and a dataset containing mostly zeroes statistical tests would not run successfully. However, it is clear that the addition of fertiliser did not notably increase the flowering response in these *Chionochloa* plants in a naturally low-flowering year one year after the addition of fertiliser.

Table 3.7. Mean number of inflorescences produced per plant in the two plots at each site (1070 and 1520 m) both before and after the fertiliser experiment (*n* = 411). Note: the before 'fertiliser' plots had not had any fertiliser applied yet.

(a) Before fertiliser addition (2016/17).

	Control	**Fertiliser**
1070m	0.880	1.013
1520m	0.347	0.089

(b) One year after fertiliser addition (2017/18).

	Control	**Fertiliser**
1070m	0	0.065
1520m	0	0

Table 3.8. The effects of treatment, site, and size (log pre-fertiliser liveBA) on pre-fertiliser flowering in *Chionochloa* at 1070 m and 1520 m on Mt Hutt before the application of fertiliser. Flowering was measured as the number of inflorescences per plant (*n* = 411). Starting model included treatment x site x size but was simplified to size + treatment x site through deletion of ns interactions. Significant effects indicated in bold.

(a) Analysis of deviance table

Predictor	**Df**	**Deviance**	**Resid. Df**	**Resid. Dev**	***F* value**	***P* value**
NULL			405	733.66		
treatment	1	6.246	404	727.41	3.244	0.072
log(pre.liveBA)	1	170.301	403	557.11	88.437	**<0.001**
site	1	28.151	402	528.96	14.619	**<0.001**

(b) Coefficients from the model

Predictor	**Estimate**	**Std. Error**	***t* value**	***P* value**
(Intercept)	-1.881	0.357	-5.271	<0.001
treatment fertiliser	-0.164	0.192	-0.852	0.395
log(pre.liveBA)	0.667	0.106	6.307	**<0.001**
site 1520m	-0.901	0.250	-3.608	**<0.001**

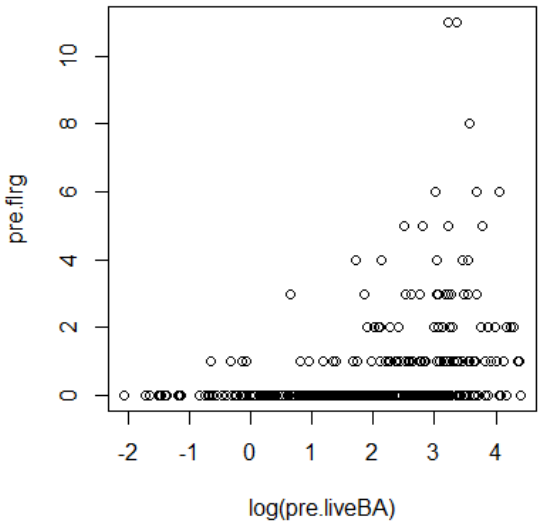


Figure 3.4. Relationship between the size of *Chionochloa* plants (log liveBA) and the number of inflorescences per plant in the 2016/17 flowering season, prior to the addition of fertiliser, for the control and fertiliser plots at 1070 m and 1520 m combined. This shows that larger plants produced more inflorescences, as expected.

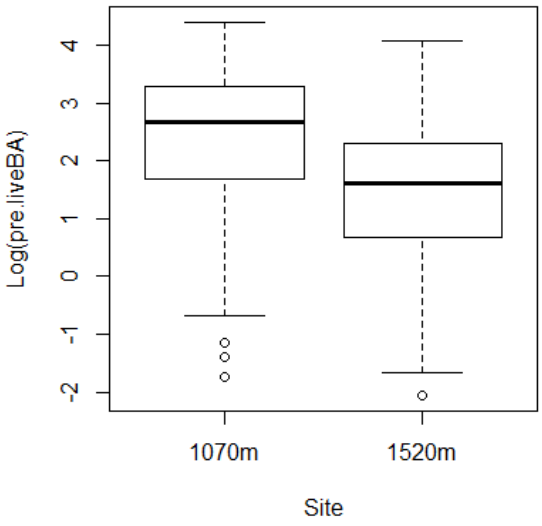


Figure 3.5. Difference in mean log liveBA of *Chionochloa* plants from the 1070 m and 1520 m sites in the 2016/17 flowering season, before the addition of any fertiliser. The average size of the plants at 1070 m was larger than at 1520 m though not significantly so. Solid black line shows the median, black box the interquartile range (from 25-75% of the data), black dotted error bars the total range minus the outliers, and black circles the outliers (individual plants whose averages fell more than 1.5 times above or below the interquartile range).

For the plants at UC, flowering was seen in the 20 *C. pallens* plants that had been transplanted to UC in the summer 2014/15 over three consecutive flowering seasons: 2016/17 prior to the addition of fertiliser, 2017/18 one year after the addition of fertiliser, and 2018/19 two years after the addition of fertiliser. (Earlier flowering at low altitudes allowed the 2018/19 flowering to be measured there before the completion of this thesis, whereas counts at Mt Hutt could not be measured that early.) The flowering was low in the first two years (mean inflorescences per plant ± sem 2016/17 = 0.85 ± 0.37, 2017/18 = 1.35 ± 0.58), but was high in the third (mean inflorescences per plant ± sem 2018/19 = 11.45 ± 2.92). As expected, prior to the addition of any fertiliser no significant difference was found between the two groups of plants in their flowering response (Table 3.9), measured as the proportion of tillers that flowered. Less expected was that, one year after the addition of fertiliser to half of the plants, there was still no significant difference between the control and fertiliser plants in their flowering response (Table 3.9), showing that fertiliser did not increase the flowering rate of plants one year after its application. However, there was a significant year (2019) x treatment

(fertiliser) interaction (Table 3.9), showing that two years after the fertiliser application there was a significant, albeit delayed, effect of fertiliser on the flowering response of plants at UC. Furthermore, flowering in the 2018/19 season was significantly greater for plants under both treatments compared to the two previous years (Table 3.9, Fig. 3.6), as was expected due to a higher DT (see Chapter 2).

Table 3.9. The effects of treatment x year on flowering in *C. pallens* plants at UC. Flowering was measured as the proportion of tillers flowering per plant, year was 2016/17 (2017, prior to fertiliser application), 2017/18 (2018, one year after fertiliser application), and 2018/19 (2019, two years after fertiliser application), *n* = 20. Significant effects in bold.

(a) Coefficients from the model

Random effects	**Variance**	**Std. Dev.**
Plant id	2.971	1.724

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-5.418	0.676	-8.020	< 0.001
as.factor (year) 2018	-0.079	0.379	-0.209	0.834
as.factor (year) 2019	1.889	0.306	6.176	**< 0.001**
treatment fertiliser	-0.930	1.044	-0.891	0.373
as.factor (year) 2018 x treatment fertiliser	1.059	0.752	1.409	0.159
as.factor (year) 2019 x treatment fertiliser	2.287	0.666	3.433	**< 0.001**

(b) Analysis of variance table

Response: cbind (flrg_tillers,(total_tillers-flrg_tillers))				
	Df	**Sum Sq**	**Mean Sq**	***F* value**
as.factor (year)	2	198.16	99.08	99.08
treatment	1	1.63	1.63	1.63
as.factor (year) x treatment	2	17.91	8.96	8.96

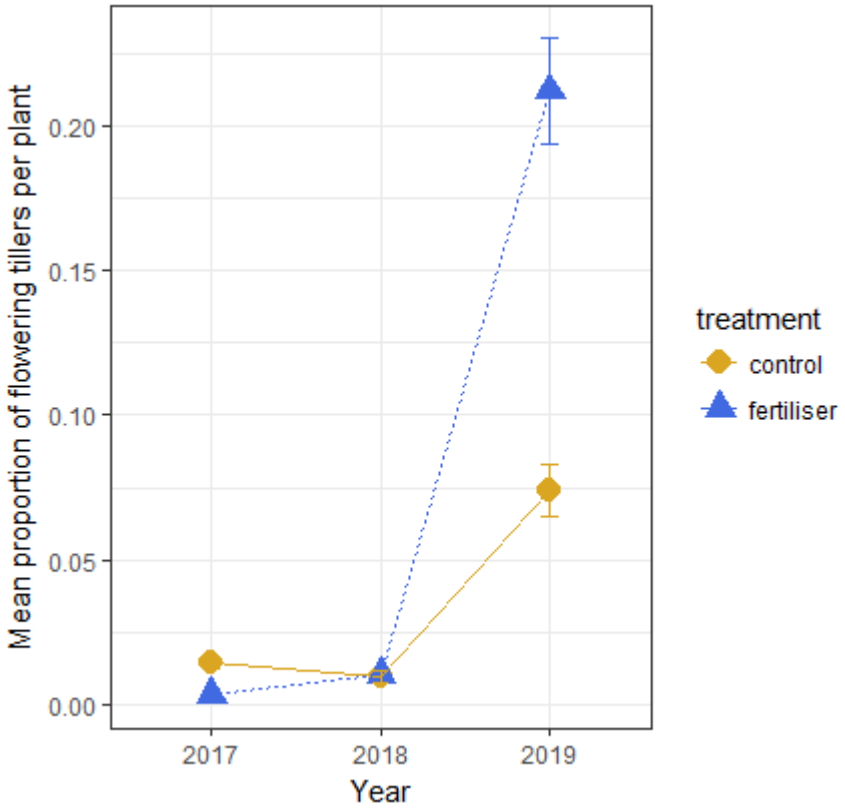


Figure 3.6. The effect of treatment over three consecutive years on the flowering response of *C. pallens* plants at UC (*n* = 20). Fertiliser was applied during the 2016/17 (2017) year and responses monitored one year later, in 2017/18 (2018), and two years later, in 2018/19 (2019). Flowering was measured as the proportion of tillers per plant that flowered. Error bars show SEM.

3.4 Discussion

The year fertiliser was applied on Mt Hutt (2016/17) was a low flowering year which would not have depleted plant resource reserves to the extent that this would have impacted on the flowering response the following year. One year after the application of fertiliser flowering was extremely low on Mt Hutt, making it clear that fertiliser had no effect on the flowering response of these *Chionochloa* plants under a low DT weather cue. At UC, where a small amount of flowering occurred in *C. pallens* both in the year fertiliser was applied and one year after, there was no significant difference between the flowering in control or fertiliser plants in either year. When Miyazaki *et al.* (2014) added nitrogen to a masting tree species, *Fagus crenata*, they found that fertilised trees expressed significantly higher quantities of flowering genes than controls, which resulted in all fertilised trees flowering the following year while no controls did. This showed that nitrogen application had an effect without any apparent temperature cue for floral induction in their study. Callahan *et al.* (2008) also found that in a non-masting year oak trees (*Quercus rubra* and *Q. velutina*) in N-treated plots produced a greater abundance of flowers compared to trees within control plots.

Furthermore, Bogdziewicz *et al.* (2017a) applied NH_4NO_3 at two rates to red oaks (*Quercus rubra*) and found that at the highest rate (150 kg/ha/year) flowering occurred in all three years compared to controls. Our rate of NH_4NO_3 application was more than twice that of Bogdziewicz *et al.* (2017a) (200 kg/ha twice in one year) yet we did not find a similar fertiliser effect on flowering in *Chionochloa* at UC or Mt Hutt. As others found an increase in flowering one year after fertiliser application, even without a temperature cue, we were expecting to see the same effect in *Chionochloa*. Connor (1966) mentioned that *Chionochloa* near sea level tend to receive enough of a floral inducting temperature every year to initiate some flowering, therefore we would have especially expected to see flowering at UC. However, the DT model predicts that plants should follow temperature cues from their new permanent site after one year, and that for the UC fertiliser experiment plants for the 2017/18 flowering season was -0.88°C. As no increase in flowering was found in *Chionochloa* at UC or Mt Hutt due to fertiliser application after one year, this may be more evidence of the importance of DT in the flowering response of *Chionochloa*.

Two years after fertiliser was applied, however, a delayed significant increase in flowering was found in fertilised *C. pallens* plants compared to controls at UC. Additionally, all *C. pallens* plants at UC flowered significantly more than in the previous two years, which was predicted from the very warm summer the previous year. If this effect is also seen in plants at Mt Hutt we may see a significant fertiliser effect in the field this coming summer (2018/19) given the predicted high masting year.

It was already known that at Mt Hutt the flowering response of *Chionochloa pallens* and *C. macra* were highly synchronised (Kelly *et al.*, 2000), and now it has been shown that the vegetative growth rates of these two species are also similar. The synchronisation of growth rates occurred under both the control and fertiliser treatments with both species showing the same significant increase in growth rate due to a fertiliser effect. This result confirms that combining the two species for analyses was appropriate and allowed for a greater number of plants to test for effects.

The close relationship between liveBA (diameter plus visually estimated percentage of live plant within the diameter) and the total tiller counts of *C. pallens* plants shows that the faster method of recording size by measuring the live BA is a reliable data collection method to use for unmanipulated plants. In the fertilised *C. pallens* plants, a significant increase was found in the average number of tillers per plant for the same liveBA, a factor which is likely the result of the significant increase in daughter tiller production in fertilised plants compared to controls. This increase in tillers for the same liveBA in fertiliser plants also demonstrates a limitation of the liveBA measurement for this kind of analysis, as the true fertiliser effect on vegetative growth was underestimated when just looking at the liveBA. Therefore, in resource-manipulation experiments

on *Chionochloa* it would be best to measure plant size in tiller counts if possible, as tiller counts would give a more accurate depiction of vegetative growth effects than liveBA.

Fertiliser was found to have no effect on the overall survival of individual tillers one year later, with the majority surviving under both treatments, although significantly more so at 1520 m. However, the effect of fertiliser on the daughter tiller production was interesting in that not only was there a significant increase due to fertiliser at all sites (UC, 1070, and 1520 m) but that this increase itself significantly increased with altitude across the three sites (see Fig. 3.3). In the unfertilised tillers, there was also a significant increase in daughter tiller production with altitude, a finding which is inconsistent with Mark (1965b), who found no significant variation in the rate of new tiller production in *C. rigida* over a range of different altitudes from 460 to 1630 m. Furthermore, in the Mark (1965a) study, no significant effect on tiller production occurred on plants that underwent transplantation to new sites compared to plants at the original sites, so the lesser daughter production seen at UC is unlikely to be a side-effect of previous transplantation.

A significant increase in vegetative growth with altitude was also seen when looking at the growth rate (live BA ratio) of whole plants within plots at 1070 and 1520 m. In the control plots at both sites there was a natural tendency for plants at 1070 m to grow at a significantly higher rate than plants at 1520 m. However, one year after plants had fertiliser added, the significant increase in growth rate at 1520 m compared to 1070 m was so great that there was no longer a difference in growth rate between these two sites (see Fig. 3.1). This was an impressive response given that, due to greater snow cover, the 1520 m plants have a shorter growing season of approx. 5 months compared to approx. 7-8 months for 1070 m plants (estimated from Greer (1979) and Mark (1965b)). Mark (1965a) additionally found that a difference in growing season length may be genetic as *C. macra* from above 1500 m retained their shorter growing period even when moved to near sea level. Mark (1965b) found that all *C. rigida* (including the later differentiated *C. macra*) plants, no matter the altitude, reached their maximum growing rate in mid-summer. Yet, interestingly, he found that the high-altitude plants on Coronet Peak had a higher growth rate at this maximum which resulted in a similar total growth to lower altitude plants for the season despite their shorter growing season (Mark, 1965b). These findings suggest that higher altitude *Chionochloa* are more resource-limited than lower altitude counterparts, but that higher altitude *Chionochloa* may be able to respond to a greater degree when resources are abundant.

Resources have been shown to be a limiting factor in flowering in natural populations of masting plants, where the ability to accumulate resources again after flowering determines the frequency of flowering (Satake and Bjørnstad, 2008; Han *et al.*, 2014). *Fagus crenata* and *Q. rubra,* two masting tree species, have been shown to significantly increase flowering in the years

succeeding fertiliser application, whether that following year was a mast year or not (Miyazaki *et al.*, 2014; Bogdziewicz *et al.*, 2017a). Furthermore, Hay *et al.* (2008) noted that in C. Burrows unpublished thesis Burrows mentioned observations that the few *Chionochloa* in the field that did flower in non-mast years were mostly found in spots with increased resources, such as around deer carcasses. Therefore, if resources are a dominant factor in the frequency of flowering in masting species we also should have seen an increase in flowering in our study species one year after fertiliser application, but this was not the case. In the summer fertiliser was applied (2016/17) there was only a very low florally inductive temperature cue, with the mean January-February temperatures being lower at all sites than the previous year so DT was negative (Table 3.10). This lack of a positive DT temperature cue is likely responsible for the resultant lack of flowering one year after fertiliser application. Monks and Kelly (2006) state that strict masting species will show switching, where a negative relationship is found between resources used for growth and resources used for reproduction within individual plants. Since essentially no flowering occurred on Mt Hutt one year after fertiliser application it can be expected that the plants which received greater quantities of resources over the year-long period will have been able to designate these resources to greater vegetative growth, as was seen. Therefore, that increased resources mainly produced increased vegetative growth one year later suggests that, for the species studied here, temperature plays a more dominant role in determining flowering frequency than do resources.

Table 3.10. Mean absolute (and respective DT) temperatures for the floral induction period of January-February, inclusive, for *Chionochloa* and *Celmisia*, where flowering will begin at the end of the same year depending on whether the temperature was warm enough to induce flowering. Fertiliser was added over summer 2016/17, with treatment effects measured during summer 2017/18 and the end of 2018.

	Mean Jan-Feb temperature (and DT) in °C		
Site	**2016**	**2017**	**2018**
1520 m	10.76 (1.03)	9.70 (-1.06)	11.69 (1.99)
1070 m	13.02 (0.94)	11.62 (-1.40)	13.31 (1.69)
UC	18.24 (0.36)	17.36 (-0.88)	18.97 (1.61)

A long-term study where nitrogen was added for more than 15 years showed that nitrogen-treated masting oak trees (*Quercus rubra* and *Q. velutina*) significantly increased in size compared to controls (Callahan *et al.*, 2008). This added vegetative growth led to increased reproductive activity in the form of increased acorn production and sizes of some individual acorns (Callahan *et al.*, 2008). Therefore, if 2018/19 is a heavy masting season on Mt Hutt, as predicted (and as was seen in UC transplants), the positive effect fertiliser had on new tiller production in *Chionochloa* at Mt Hutt may lead to significantly greater flowering, especially since fertiliser addition increased the tendency of tillers to flower in UC *C. pallens* (Fig. 3.3). The 2-year lag in increased flowering after fertiliser

application shows that resources do not play a dominant role in controlling flowering in *C. macra* or *C. pallens*, and that the control of flowering frequency for these species is dominated by another factor. The results found here, and those from Chapter 2, suggest that temperature plays a more dominant role on flowering frequency in *Chionochloa* than do resources, and that the difference in temperature from the DT model is better at explaining flowering events in these plants than either the T1 model or resources. Finally, these findings suggest that not all masting species respond similarly to increased resources, such as nitrogen.

Chapter 4. Fertiliser effects on *Celmisia*

4.1. Introduction

As explained in the Chapter 3 objectives for the effects of fertiliser on *Chionochloa*, resources play a major role in the flowering of plants. The time it takes masting plants, in particular, to accumulate enough resources to flower again after a heavy masting year may be a controlling factor that determines the gap between large flowering events seen in these species. This chapter followed the same objectives as those for *Chionochloa* in Chapter 3 except that the study species was a herbaceous dicot, the New Zealand endemic alpine daisy, *Celmisia lyallii*. Two sites were used on Mt Hutt and one at University of Canterbury (UC). The responses tested between fertilised and control plants included vegetative and flowering responses, and these were used to answer the following key questions:

1) Does the addition of nutrients, via fertiliser, increase vegetative growth by increasing the number of daughter rosettes produced,
    a. one year after fertiliser application?
    b. Two years after fertiliser application?
2) Does the addition of nutrients increase the number of *C. lyallii* rosettes within patches at Mt Hutt?
3) Does the addition of nutrients increase the flowering response of *C. lyallii*,
    a. one year after fertiliser application?
    b. two years after fertiliser application?

4.2. Methods

Celmisia lyallii were monitored in fertiliser and control plots at both 1350 m and 1520 m on Mt Hutt, and in potted plants at UC. The UC plants were transplanted from 1350 m on Mt Hutt in 2015, and initially placed into fertilised potting mix. Half of these pots were given additional fertiliser for this experiment. Table 4.1. lists details of the *C. lyallii* plants under each treatment at all sites and the data collected for each. The 1520 m control and fertiliser plots were both 20 x 2 m, whereas the plot at 1350 m was a single 20 x 2 m plot running lengthways down a slope where the upper ~half (11 x 2 m) was the unfertilised control plot and the bottom ~half (9 x 2 m) the fertiliser plot. Control plants for the RNA-sampled *C. lyallii* at 1520 m ran along the same control line as the RNA-sampled *C. pallens*, running parallel to, and 5 m from, each of the fertiliser and control plots at this site. As per the Chapter 3 methods section, fertiliser was applied at a rate of 20 g of $CaNH_4NO_3$ and 50 g of

superphosphate per m^2 in December 2016 and again in March 2107 (see Table 3.1). Data were first collected during the 2016/17 summer season, before any effects of the applied fertiliser would have taken effect, then again one year after fertiliser application in 2017/18 summer, with some data also collected during November and December 2018. *Celmisia* patches were counts of the number of rosettes within contiguous areas (patches) within each plot. Flowering was counts of the number of inflorescences among all rosettes within each patch in the plots on Mt Hutt, or on each rosette in pots at UC and on the 1520 m control line.

Prior to the addition of fertiliser, individual rosettes within plots on Mt Hutt and at UC were tagged for leaf sampling for an RNA-based study looking into the effect of fertiliser application on gene dynamics. Vegetative growth in *Celmisia* was assessed by counting the number of daughter rosettes produced from within each previously tagged parent rosette at the UC, 1350 m and 1520 m sites. As daughter rosettes grow from within the parent before extending sideways they can be identified by closely inspecting each parent rosette. Furthermore, as there had previously been several years with very little flowering, and therefore little seed production, newly established plants via seed were likely uncommon during the period of study. Establishment from seed in this alpine environment is probably very rare for *Celmisia*, as it is for *Chionochloa* (Rees *et al.*, 2002).

It was not feasible to count the total number of daughter rosettes produced within the whole plots as there were over 1400 rosettes in total among the two plots at each of 1350 m and 1520 m. The patch counts that were made in these plots counted only larger, adult rosettes as these are easy to identify in a sweeping count of each patch within each plot. Therefore no daughter rosette counts were included in this analysis. The number of rosettes within each patch can fluctuate from season to season with some rosettes dying and some smaller rosettes growing large enough to recruit to the adult population. The variation in rosette counts within each patch over time show the net change in numbers of adult rosettes within each plot during the one year period after fertiliser was applied to some, and whether these patches increased or decreased in total rosette number. The net increase in rosettes within patches throughout the plots was analysed by creating a ratio using the equation:

Celmisia rosette increase ratio = rosettes per patch post-fertiliser/rosettes per patch pre-fertiliser

The log of this rosette increase ratio was then used as a continuous variable to assess the survival rate of rosettes within plots between the summers of 2016/17 and 2017/18, and for a treatment effect, i.e. did the addition of fertiliser increase the rosette increase rate within patches, one year after fertiliser application, compared to the control patches in plots at 1350 m and 1520 m. The assumptions of linearity and equal variances were not met for the rosette increase ratio per

patch, so the rosette increase ratio was log-transformed using natural log (ln) for all analyses. The rosette increase rate of adult *C. lyallii* rosettes within patches in each plot on Mt Hutt were analysed using a multi-factor ANOVA testing for an interaction effect of treatment (control or fertiliser) x site (1350 m or 1520 m) on the rosette increase ratio between the 2016/17 and 2017/18 summers. All the original rosettes in the pots at UC were still alive over this same period, with no additional adult rosettes, so no statistical test was run on the rosette increase rate of adult rosettes at UC.

For the vegetative growth analyses of *C. lyallii*, counts of daughter rosettes produced by tagged rosettes at UC and 1520 m were made one year after the application of fertiliser, then at UC, 1350 m, and 1520 m two years after. Counts of daughter rosettes after the first year were not able to be collected from the 1350 m site before the snow came. Generalised linear models were run using poisson error distributions to test for an effect of treatment, site, and treatment x site on the number of daughter rosettes produced. The first test used daughter rosettes counts at UC and 1520 m one year after fertiliser application as the response variable. Because no 1520 m control rosettes produced any daughter rosettes one year after fertiliser addition the analysis gave a "complete separation" error. I found that I needed to change one of these zeroes to a 1 to get the analysis to run properly in R, so the results from that analysis underestimate the true difference. The second test used the rate of daughter tiller production between one year after and two years after the application of fertiliser (i.e. daughter rosettes after two years – daughter rosettes after one year) at UC and 1520 m as the response variable. I ran this test as I was interested in knowing whether any effects of fertiliser on daughter rosette production one year after fertiliser application would continue into a second consecutive growing season. The third poisson GLM used the total number of daughter rosettes produced across the whole two-year period since fertiliser application at all three sites (UC, 1350 m, and 1520 m) as the response variable.

My initial analysis of the daughter rosettes one year after fertiliser application led me to think I had made an error with my sample selection at 1520 m, as no control rosettes had any daughters whereas a lot of fertiliser rosettes did. The 1520 m control line rosettes used for this analysis tended to grow in smaller patches, or singly, while the fertiliser plot rosettes were in patches of varying size. We went back two years after fertiliser addition (December 2018) to take another count of each, and to add 10 random rosettes from within the control plot where they also grow in patches of varying size, as well as counts from the 1350 m plot. It was clear from this second collection of daughter rosette counts that my initial data collection was not affected by measuring single rosettes rather than those in clumps. Rosettes within the 1520 m control plot looked identical to the control line rosettes and only a few control rosettes from either had any daughter rosette production after two years, and those were so small as to obviously be recent growth. Furthermore,

as only one newly tagged 1520 m control plot rosette had daughter rosettes, and these were only very small and clearly a recent addition, I included these 10 rosettes as all having no daughter rosettes the first year after fertiliser was applied in my dataset for 1520 m controls. We also noticed that considerable further vegetative growth had occurred in some plants and decided to add this second year of growth to the data analyses and recounted all tagged rosettes at all three sites.

The flowering data for *C. lyallii* at UC were counts of inflorescences per rosette so these data were analysed using a poisson error distribution. However, to account for the fact that some pots contained more than one rosette, I ran these as a GLMM with a random term for the pot number each rosette was in. Using the lme4 package in R, the code for the analysis was : <-glmer ((flrg ~ as.factor (year) * treatment + (1|pot), family=poisson). The year factor included the flowering the year fertiliser was applied and the two years following (from 2016/17 to 2018/19), while treatment was control or fertiliser. This test allowed me to identify whether any difference in flowering response was present between the two treatment groups before fertiliser addition, as well as whether any difference occurred between them due to fertiliser in the following two years. Additionally, the test catered for any variation in flowering across the years as well as all combinations of year x treatment interactions.

No flowering occurred in any *C. lyallii* on Mt Hutt during the 2017/18 flowering season, so no analysis could be run on Mt Hutt flowering responses after fertiliser application.

Table 4.1. Data recorded for *Celmisia lyallii* showing the number of plant patches, and individually monitored plants, at each site under each treatment and the data gathered both before the commencement of the fertiliser experiment (2016/17), one year after the fertiliser experiment (2017/18), and two years after (December 2018). *Rosettes* signifies the counting of rosettes per patch; *Daughters* signifies the counting of daughter rosettes produced within individual parent rosettes; *Infl.* signifies the counting of inflorescences per patch or per plant where individual rosettes were monitored. Note: RNA-sampled plants and daughter-counted plants were individually tagged plants within plot patches.

Species and site	Group label	Treatment	No. patches	2016/17 summer		2017/18 summer			December 2018	
				Rosettes	Infl.	Rosettes	Daughters	Infl.	Infl.	Daughters
Celmisia lyallii										
1520 m	Fertiliser plot	Fertilised	34	✓	✓	✓		✓		
	-includes RNA-sampled plants	Fertilised	10 plants	✓	✓	✓	✓	✓		✓
	Control plot	Unfertilised	27	✓	✓	✓		✓		
	-includes daughter-counted plants	Unfertilised	10 plants	✓	✓	✓	✓	✓		✓
	Control line	Unfertilised	10 plants	✓	✓	✓	✓	✓		✓
1350 m	Fertiliser plot	Fertilised	12	✓	✓	✓		✓		
	-includes RNA-sampled plants	Fertilised	20 plants	✓	✓	✓	✓	✓		✓
	Control plot	Unfertilised	14	✓	✓	✓		✓		
	-includes RNA-sampled plants	Unfertilised	20 plants	✓	✓	✓	✓	✓		✓
UC	Fertiliser plants	Fertilised	28 plants		✓	✓	✓	✓	✓	✓
	Control plants	Unfertilised	28 plants		✓	✓	✓	✓	✓	✓

4.3 Results

Effect of fertiliser on vegetative growth and rosette survival in Celmisia lyallii

Using the rosette increase ratio of post-fertiliser patch rosette count/pre-fertiliser patch rosette count, I tested the net increase in adult rosettes within plot patches on Mt Hutt and whether the addition of fertiliser had any effect on this increase. There was a significant interaction of treatment x site on the rosette increase rate of *C. lyallii* within the plots (Table 4.2). This interaction was due to a greater effect of fertiliser on the rosette increase of adult rosettes within patches at the highest site, 1520 m (Table 4.2b, Fig. 4.1). At 1350 m both control and fertiliser patches showed a similar small increase in net rosette counts, whereas at 1520 m control patches had essentially no change in counts whereas fertiliser patches had a large increase. It is not known why fertiliser boosted net rosette counts at 1520 m but not at 1350 m.

Table 4.2. The effects of treatment (control or fertiliser) and site (1350 m or 1520 m) on the log rosette increase rate (post-fertiliser patch count/pre-fertiliser patch count) of adult *C. lyallii* rosettes (*n* = 84 patches) at Mt Hutt in the 2017/18 summer season, one year after the addition of fertiliser. Significant effects indicated in bold.

(a) Analysis of variance table

Response: log (survival ratio)					
	Df	**Sum Sq**	**Mean Sq**	***F* value**	***P* value**
treatment	1	1.26	1.26	10.65	**0.002**
site	1	0.00	0.00	0.04	0.841
Treatment x site	1	1.06	1.06	8.97	**0.004**
Residuals	80	9.47	0.12		

(b) Coefficients from the model

Predictor	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	0.189	0.092	2.058	0.043
treatment fertiliser	-0.093	0.135	-0.686	0.495
site 1520 m	-0.220	0.113	-1.939	0.056
treatment fertiliser x site 1520 m	0.488	0.063	2.995	**0.004**

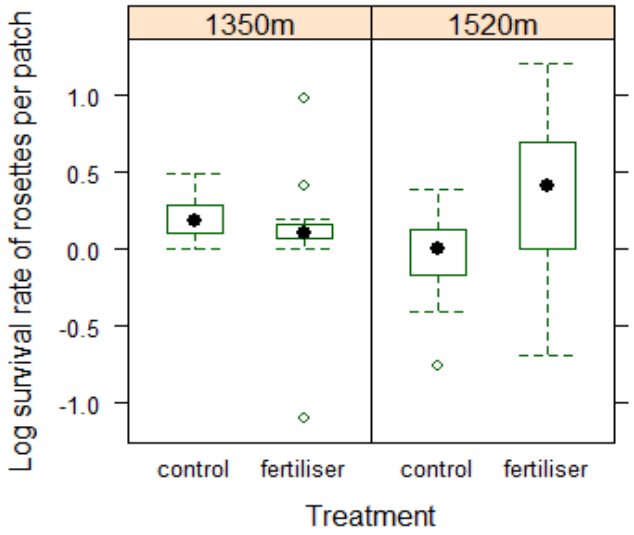


Figure 4.1. Varying effects of treatment between the 1350 m and 1520 m sites on Mt Hutt on the log (ln) survival rate (post-fertiliser patch count/pre-fertiliser patch count) of *C. lyallii* rosettes during the 2017/18 summer season, one year after the application of fertiliser. Black dots show the median, solid green boxes the interquartile range (from 25-75% of the data), dotted green error bars the total range minus the outliers, and green circles the outliers (points more than 1.5 times above or below the interquartile range).

The production of daughter rosettes in *C. lyallii* at UC and 1520 m, one year after the application of fertiliser, was found to significantly depend on a treatment x site interaction (Table 4.3) even though the statistics underestimate the true differences due to having to change a '0' to a '1' to avoid complete separation errors (see methods). Fertiliser significantly increased the number of daughter rosettes produced at both sites, however the rate of increase was significantly higher at the higher 1520 m site (Fig. 4.2), reminiscent of the effect fertiliser had at the same site in daughter tiller production in *Chionochloa* one year after fertiliser was applied (Chapter 3).

Next, I analysed the difference in daughter rosette production between one year after and two years after the application of fertiliser at UC and 1520 m, i.e. the vegetative growth rate of individual tagged parent rosettes between the one to two year period after fertiliser was added. The interaction between treatment x site was almost significant, while there was a significant individual effect of site (Table 4.4). The effect of site appears to be due to the 1520 m fertiliser plants still showing significantly more daughter rosette production (Table 4.4b), and is likely the reason for the almost significant interaction of treatment x site (Fig. 4.3).The rate of daughter rosette production increased slightly in the control plants but slowed down in the fertiliser plants over the second-year period (Fig. 4.3 cf. Fig 4.2, note the different y-axis scales).

Table 4.3. The effect of treatment and site on the production of daughter rosettes in *C. lyallii* at UC and 1520 m on Mt Hutt one year after the application of fertiliser. Significant effects indicated in bold.

(a) Analysis of deviance table. Evaluated using type III sums of squares when entering each term into the model last.

Response: year one daughter rosettes					
	Df	**Deviance**	**Resid. Df**	**Resid. Dev.**	***P* value**
NULL			84	201.66	
treatment	1	55.84	83	132.40	**< 0.001**
site	1	24.84	82	132.40	**< 0.001**
treatment x site	1	15.12	81	117.28	**< 0.001**

(b) Coefficients from the model

Predictor	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-2.996	1.000	-2.996	0.003
treatment fertiliser	4.264	1.016	4.199	**< 0.001**
site UC	1.609	1.069	1.505	0.132
treatment fertiliser x site UC	-3.377	1.110	-3.041	**0.002**

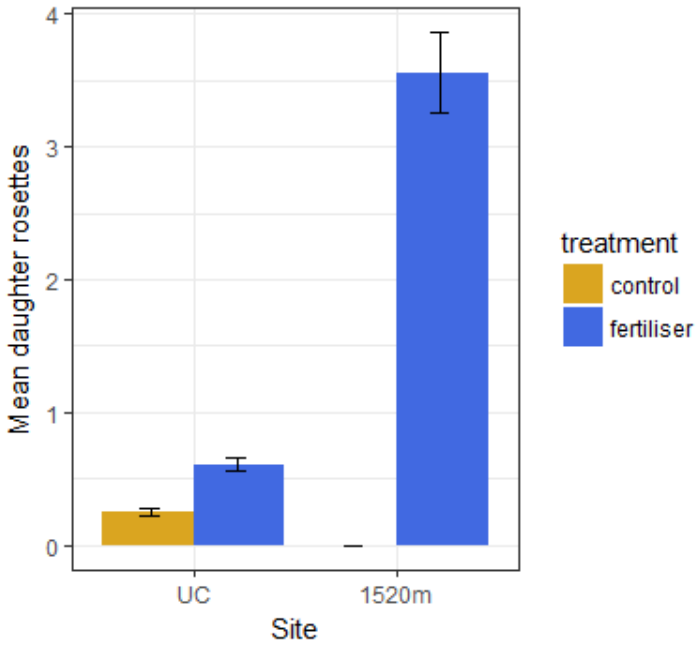


Figure 4.2. Raw mean ± SE daughter rosettes produced per parent rosette one year after the application of fertiliser for control and fertiliser *C. lyallii* plants at UC and 1520 m on Mt Hutt (*n* = 28 per treatment at UC, *n* = 20 for 1520 m control, and *n* = 9 for 1520 m fertiliser).

Table 4.4. The effects of treatment and site on the production of daughter rosettes in *C. lyallii* at UC and 1520 m on Mt Hutt between the one to two year period after the application of fertiliser. Significant effects indicated in bold.

(a) Analysis of deviance table. Evaluated using type III sums of squares when entering each term into the model last.

Response: year two daughter rosettes + 1					
	Df	**Deviance**	**Resid. Df**	**Resid. Dev.**	***P* value**
NULL			83	112.19	
treatment	1	1.94	82	107.23	0.164
site	1	3.92	81	107.23	**0.048**
treatment x site	1	3.50	80	103.73	0.061

(b) Coefficients from the model

Predictor	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-1.204	0.408	-2.949	0.003
treatment fertiliser	1.204	0.527	2.284	**0.022**
site UC	-0.012	0.540	-0.023	0.982
treatment fertiliser x site UC	-1.374	0.739	-1.860	0.063

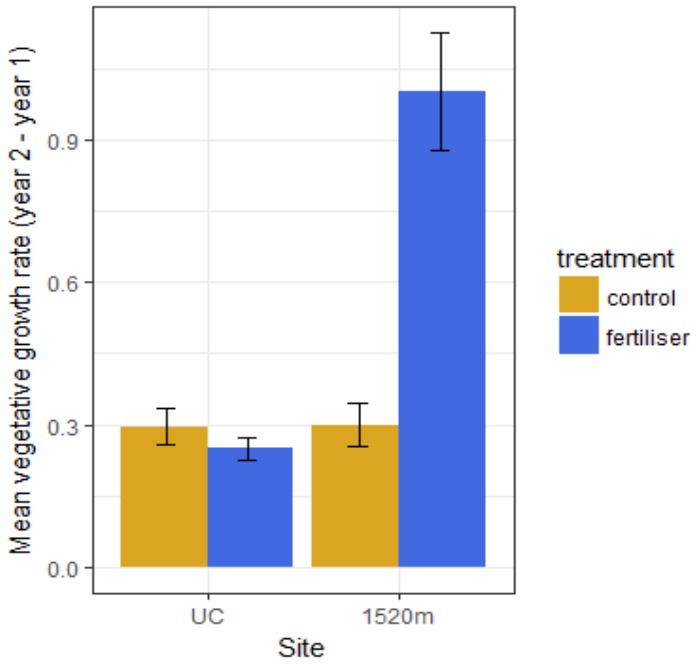


Figure 4.3. Raw mean ± SE vegetative growth rate of daughter rosettes per parent rosette between the period of one and two years after the application of fertiliser for control and fertiliser *C. lyallii* plants at UC and 1520 m on Mt Hutt (*n* = 28 per treatment at UC, *n* = 20 for 1520 m control, and *n* = 9 for 1520 m fertiliser).

As counts of daughter rosettes at 1350 m were not made one year after fertiliser application, but only after two years, I separately analysed the total number of daughter rosettes produced at all three sites two years after fertiliser application. A significant interaction of treatment x site was

found on the total number of daughter rosettes produced in *C. lyallii* plants (Table 4.5). Interestingly, the plants at UC and 1350 m responded similarly in their two-year total daughter rosette production under both treatments, whereas the fertiliser plants at 1520 m produced significantly more rosettes compared to the other two sites (Fig. 4.4). Significant individual effects of treatment and site were also found, with fertiliser significantly increasing the total production of daughter rosettes after two years in plants at all sites to varying degrees (Table 4.5, Fig. 4.4).

Table 4.5. The effect of treatment and site on the total production of daughter rosettes in *C. lyallii* at UC, 1350 m and 1520 m on Mt Hutt two years since the application of fertiliser. Significant effects indicated in bold.

(a) Analysis of deviance table. Evaluated using type III sums of squares when entering each term into the model last.

Response: year two daughter rosettes					
	Df	**Deviance**	**Resid. Df**	**Resid. Dev.**	***P* value**
NULL			124	343.79	
treatment	1	49.23	123	270.61	**< 0.001**
site	2	35.45	121	270.61	**< 0.001**
treatment x site	2	26.92	119	243.70	**< 0.001**

(b) Coefficients from the model

Predictor	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-0.598	0.302	-1.983	0.047
treatment fertiliser	0.492	0.383	1.287	0.198
site 1520 m	-0.606	0.508	-1.194	0.232
site UC	0.010	0.397	0.025	0.980
treatment fertiliser x site 1520 m	2.280	0.578	3.946	**< 0.001**
treatment fertiliser x site UC	-0.059	0.505	-1.117	0.907

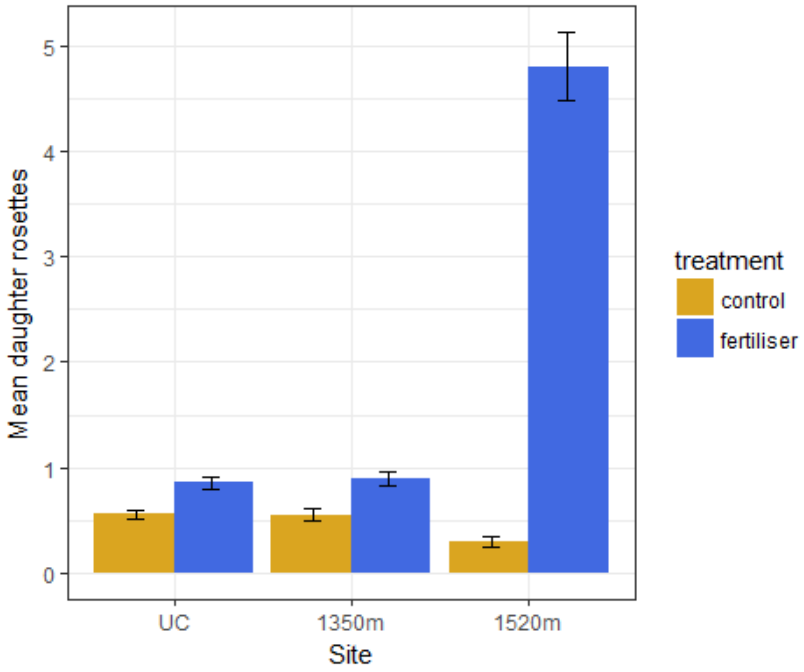


Figure 4.4. Raw mean ± SE daughter rosettes produced per parent rosette two years after the application of fertiliser for control and fertiliser *C. lyallii* plants at UC, 1350 m, and 1520 m on Mt Hutt (*n* = 28 per treatment at UC, *n* = 20 per treatment at 1350 m, *n* = 20 for 1520 m control, and *n* = 10 for 1520 m fertiliser).

Effect of fertiliser on flowering in Celmisia lyallii

Flowering in *C. lyallii* at UC was reasonably high the year before any fertiliser was applied as well as one year and two years after fertiliser application. No significant difference was found between the flowering responses of the two groups of *C. lyallii* plants at UC prior to the addition of any fertiliser in the 2016/17 summer (Table 4.6). Treatment was not found to have a significant effect on flowering between control and fertiliser plants either the year before or one year after fertiliser was added (Table 4.6). However, flowering significantly increased in both control and fertiliser plants during the 2017/18 summer compared to the 2016/17 summer (Table 4.6 and Fig. 4.5). Therefore, the expected fertiliser-induced increase in flowering one year after fertiliser application was not seen in *C. lyallii* at UC in this study.

A significant year x treatment interaction was found for the 2018/19 season on the number of inflorescences produced in *C. lyallii* at UC (Table 4.6). This interaction showed that fertiliser plants produced significantly more inflorescences than control plants two years after the application of fertiliser (Fig. 4.5).

Table 4.6. The effect of year and treatment on the number of inflorescences per rosette in *C. lyallii* plants at UC for three consecutive flowering seasons, starting from 2016/17 (2017) when fertiliser was applied. (*n* = 28 per treatment). Significant effects indicated in bold.

Random effects	**Variance**	**Std. Dev.**
pot	0.478	0.691

Fixed effects	**Estimate**	**Std. Error**	***z* value**	***P* value**
(Intercept)	-0.014	0.287	-0.050	0.960
as.factor (year) 2018	0.486	0.222	2.191	**0.029**
as.factor (year) 2019	0.061	0.243	0.250	0.803
treatment fertiliser	-0.142	0.419	-0.339	0.735
as.factor (year) 2018 x treatment fertiliser	0.303	0.325	0.931	0.352
as.factor (year) 2019 x treatment fertiliser	0.672	0.342	1.965	**0.049**

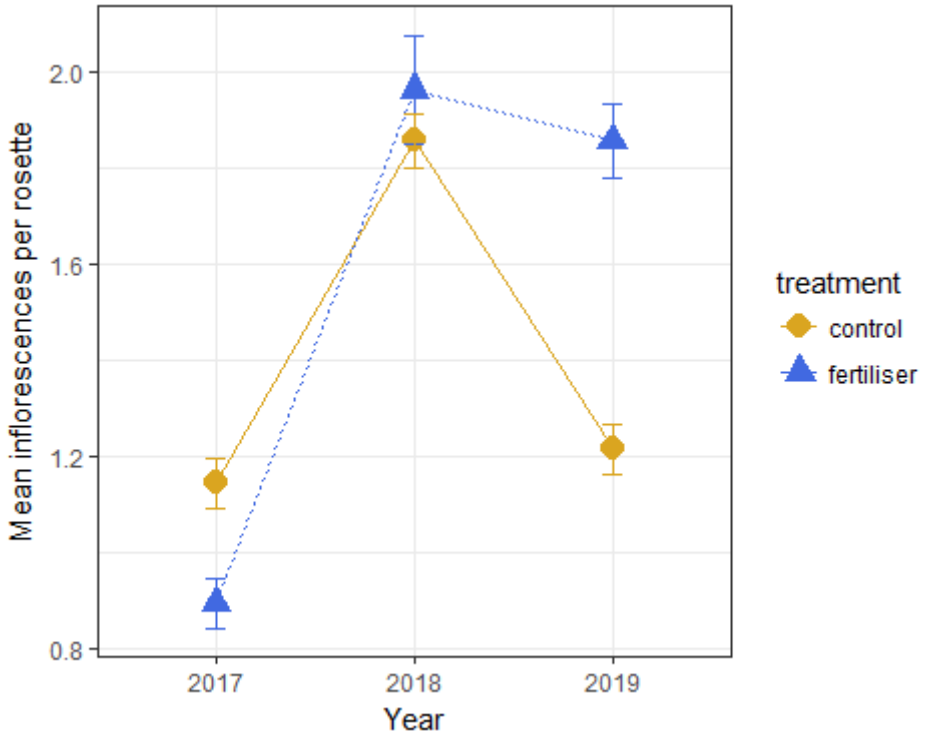


Figure 4.5. Raw mean ± SE number of inflorescences per *C. lyallii* rosette at UC for plants under control and fertiliser treatments before (2016/17 season (*2017*)), one year after (2017/18 season (*2018*)), and two years after (2018/19 season (*2019*)) fertiliser was applied (*n* = 56). A significant increase was found in the flower production for both treatments between 2017 and 2018, and a significant effect of fertiliser treatment was found in 2019.

At Mt Hutt pre-fertiliser flowering during the 2016/17 summer season within the *C. lyallii* rosette patches in all plots was extremely low (Table 4.7), and with so many zeroes in the dataset statistical analyses would not run properly. However, it can be assumed that plant resources were not depleted through flowering prior to the addition of fertiliser, so this factor would not have affected

the plants abilities to flower the following flowering season after the addition of fertiliser. There was no flowering at all from any *C. lyallii* within plots (or the surrounding area) during the 2017/18 summer season (Table 4.7b), therefore fertiliser certainly did not increase the flowering response in any of these plants one year after its addition.

Table 4.7. Mean number of *C. lyallii* inflorescences per rosette patch ± SEM within the plots at both 1350 m and 1520 m on Mt Hutt prior to the addition of any fertiliser (a) and one year after the addition of fertiliser (b).

(a) 2016/17 summer season

	Control	**Fertiliser**
1350 m	0.071 ± 0.071	0.833 ± 0.405
1520 m	0.000 ± 0.000	0.029 ± 0.029

(b) 2017/18 summer season

	Control	**Fertiliser**
1350 m	0.000 ± 0.000	0.000 ± 0.000
1520 m	0.000 ± 0.000	0.000 ± 0.000

4.4. Discussion

Flowering in *Celmisia lyallii* on Mt Hutt during the 2016/17 summer, when fertiliser was added, was extremely low, and one year later there was no flowering in any monitored plants at Mt Hutt or the surrounding areas. Therefore, fertiliser did not increase the flowering in this species at Mt Hutt one year after its application, which is different from other masting species, e.g. *Fagus crenata* (Miyazaki *et al.*, 2014). Low flowering is not unexpected in *C. lyallii* in situ as they consistently produce fewer flowers in low-masting years than other masting species at the same site, e.g. *Chionochloa pallens* at Mt Hutt (Kelly *et al.*, 2013; Pearse *et al.*, 2016), and both summer 2016/17 and 2017/18 were low-flowering years generally at this site.

The *C. lyallii* previously transplanted to UC showed some flowering in all three years of observation for this study, from before fertiliser addition to two years after. Abundant flowering is a reported factor for this species when cultivated in warmer environments where less abortion of summer-initiated floral primordia occurs (Mark, 1970). However, as with the Mt Hutt *C. lyallii*, no significant difference was found one year after fertiliser application (2017/18) between the flowering responses of plants in the control or fertiliser groups. For both treatment groups the 2017/18 flowering season was significantly heavier than the previous year, when the fertiliser was applied. These results show that the reported effect fertiliser has on flowering in other masting species one year after its application is not seen in *C. lyallii*.

Interestingly, two years after fertiliser addition, flowering at UC did significantly increase in the fertiliser plants compared to the controls. The level of flowering in the *C. lyallii* controls dropped

back near their level two years previous, while the fertiliser plants maintained a similar high level to the year before (Fig. 4.5). This significant fertiliser-affected flowering seen two years after treatment may be indicative of what could occur on Mt Hutt this coming summer (2018/19) as a high flowering year is predicted.

The rosette increase rate of adult rosettes within patches in plots at 1350 m and 1520 m on Mt Hutt showed a significant increase due to fertiliser addition at 1520m. The plants within the 1520 m fertiliser plot were observed to look far healthier than plants in the counterpart control plot, an effect that was much more visible at the highest site compared to any other site. The fertiliser appeared to make the leaves of the plants longer and thicker.

A similar vegetative-growth pattern was found when analysing the daughter rosette counts at UC and 1520 m one year after fertiliser addition. The daughter rosette production in the 1520 m fertiliser plants was significantly greater than elsewhere, although fertiliser did also significantly increase daughter rosette production at UC to a lesser degree (see Fig. 4.2). No daughter rosette production was seen in the control plants at 1520 m during summer 2017/18 suggesting plants at this site may be resource-limited.

Between the one to two year period after fertiliser application, the rate of additional daughter rosette production lessened in the fertiliser plants at UC and 1520 m compared to the first year following fertiliser. However, the 1520 m fertiliser plants were still producing significantly more daughter rosettes than any other group (see Fig. 4.3). Control plants at both sites had a slight increase in daughter rosette production, a response which may be related to the warmer than average growing season during 2017/18 resulting in greater mineralisation and natural availability of nitrogen and phosphorus for these plants (Jonasson *et al.*, 1999). Fertiliser addition, along with increased temperatures, has been reported to have a proportionally greater effect on vegetative growth in higher altitude plants that normally live in colder climes with shorter growing seasons (Jonasson *et al.*, 1999), an effect which may explain the increases in daughter production by both *Celmisia* and *Chionochloa* at the highest altitude in this study.

The total daughter rosette counts two years after fertiliser addition at UC, 1350 m, and 1520 m showed that fertiliser significantly affected daughter rosette production at all three sites, yet with the effect at 1520 m significantly greater. Interestingly, there was no difference in daughter rosette production between the control and fertiliser plants at UC and 1350 m (see Fig. 4.4). This is a different pattern to what was seen in the *C. pallens* daughter tiller production, where an obvious increase occurred along a rising altitude gradient. As the *C. lyallii* plants at UC had previously been transplanted from 1350 m, perhaps there is some genetic control responsible for their vegetative growth response based on internal resource allocation. In comparison, the 1520 m plants may have

slightly different genes that allow for a greater response to increased resources, as may be the case in *C. pallens* too. Several *C. lyallii* rosettes were transplanted to UC from 1520 m on Mt Hutt in January 2018, it would be interesting to see if these plants respond differently to the same fertiliser treatment.

In conclusion, *C. lyallii* have responded similarly to *Chionochloa* in respect to applied fertiliser, in that no significant increase in flowering occurred one year later, in a year which was low-flowering generally. This shows that flowering in these alpine masting species is not controlled by nitrogen addition in the same way as that reported for other masting species (Callahan *et al.*, 2008; Miyazaki *et al.*, 2014; Bogdziewicz *et al.*, 2017a). Both *C. lyallii* and *Chionochloa* had significant increases in vegetative growth with fertiliser, especially at the highest altitude site, 1520 m, which may indicate a higher level of resource-limitation for plants at high altitude. However, some differences between *C. lyallii* and *Chionochloa* were also evident. Firstly, *Celmisia lyallii* at UC flowered heavily under both treatments in summer 2017/18, while *C. pallens* had only a small amount of flowering in the same season. However, this may be because *C. lyallii* was initially put into a fertilised potting mix when transplanted in 2015, whereas *C. pallens* was not when transplanted in 2014. It is also possible that near sea level these two species respond differently to temperature and resource cues in relation to the amount of flowering that occurs in a given season. Secondly, *C. lyallii* did not show a gradual increase in vegetative growth with altitudinal gain as seen in *Chionochloa,* but instead was quite similar from 15 m to 1350 m then leapt in production at the high site of 1520 m. This finding suggests the environment may be playing more of a role in the change of growth with altitude in *Chionochloa,* while genetics may have more of an effect in *C. lyallii*. The findings for *C. lyallii* in this chapter will be related to the overall question looking at whether temperature or resources control flowering in masting species in Chapter 5.

Chapter 5. Discussion

5.1. Effect of fertiliser on relative growth and flowering

Fertiliser was added to *C. pallens, C. macra* and *C. lyallii* in this study with the expectation that it would significantly increase the amount of flowering in these three species one year after its application. This expectation was based on findings that fertiliser addition to masting plants has resulted in fertilised plants showing significant increases in flowering and seeding compared to control plants in the year following fertiliser application (Miyazaki *et al.*, 2014; Bogdziewicz *et al.*, 2017a). Fertiliser experiments have been undertaken because it had been shown that after a heavy mast year plants expend a lot of resources and require at least a one year delay, where resource accumulation takes place, before being able to flower or reproduce again (Sala *et al.*, 2012; Han *et al.*, 2014). Resource budget models also show masting plants need this resource accumulation period to flower heavily again (Isagi *et al.*, 1997; Satake and Bjørnstad, 2008). Therefore, it seems that resources are a major limiting factor in the periodicity of mast flowering. The aim of this study was to use the expected flowering response to determine how much control resources have on flowering in masting plants and whether resources are more important in determining the period between flowering events than temperature cues. However, what was found one year after fertiliser application was either little to no flowering in plants in their natural environment on Mt Hutt or a non-significant difference between flowering of control and treatment plants that had previously been transplanted to UC, near sea level.

Another finding one year after fertiliser application was a significant increase in the production of daughter tillers in *C. pallens* and daughter rosettes in *C. lyallii*. Therefore, despite there being no fertiliser effect on flowering, fertiliser still clearly had a significant effect on the plants it was applied to. This result suggests that without a large enough temperature cue to promote flowering the plants switched their resource allocation from reproductive effort to increasing their vegetative biomass (Monks and Kelly, 2006). A lack of temperature cue was suggested to be responsible for the increase in foliage, determined by leaf litter quantity, over seeding in *Nothofagus solandri* (mountain beech) one year after nitrogen application also (Davis *et al.*, 2004).

Oddly, Mark (1965b) found no significant variation in the rate of new tiller production in *C. rigida* over a range of different altitudes from 460 m to 1630 m, which is inconsistent with my findings for the control *C. pallens* plants from UC, 1070 m and 1520 m. In my study, the daughter tiller production in control plants increased significantly with altitude between the three sites. The addition of fertiliser greatly increased the vegetative growth of plants (as both production rate of

daughter tillers and growth ratio) with the highest altitude increasing the most and the lowest altitude the least. Fertiliser addition, along with increased temperatures, has been reported to have a proportionally greater effect on vegetative growth in higher altitude plants that live in colder climes with shorter growing seasons (Jonasson *et al.*, 1999). Körner (1989) also found that high altitude plants had higher concentrations of stored resources than lower altitude plants, and that this was due to having thicker leaves which increased the leaf weight per area. *Chionochloa* and *C. lyallii* plants in our fertiliser plots were observed to have thicker leaves than control plants at the same sites, with this effect increasing with altitude among sites (pers. ob.). The Jonasson *et al.* (1999) and Körner (1989) findings may explain the significant increase in daughter rosette production by *Celmisia* and tiller production by *Chionochloa* at the highest altitude (1520 m) in this study.

Two years after the application of fertiliser, and with one of the highest natural DT temperature cues since flowering observations began for this study, a significant positive effect of fertiliser was found on the flowering of *C. pallens* and *C. lyallii* at UC. All plants at UC flowered heavily, but significantly more so in the fertiliser plants compared to the control plants. Similar effects have been seen in the New Zealand masting beech tree *N. solandri*. Davis *et al.* (2004) and Smaill *et al.* (2011) found that adding N fertiliser to *N. solandri* increased seedfall compared to unfertilised trees in the years where there was a temperature cue to initiate flowering, and not in years without an appropriate temperature cue. This delay of a floral fertiliser response suggests that flowering is more likely under the primary control of temperature, particularly the DT for the previous summer seasons, than resources. It will remain to be seen whether this effect is emulated on Mt Hutt in this current 2018/19 season or not.

It is possible that the delay in flowering response could be an artefact of the age of the tillers when fertiliser was added, and whether they had reached maturity enabling them to flower. *Chionochloa rigida* raised from seed at Lincoln (near sea level) took 4-5 years to reach maturity and be able to flower (Connor, 1966). Connor (1966) also mentions that secondary tillers (daughters) remain vegetative in their first year and are not “inductible”, and that it is possible this may be true of their second years too. However, we have seen daughter tillers flower the year following their production in *C. pallens* at UC and also in the field at Mt Hutt (pers. ob.). Therefore, the age of the tillers is unlikely to be a reason why flowering did not occur one year after fertiliser application.

We know that there were still a lot of tillers remaining in the 14warm *C. pallens* plants at UC after heavy flowering in 2015/16, and most of these tillers were still alive the following summer as well as there being a large proportion of new tillers produced. The summer after the heavy flowering, 2016/17, was a low flowering year plus fertiliser was added to some, yet this still did not

lead to good flowering under either the control or fertiliser treatments in the summer following fertiliser application. This shows that even under 'prime' conditions, with temperatures warm enough to induce flowering and a year between to re-accumulate resources, the expected effect of fertiliser did not occur. Therefore, resources do not appear to be the driving factor behind the irregularity of masting events in *C. pallens*.

In conclusion, the alpine masting species used in this study have not replicated the responses of other masting species in terms of flowering. Where other masting species significantly increased flowering effort one year after having fertiliser added (Miyazaki *et al.*, 2014; Bogdziewicz *et al.*, 2017a), the two *Chionochloa* species and one *Celmisia* species used in this study showed no floral increase due to fertiliser addition. This result implies the hypothesis that resource-limitation controls flowering frequency in masting plants (Isagi *et al.*, 1997; Han *et al.*, 2014; Miyazaki *et al.*, 2014) does not hold true for all masting species, especially the alpine species used in this study.

5.2. Manipulative temperature studies

Crone and Rapp (2014) mention that not enough temperature manipulation experiments have been attempted to really assess the effect temperature has on masting. However, two studies not known to Crone and Rapp (2014), the Mark (1965a) and Mark (1968) transplantation studies on several *Chionochloa* species showed quite well the effect changes of temperature, due to shifts in altitude, had on flowering responses in these masting plants. The work in this thesis further complements the previous *Chionochloa* transplant work and adds to it *C. lyallii* transplant responses to temperature change as well.

Chionochloa transplant experiments have shown that the change in temperature experienced by plants moved to different altitudes directly affects their flowering and vegetative growth (Mark, 1965a; Mark, 1968). When moved upwards to cooler temperatures *Chionochloa* plants flower less than plants from their home site, and similarly, plants moved downwards to warmer temperatures flower much more than home site plants, while plants at home sites only flower heavily in mast years (Mark, 1965a; Mark, 1968), as was found in this study too. The manipulation of temperature that occurs with transplantation to varying altitudes is enough to induce similar changes in flowering to what occurs naturally over time with changing temperatures at a single site. For smaller sized plants (i.e. not trees), this is an easily replicated way to effectively study the effects of temperature change on masting, or flowering in general.

However, some responses resulting from transplantation may not be an accurate depiction of what plants would do in their natural environments. In the Mark (1965a) study, *Chionochloa* moved down near sea-level (to Dunedin) flowered every year for the three years of study, and we

have seen flowering in UC transplants every year since they were moved too. The majority of the Mark (1965a) transplants were *C. rigida*, which have a natural distribution from the high-alpine zone down to sea level, although they predominantly grow in the low-alpine zone. It seems this large altitudinal range means the species has a more tolerant response to large changes in temperature (Mark, 1969). In contrast, *C. macra* only occur at high altitudes among other high-alpine vegetation (Greer, 1979), and *C. pallens* is found naturally on better soils in the alpine zone from 950–1600 m, meaning they are less likely to be adapted to large altitudinal and temporal changes like *C. rigida* (Mark, 1969), so therefore cannot be expected to react the same when moved. Mark (1965a) found distinct differences between *C. rigida*, from lower altitude populations, and *C. macra*, from high altitude populations, especially in growth rates (e.g. a period of dormancy was maintained in *C. macra* plants even at lower altitudes). Therefore, if *C. macra* grow less at lower altitudes due to a possible genetic response it is likely that *C. pallens* would do the same and may explain why it is often a struggle to keep transplants alive at low altitude.

Mark (1965a) noted that *C. macra* plants from the highest altitude (1590 m) struggled to survive when moved down near sea level for several consecutive years, and after 10 years at this site all 1590 m *C. macra* plants had died (Greer, 1979). It is clear that moving some *Chionochloa* species near sea level, far below their natural range, has a detrimental impact on plant health and survival. Maybe above a certain temperature increase more effort goes into reproduction than vegetative growth. *Chionochloa* species, such as *C. macra* and *C. pallens*, do not appear to be able to adjust to large increases in temperature as their natural response causes them to flower more while producing very few replacement daughter tillers. This response suggests that these species of *Chionochloa* will not cope with climate change if the temperature at higher altitudes get up to those near sea level. An example of this rapid detrimental effect was seen at UC in the 15Warm *C. pallens* transplants. Early in summer 2017/18 the 10 plants had a lot of healthy tillers. However, that summer was the hottest since 1997/98, and two months later most of these plants were dead with any remaining plants having very few surviving tillers. The 15Warm *C. pallens* had been placed in fertilised potting mix and had replaced a lot of the tillers that had flowered the previous season, maintaining a large size (mean = 193.2 ± 39.0 tillers per plant). The 14Warm *C. pallens* plants, however, did not have as many tillers remaining after the previous season's flowering (mean = 106.8 ± 17.9 tillers per plant for controls and 144.0 ± 21.4 for fertiliser plants), likely due to being placed in unfertilised potting mix. The smaller size of the 14warm plants may have saved them as it seems the 15Warm plants suffered greatly from heat stress and could not maintain their greater foliage under hot, dry conditions, even though they were irrigated, while the 14Warm plants managed to keep their smaller mass alive. The 14Warm plants have produced noticeably fewer replacement daughter

tillers over the years, so will likely eventually die too. None of these effects were seen in the *C. lyallii* plants at UC, but as their lifeform means the original rosette survives and can produce future inflorescences, in comparison to *Chionochloa* tillers dying after flowering, this is not surprising. The leaves of *C. lyallii* are also a lot thicker than *Chionochloa* leaves and likely resist severe weather effects of better.

The terminal investment hypothesis states that long-lived, iteroparous organisms will put more resources into reproduction in a trade-off against growth as they get closer to death (Koenig *et al.*, 2017). This hypothesis was investigated by Koenig *et al.* (2017) in several masting species of California Oaks (genus *Quercus*) with long-term datasets including 70 apparently natural tree deaths. They found no evidence suggesting these species show any terminal investment, and in fact found a slight yet insignificant decrease in both radial growth and acorn production in the years leading up to tree death (Koenig *et al.*, 2017). This may be a worthwhile hypothesis to investigate for alpine *Chionochloa* species at unnaturally low altitudes to see if this may be what is causing plants deaths near sea level. However, large sections of *Chionochloa* at Mt Hutt were observed to die off after very heavy flowering at the 1620 m site in 1999 (D. Kelly, Pers. comm.). Plant deaths at UC are more likely the result of a large number of tillers dying after heavy flowering and a temperature-related lack of daughter tiller production than a natural response to impeding death as suggested by the terminal investment hypothesis.

A variation of transplantation was used in this study where *C. pallens* halves were moved down to two different altitudes (450 m and UC) in November for a summer period before being returned to their home site in March. One result of this transplantation experiment was the identification of the fact that *C. pallens* do not require vernalisation to flower, as several inflorescences were observed on some of these plants after their return in May of that same year. Mark (1968) had previously noted that *C. crassiuscula, C. flavescens, C. oreophila,* and *C. rigida* all displayed autumn initiation of inflorescences when transplanted to Dunedin, near sea level, but these plants had all remained at that site indefinitely. Interestingly, ours were all back at their colder 1070 m home site after the floral induction period yet still flowered earlier. Connor (1966) mentions false springs and mild winters were seen to result in early culm elongation of panicles in *C. rigida*, but these later were damaged by normal frosts. However, our 16Warm and 16Hot transplants appeared to complete their flowering, although the fates of florets through to viable seeds was not followed.

The results of our transplantation experiments, and those of Mark (1965a) and Mark (1968), show that these experiments are a useful way to look at effects of changing temperature on masting species. The response of plants to extreme temperatures outside their natural range may help

understand how plants will be affected by climate change. In terms of further identifying how well the DT model explains flowering responses of *Chionochloa* within their natural range, it would be good to transplant plants within this range more often (e.g. from 1620 m to 1070 m and vice versa). For *C. lyallii*, as they flower less often than *Chionochloa,* studies would need to be run for longer before gaining any clear understanding of how temperature change affects flowering in this species.

5.3. Temperature cues vs. resources

The results of our fertiliser experiment suggest that resources are not a main controlling factor for flowering in *Chionochloa* or *Celmisia*, rather that temperature is the dominant factor in mast flowering for these species. The importance of temperature over resources on flowering in *Chionochloa* and *Celmisia* were shown in three ways.

1) By the lack of fertiliser-induced flowering on Mt Hutt the year following fertiliser application. This was, however, the predicted result given the respective T1 and DT temperature cues.

2) Flowering did occur one year after fertiliser application at UC due to the warmer temperatures at this low elevation which was clearly enough to induce some flowering. Yet, no fertiliser-induced increase in flowering was found in either *C. pallens* or *C. lyallii*.

3) Heavy flowering only occurred at UC two years after fertiliser application and after high T1 and DT temperature cues. Both control and fertiliser plants flowered heavily. The fertiliser *C. pallens* and *C. lyallii* did show significant increases in flowering compared to control plants after this two-year lag, although this result was clearly dependent on the temperature cue to initiate it.

These results show the suggestion that the DT model is just a proxy for T_{-1} + resources (Monks *et al.*, 2016) does not appear to be the case for our study species. Resources budget models are also not as important as temperature on flowering in the species used in our study.

In a study by Abe *et al.* (2016) on *F. crenata* they stated that both resource (nitrogen) dynamics and temperature cues were required to explain the observed flowering response. However, their study did not show to what level each of these two factors dominated the observed flowering response, only that they are both needed. Abe *et al.* (2016) found that small clumps of *F. crenata* trees within an area responded more synchronously than the population as a whole in that area, a phenomenon earlier mentioned by Satake and Iwasa (2000). This finding may be due to the microclimate within each clump better explaining the flowering response of those trees than the climate for the entire population as a whole (Koenig *et al.*, 2015). It is common knowledge that resources are required for flowering to occur (Bazzaz *et al.*, 1987), but this does not necessarily

mean they are a main controlling factor in the periodicity of flowering in masting plants. The availability of resources to plants is also dependent on climate, as precipitation and temperature are required in order for organic matter and parent (rock) material to break down (White and Blum, 1995) into substances plants can take up and use. Therefore, it is possible that temperature is doubly important for flowering in masting species as it directly impacts floral induction and the availability of resources for plants.

Temperature has been shown to significantly correlate with flowering and seeding in *Q. petraea* and *Q. robur* over a 14-year period where notable yearly increases in temperature due to climate change have occurred (Caignard *et al.*, 2017). The size and frequency of reproductive responses in these oaks increased with rising spring temperatures, and longer growing seasons meant oak trees both grew and reproduced more across the years (Caignard *et al.*, 2017). As nitrogen deposition increases with increasing temperature (Reay *et al.*, 2008), other studies have investigated the effects of added nitrogen to forests to simulate the effect of rising temperatures. In a study on red (*Q. borealis*) and black (*Q. velutina*) oaks where nitrogen had been added for 20 years, it was found that nitrogen increased the size of plants and this further lead to an increase in acorn production and frequency of seeding events compared to controls (Callahan *et al.*, 2008). Both Callahan *et al.* (2008) and Caignard *et al.* (2017) mention that this climate change response could lead to negative impacts on oak fitness as the benefits of seed predator satiation, due to masting, may be released. A loss of predator satiation benefits as a result of increased periodicity of mast seeding was shown experimentally in *Q. rubra* by Bogdziewicz *et al.* (2017a) when also looking at the long-term effect increases in nitrogen will have on masting. Therefore, it seems the loss of predator satiation benefits will likely be a negative consequence of increased climate and nitrogen deposition on some masting plants.

Drobyshev *et al.* (2010) had previously found a distinctive weather pattern that preceded mast years in *Fagus sylvatica* (European beech) in the Swedish province of Halland, it was that of a cold growing season two years before followed by a warm growing season one year before the mast event. Later, this DT-like temperature pattern was shown to match mast years for *F. sylvatica* in a 253-year long historical study by Drobyshev *et al.* (2014). The latter study also showed that long-term changes in the mean of average summer temperatures meant that, even though there were periods where the frequency of masting got closer or further apart due to temperature fluctuations in time, *F. sylvatica* still maintained a varied but synchronous masting regime (Drobyshev *et al.*, 2014), as was suggested by Kelly *et al.* (2013) when predicting the response of masting plants to climate change.

Further improvements can still be made to the DT model to increase its predictive power. Looking at summer air temperatures from Connor (1966), which show induction and flowering patterns across years for *C. rigida* and *Nothofagus* species for the December-January period, the mean maximum temperature fits the responses more accurately than the mean minimum does. In Allen *et al.* (2014), it is suggested that the mean maximum temperature explains beech seeding better. Hence, testing DT using the mean maximum temperature may improve model fits even more than using mean temperatures. Testing the DT model using the mean maximum temperature against the mean temperature was initially planned for this study but time constraints meant it could not be done. This would be an interesting idea to test in the future.

A benefit of the DT model is that it indirectly incorporates the resource state, through the effect of temperature on the breakdown and mineralisation of surrounding matter leading to resource availability, but without the need to collect individual site resource data. This makes the DT model a more user-friendly, less time-consuming, and more cost-effective way to predict the flowering response of masting plants. Clearly the temperature, and the effect the temperature has on resource availability, plays a far more dominant role in controlling the flowering response in the species used in this study than resources alone. Therefore, it makes far more sense to make predictions on future flowering responses of masting plants, where the DT model temperature pattern is also shown to explain masting well, using this dominant temperature cue. For the masting species where the DT temperature pattern does explain flowering (*Fagus sylvatica* (Piovesan and Adams, 2001; Drobyshev et al., 2010; Drobyshev et al., 2014; Hacket-Pain et al., 2015; Vacchiano et al., 2017; Lebourgeois et al., 2018); *F. grandifolia* (Piovesan and Adams, 2001); *Picea abies* (Selås *et al.*, 2002; Bisi *et al.*, 2016); *Celmisia lyallii, Chionochloa australis, C. crassiuscula, C. macra, C. pallens, C. rigida, C. rubra, C. teretifolia, Elaeocarpus dentatus, Nothofagus fusca, N. menziesii, N. solandri, N. truncata, Phormium cookianum, P. tenax* (Kelly et al., 2013)) the DT model will be a useful tool for predicting future masting events, especially from a conservation point of view (Holland *et al.*, 2015; Elliott and Kemp, 2016; Bisi *et al.*, 2018).

If a similar response to that found in *C. pallens* at UC, and *C. macra* at Dunedin (Greer, 1979), is found in other masting species whose flowering is also explained by the DT model, then the survival of these species could suffer as a consequence of climate change. Such great increases in temperature seem to induce a heavier than normal flowering response at the cost of new vegetative growth, which over time will reduce plant health and lead to plant death and possible species extinction. Essentially they could flower themselves to death.

The results of this study looking at three alpine masting species will not pertain to all masting species. Masting is a convergent evolutionary trait which leads to greater species survival

and fitness in multiple species and has come about in a variety of ways. Climate alone has been shown to effect flowering in quite different ways, for example floral initiation in *F. crenata* relies on low minimum night-time temperatures between April and May (Kon and Noda, 2007), whereas *F. sylvatica* and *F. grandifolia* require a drought event the summer before to initiate masting (Piovesan and Adams, 2001). As Crone and Rapp (2014) concluded "given the many ways in which mast seeding increases fitness, it is not surprising that it would arise in different ways in different evolutionary lineages." One example of this can be seen in Kelly and Sork (2002) where they mention a variety of selection pressures due to the habits of seed predators resulting in variation in masting and its synchrony. Add to this the findings of Nussbaumer *et al.* (2018), who found in some species masting was explained well by DT, but other species were better explained in different ways. This demonstrates the great variation in controlling cues for different masting species (Nussbaumer *et al.*, 2018), a point also noted by Moreira *et al.* (2015). Therefore, the many different reasons that masting as a strategy can occur mean there are just as many cues masting plants can respond to, and predicting their responses to climate change will never result in a 'one size fits all' answer.

References

Abe, T., Tachiki, Y., Kon, H., Nagasaka, A., Onodera, K., Minamino, K., Han, Q. & Satake, A. (2016). Parameterisation and validation of a resource budget model for masting using spatiotemporal flowering data of individual trees. *Ecology Letters* 19(9): 1129-1139.

Allen, R. B., Hurst, J. M., Portier, J. & Richardson, S. J. (2014). Elevation-dependent responses of tree mast seeding to climate change over 45 years. *Ecology and evolution* 4(18): 3525-3537.

Antonovics, J. & Levin, D. A. (1980). The ecological and genetic consequences of density-dependent regulation in plants. *Annual review of ecology and systematics* 11(1): 411-452.

Bazzaz, F. A., Chiariello, N. R., Coley, P. D. & Pitelka, L. F. (1987). Allocating resources to reproduction and defense. *BioScience* 37(1): 58-67.

Bisi, F., Chirichella, R., Chianucci, F., Von Hardenberg, J., Cutini, A., Martinoli, A. & Apollonio, M. (2018). Climate, tree masting and spatial behaviour in wild boar (*Sus scrofa* L.): insight from a long-term study. *Annals of Forest Science* 75(2): 46.

Bisi, F., von Hardenberg, J., Bertolino, S., Wauters, L., Imperio, S., Preatoni, D., Provenzale, A., Mazzamuto, M. & Martinoli, A. (2016). Current and future conifer seed production in the Alps: testing weather factors as cues behind masting. *European Journal of Forest Research* 135(4): 743-754.

Bogdziewicz, M., Crone, E. E., Steele, M. A. & Zwolak, R. (2017a). Effects of nitrogen deposition on reproduction in a masting tree: benefits of higher seed production are trumped by negative biotic interactions. *Journal of Ecology* 105(2): 310-320.

Bogdziewicz, M., Fernández-Martínez, M., Bonal, R., Belmonte, J. & Espelta, J. M. (2017b). The Moran effect and environmental vetoes: phenological synchrony and drought drive seed production in a Mediterranean oak. *Proc. R. Soc. B* 284(1866): 20171784.

Bogdziewicz, M., Steele, M. A., Marino, S. & Crone, E. E. (2018). Correlated seed failure as an environmental veto to synchronize reproduction of masting plants. *New Phytologist* 219(1): 98-108.

Caignard, T., Kremer, A., Firmat, C., Nicolas, M., Venner, S. & Delzon, S. (2017). Increasing spring temperatures favor oak seed production in temperate areas. *Scientific reports* 7(1): 8555.

Callahan, H. S., Del Fierro, K., Patterson, A. E. & Zafar, H. (2008). Impacts of elevated nitrogen inputs on oak reproductive and seed ecology. *Global Change Biology* 14(2): 285-293.

Campbell, A. D. (1981). Flowering records for *Chionochloa*, *Aciphylla*, and *Celmisia* species in the Craigieburn Range, South Island, New Zealand. *New Zealand journal of botany* 19(1): 97-103.

Connor, H. E. (1966). Breeding systems in New Zealand grasses: VII. Periodic flowering of Snow Tussock, *Chionochloa Rigida*. *New Zealand journal of botany* 4(4): 392-397.

Crone, E. E., Miller, E. & Sala, A. (2009). How do plants know when other plants are flowering? Resource depletion, pollen limitation and mast-seeding in a perennial wildflower. *Ecology Letters* 12(11): 1119-1126.

Crone, E. E. & Rapp, J. M. (2014). Resource depletion, pollen coupling, and the ecology of mast seeding. *Annals of the New York Academy of Sciences* 1322(1): 21-34.

Davi, H., Cailleret, M., Restoux, G., Amm, A., Pichot, C. & Fady, B. (2016). Disentangling the factors driving tree reproduction. *Ecosphere* 7(9).

Davis, M. R., Allen, R. B. & Clinton, P. W. (2004). The influence of N addition on nutrient content, leaf carbon isotope ratio, and productivity in a *Nothofagus* forest during stand development. *Canadian Journal of Forest Research* 34(10): 2037-2048.

Drobyshev, I., Niklasson, M., Mazerolle, M. J. & Bergeron, Y. (2014). Reconstruction of a 253-year long mast record of European beech reveals its association with large scale temperature variability and no long-term trend in mast frequencies. *Agricultural and forest meteorology* 192: 9-17.

Drobyshev, I., Övergaard, R., Saygin, I., Niklasson, M., Hickler, T., Karlsson, M. & Sykes, M. T. (2010). Masting behaviour and dendrochronology of European beech (*Fagus sylvatica* L.) in southern Sweden. *Forest Ecology and Management* 259(11): 2160-2171.

Elliott, G. & Kemp, J. (2016). Large-scale pest control in New Zealand beech forests. *Ecological Management & Restoration* 17(3): 200-209.

Fenner, M., Lee, W. G. & Pinn, E. H. (2001). Reproductive features of *Celmisia* species (Asteraceae) in relation to altitude and geographical range in New Zealand. *Biological Journal of the Linnean Society* 74(1): 51-58.

Greer, D. H. (1979). Effects of long-term preconditioning on growth and flowering of some snow tussock (*Chionochloa* spp.) populations in Otago, New Zealand. *Australian journal of botany* 27(5): 617-630.

Hacket-Pain, A. J., Friend, A. D., Lageard, J. G. & Thomas, P. A. (2015). The influence of masting phenomenon on growth–climate relationships in trees: explaining the influence of previous summers' climate on ring width. *Tree Physiology* 35(3): 319-330.

Han, Q., Kabeya, D., Iio, A., Inagaki, Y. & Kakubari, Y. (2014). Nitrogen storage dynamics are affected by masting events in *Fagus crenata*. *Oecologia* 174(3): 679-687.

Han, Q., Kabeya, D., Iio, A. & Kakubari, Y. (2008). Masting in *Fagus crenata* and its influence on the nitrogen content and dry mass of winter buds. *Tree Physiology* 28(8): 1269-1276.

Hay, J., Kelly, D. & Holdaway, R. J. (2008). Causes and consequences of frequent flowering on edges in the mast-seeding genus *Chionochloa* (Poaceae). *New Zealand Journal of Ecology* 32(1): 80-91.

Hoch, G., Siegwolf, R. T., Keel, S. G., Körner, C. & Han, Q. (2013). Fruit production in three masting tree species does not rely on stored carbon reserves. *Oecologia* 171(3): 653-662.

Holland, E. P., James, A., Ruscoe, W. A., Pech, R. P. & Byrom, A. E. (2015). Climate-based models for pulsed resources improve predictability of consumer population dynamics: outbreaks of house mice in forest ecosystems. *PloS one* 10(3): e0119139.

Ichie, T. & Nakagawa, M. (2013). Dynamics of mineral nutrient storage for mast reproduction in the tropical emergent tree *Dryobalanops aromatica*. *Ecological research* 28(2): 151-158.

Ims, R. A. (1990). The ecology and evolution of reproductive synchrony. *Trends in Ecology & Evolution* 5(5): 135-140.

Isagi, Y., Sugimura, K., Sumida, A. & Ito, H. (1997). How does masting happen and synchronize? *Journal of Theoretical Biology* 187(2): 231-239.

Jonasson, S., Michelsen, A., Schmidt, I. K. & Nielsen, E. V. (1999). Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. *Ecology* 80(6): 1828-1843.

Kelly, D. (1994). The evolutionary ecology of mast seeding. *Trends in Ecology & Evolution* 9(12): 465-470.

Kelly, D., Geldenhuis, A., James, A., Penelope Holland, E., Plank, M. J., Brockie, R. E., Cowan, P. E., Harper, G. A., Lee, W. G. & Maitland, M. J. (2013). Of mast and mean: differential-temperature cue makes mast seeding insensitive to climate change. *Ecology Letters* 16(1): 90-98.

Kelly, D., Harrison, A. L., Lee, W. G., Payton, I. J., Wilson, P. R. & Schauber, E. M. (2000). Predator satiation and extreme mast seeding in 11 species of *Chionochloa* (Poaceae). *Oikos* 90(3): 477-488.

Kelly, D., Hart, D. E. & Allen, R. B. (2001). Evaluating the wind pollination benefits of mast seeding. *Ecology* 82(1): 117-126.

Kelly, D. & Sork, V. L. (2002). Mast seeding in perennial plants: why, how, where? *Annual review of ecology and systematics* 33(1): 427-447.

Kelly, D. & Sullivan, J. J. (1997). Quantifying the Benefits of Mast Seeding on Predator Satiation and Wind Pollination in *Chionochloa pallens* (Poaceae). *Oikos* 78(1): 143-150.

Kelly, D., Turnbull, M. H., Pharis, R. P. & Sarfati, M. S. (2008). Mast seeding, predator satiation, and temperature cues in *Chionochloa* (Poaceae). *Population Ecology* 50(4): 343-355.

Koenig, W. D., Knops, J. M. H., Carmen, W. J. & Pearse, I. S. (2015). What drives masting? The phenological synchrony hypothesis. *Ecology* 96(1): 184-192.

Koenig, W. D., Knops, J. M. H., Carmen, W. J. & Pesendorfer, M. B. (2017). Testing the terminal investment hypothesis in California oaks. *The American Naturalist* 189(5): 564-569.

Kon, H. & Noda, T. (2007). Experimental investigation on weather cues for mast seeding of *Fagus crenata*. *Ecological research* 22(5): 802-806.

Kon, H. & Saito, H. (2015). Test of the temperature difference model predicting masting behavior. *Canadian Journal of Forest Research* 45(12): 1835-1844.

Körner, C. (1989). The nutritional status of plants from high altitudes. *Oecologia* 81(3): 379-391.

Körner, C. & Hiltbrunner, E. (2017). The 90 ways to describe plant temperature. *Perspectives in Plant Ecology, Evolution and Systematics*.

Lambers, J. H. R., Clark, J. S. & Beckage, B. (2002). Density-dependent mortality and the latitudinal gradient in species diversity. *Nature* 417(6890): 732.

Lebourgeois, F., Delpierre, N., Dufrêne, E., Cecchini, S., Macé, S., Croisé, L. & Nicolas, M. (2018). Assessing the roles of temperature, carbon inputs and airborne pollen as drivers of fructification in European temperate deciduous forests. *European Journal of Forest Research*: 1-17.

Mark, A. F. (1965a). Ecotypic differentiation in Otago populations of narrowleaved snow tussock, *Chionochloa rigida*. *New Zealand journal of botany* 3(4): 277-299.

Mark, A. F. (1965b). The environment and growth rate of narrow-leaved snow tussock, *Chionochloa rigida*, in Otago. *New Zealand journal of botany* 3(2): 73-103.

Mark, A. F. (1965c). Flowering, seeding, and seedling establishment of narrow-leaved snow tussock, *Chionochloa rigida*. *New Zealand journal of botany* 3(3): 180-193.

Mark, A. F. (1968).Factors controlling irregular flowering in four alpine species of *Chionochloa*. In *Proceedings (New Zealand Ecological Society)*, Vol. 15, 55-60: JSTOR.

Mark, A. F. (1969). Ecology of snow tussocks in the mountain grasslands of New Zealand. *Vegetatio* 18(1-6): 289-306.

Mark, A. F. (1970). Floral initiation and development in New Zealand alpine plants. *New Zealand journal of botany* 8(1): 67-75.

Mason, E. G., Salekin, S. & Morgenroth, J. A. (2017). Comparison between meteorological data from the New Zealand National Institute of Water and Atmospheric Research (NIWA) and data from independent meteorological stations. *New Zealand Journal of Forestry Science* 47(1): 7.

Miyazaki, Y., Maruyama, Y., Chiba, Y., Kobayashi, M. J., Joseph, B., Shimizu, K. K., Mochida, K., Hiura, T., Kon, H. & Satake, A. (2014). Nitrogen as a key regulator of flowering in *Fagus crenata*:

understanding the physiological mechanism of masting by gene expression analysis. *Ecology Letters* 17(10): 1299-1309.

Monks, A. & Kelly, D. (2006). Testing the resource-matching hypothesis in the mast seeding tree *Nothofagus truncata* (Fagaceae). *Austral Ecology* 31(3): 366-375.

Monks, A., Monks, J. M. & Tanentzap, A. J. (2016). Resource limitation underlying multiple masting models makes mast seeding sensitive to future climate change. *New Phytologist* 210(2): 419-430.

Montesinos, D., García-Fayos, P. & Verdú, M. (2012). Masting uncoupling: mast seeding does not follow all mast flowering episodes in a dioecious juniper tree. *Oikos* 121(11): 1725-1736.

Moreira, X., Abdala-Roberts, L., Linhart, Y. B. & Mooney, K. A. (2015). Effects of climate on reproductive investment in a masting species: assessment of climatic predictors and underlying mechanisms. *Journal of Ecology* 103(5): 1317-1324.

Norton, D. A. (1985). A multivariate technique for estimating New Zealand temperature normals. *Weather and Climate*: 64-74.

Norton, D. A. & Kelly, D. (1988). Mast seeding over 33 years by *Dacrydium cupressinum* Lamb.(rimu)(Podocarpaceae) in New Zealand: the importance of economies of scale. *Functional ecology*: 399-408.

Nussbaumer, A., Waldner, P., Apuhtin, V., Aytar, F., Benham, S., Bussotti, F., Eichhorn, J., Eickenscheidt, N., Fabianek, P. & Falkenried, L. (2018). Impact of weather cues and resource dynamics on mast occurrence in the main forest tree species in Europe. *Forest Ecology and Management* 429: 336-350.

Övergaard, R., Gemmel, P. & Karlsson, M. (2007). Effects of weather conditions on mast year frequency in beech (*Fagus sylvatica* L.) in Sweden. *Forestry* 80(5): 555-565.

Pearse, I. S., Koenig, W. D. & Kelly, D. (2016). Mechanisms of mast seeding: resources, weather, cues, and selection. *New Phytologist* 212(3): 546-562.

Pearse, I. S., Koenig, W. D. & Knops, J. M. H. (2014). Cues versus proximate drivers: testing the mechanism behind masting behavior. *Oikos* 123(2): 179-184.

Piovesan, G. & Adams, J. M. (2001). Masting behaviour in beech: linking reproduction and climatic variation. *Canadian Journal of Botany* 79(9): 1039-1047.

Platt, K. H., Allen, R. B., Coomes, D. A. & Wiser, S. K. (2004). Mountain beech seedling responses to removal of below-ground competition and fertiliser addition. *New Zealand Journal of Ecology*: 289-293.

Reay, D. S., Dentener, F., Smith, P., Grace, J. & Feely, R. A. (2008). Global nitrogen deposition and carbon sinks. *Nature Geoscience* 1(7): 430.

Rees, M., Kelly, D. & Bjørnstad, O. N. (2002). Snow tussocks, chaos, and the evolution of mast seeding. *The American Naturalist* 160(1): 44-59.

Richardson, S. J., Allen, R. B., Whitehead, D., Carswell, F. E., Ruscoe, W. A. & Platt, K. H. (2005). Climate and Net Carbon Availability Determine Temporal Patterns of Seed Production by *Nothofagus*. *Ecology* 86(4): 972-981.

Sala, A., Hopping, K., McIntire, E. J., Delzon, S. & Crone, E. E. (2012). Masting in whitebark pine (Pinus albicaulis) depletes stored nutrients. *New Phytologist* 196(1): 189-199.

Satake, A. & Bjørnstad, O. N. (2008). A resource budget model to explain intraspecific variation in mast reproductive dynamics. *Ecological research* 23(1): 3-10.

Satake, A. & Iwasa, Y. (2000). Pollen coupling of forest trees: forming synchronized and periodic reproduction out of chaos. *Journal of Theoretical Biology* 203(2): 63-84.

Schauber, E. M., Kelly, D., Turchin, P., Simon, C., Lee, W. G., Allen, R. B., Payton, I. J., Wilson, P. R., Cowan, P. E. & Brockie, R. E. (2002). Masting by Eighteen New Zealand Plant Species: The Role of Temperature as a Synchronizing Cue. *Ecology* 83(5): 1214-1225.

Selås, V., Piovesan, G., Adams, J. M. & Bernabei, M. (2002). Climatic factors controlling reproduction and growth of Norway spruce in southern Norway. *Canadian Journal of Forest Research* 32(2): 217-225.

Smaill, S. J., Clinton, P. W., Allen, R. B. & Davis, M. R. (2011). Climate cues and resources interact to determine seed production by a masting species. *Journal of Ecology* 99(3): 870-877.

Tait, A. & Macara, G. (2014). Evaluation of interpolated daily temperature data for high elevation areas in New Zealand. *Weather and Climate* 34: 36-49.

Tanentzap, A. J., Lee, W. G. & Coomes, D. A. (2012). Soil nutrient supply modulates temperature-induction cues in mast-seeding grasses. *Ecology* 93(3): 462-469.

Tanentzap, A. J., Lee, W. G., Coomes, D. A. & Mason, N. W. H. (2014). Masting, mixtures and modes: are two models better than one? *Oikos* 123(9): 1144-1152.

Vacchiano, G., Hacket-Pain, A., Turco, M., Motta, R., Maringer, J., Conedera, M., Drobyshev, I. & Ascoli, D. (2017). Spatial patterns and broad-scale weather cues of beech mast seeding in Europe. *New Phytologist* 215(2): 595-608.

Webb, C. J. & Kelly, D. (1993). The reproductive biology of the New Zealand flora. *Trends in Ecology & Evolution* 8(12): 442-447.

White, A. F. & Blum, A. E. (1995). Effects of climate on chemical weathering in watersheds. *Geochimica et Cosmochimica Acta* 59(9): 1729-1747.

Zotov, V. D. (1970). *Chionochloa macra* (Gramineae): a new species. *New Zealand journal of botany* 8(1): 91-93.

Appendix A: Potting mix analysis determining whether 2014 and 2015 UC transplants were placed in fertilised or unfertilised potting mix

In writing up the methods, it was discovered that the type of potting mix plants transplanted to UC from Mt Hutt were put into, either fertilised or unfertilised, was not recorded for 2014 or 2015. As the pattern in flowering response of these plants two years after transplantation unexpectedly differed it was important to know this detail as it may explain the difference. The 14Warm plants, moved in 2014, responded as expected with high flowering the summer season following transplantation and very little flowering the second summer season after being moved. Whereas, the 15Warm plants, moved in 2015, flowered high in the first summer after being moved, as expected, but still flowered reasonable high in the second year after transplantation too. If the 14Warm plants were put into unfertilised potting mix but the 15Warm were put into fertilised potting mix this anomaly may be explained.

To test which potting mix plants were put into, soil samples were collected in September 2018 from six plants within four UC *Chionochloa* plant groups (14Warm unfertilised, 14Warm fertilised, 15Warm, and 17Hot) plus a fertilised and unfertilised potting mix control. Table 1.1 shows each of the sample groups that had six soil samples collected, including plants in known and unknown potting mixes and controls. Fertiliser balls were observed in the soil of some 15Warm plants upon collection. Potting mix supplies have been obtained from the same supplier for the past 20 years, with the fertilised potting mix containing slow release fertiliser (Pers. comm. D. Conder). Soil samples were dried in an oven at 60°C before being ground in a ball mill. The 36 samples were then sent to the Waikato Stable Isotope Unit, Department of Biological Sciences at the University of Waikato, New Zealand, for nitrogen analysis. This analysis tested natural abundance levels of nitrogen-15 (^{15}N) to obtain the percentage of nitrogen in the samples and the ^{15}N signature in order to identify, *a posteriori*, which potting mixes were used for each group of transplants.

Results from this analysis show that the 15Warm plants were placed into fertilised potting mix, as similar readings to the control fertiliser potting mix were found (Table 1.1). The nitrogen results for the 14Warm plants show they were initially placed into unfertilised potting mix. Interestingly, these results also show that the (non-slow-release) fertiliser applied to half of the 14Warm plants in summer 2016/17 no longer remains, as the nitrogen levels are similar to the unfertilised 14Warm plants. Therefore, the nitrogen in the slow release fertiliser potting mix lingers far longer, and has a longer lasting effect, than the nitrogen in the applied fertiliser used for this study.

It was also unknown whether the *Celmisia lyallii* brought down from Mt Hutt in 2015 were placed into fertilised or unfertilised potting mix. Soil samples were not collected from these plants

for analysis. However, I slid plants from their pots and observed slow-release fertiliser balls in their soil also, showing that they were too placed into fertilised potting mix similar to the *Chionochloa* plants transplanted at the same time.

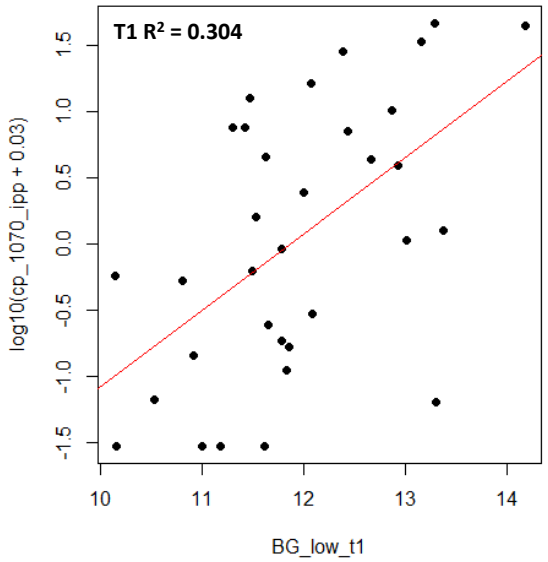
Table 1.1. Potting mix samples and results for nitrogen content percentage and ^{15}N signature from separate plants at the UC greenhouses (*n* = 6 for each sample group). The potting mix two groups of plants (14Warm and 15Warm) were put into was unknown so samples were taken from individuals of a group of plants known to be in unfertilised potting mix (17Hot), and from both fertilised and unfertilised potting mixes as controls, to measure the nitrogen and identify the unknown potting mixes.

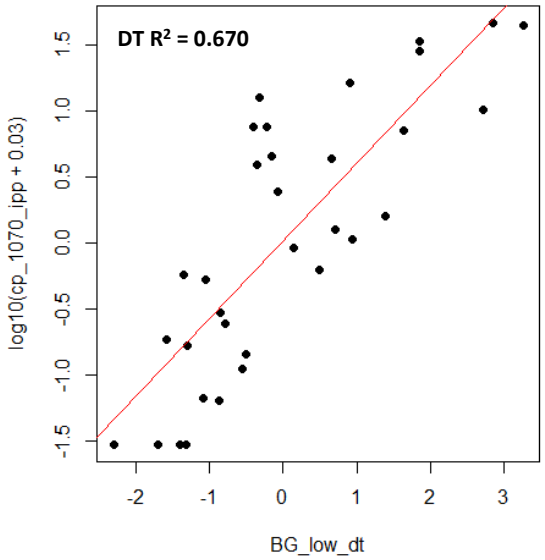
Sample group	Date potted	Potting mix	Later treatment	% N	Delta ^{15}N	Result
Fertilised potting mix - control	Oct 2018	fertilised	none	0.96	1.51	fertilised
Unfertilised potting mix - control	Oct 2018	unfertilised	none	0.39	0.67	unfertilised
14Warm	Dec 2014	unknown	fertilised	0.55	-0.44	unfertilised
14Warm	Dec 2014	unknown	none	0.53	-0.22	unfertilised
15Warm	Nov 2015	unknown	none	0.77	1.79	fertilised
17Hot	Jan 2018	unfertilised	none	0.50	-0.39	unfertilised

Appendix B: Regression graphs and R^2s for individual species annual flowering (log10) at various sites comparing the T1 model to the DT model across each temperature estimate type.

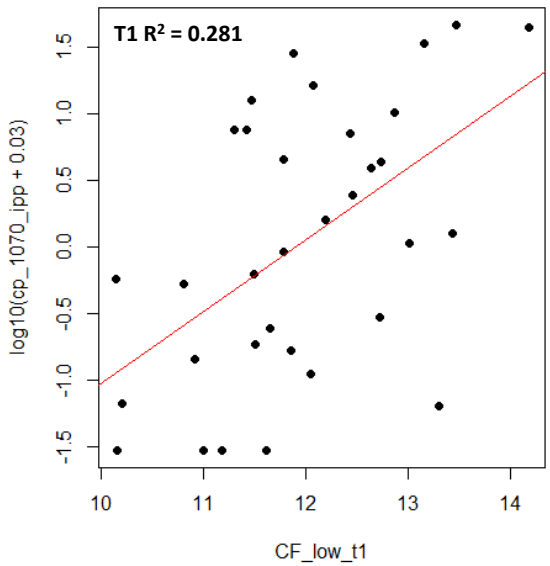
Chionochloa pallens

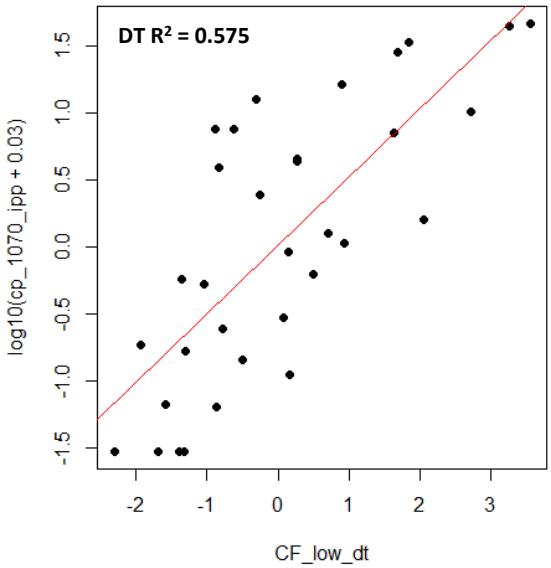
1070 m – CliFlo Botanical Gardens for missing temperatures



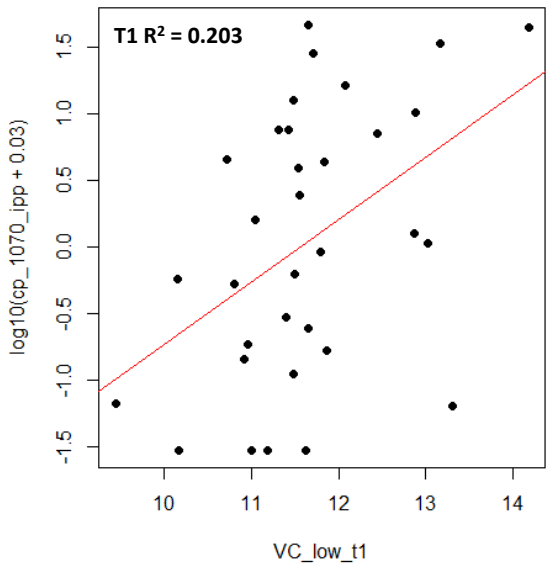


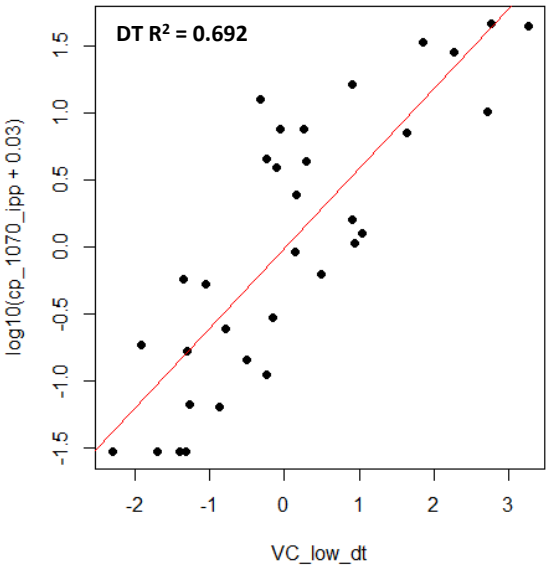
1070 m – CliFlo Darfield for missing temperatures



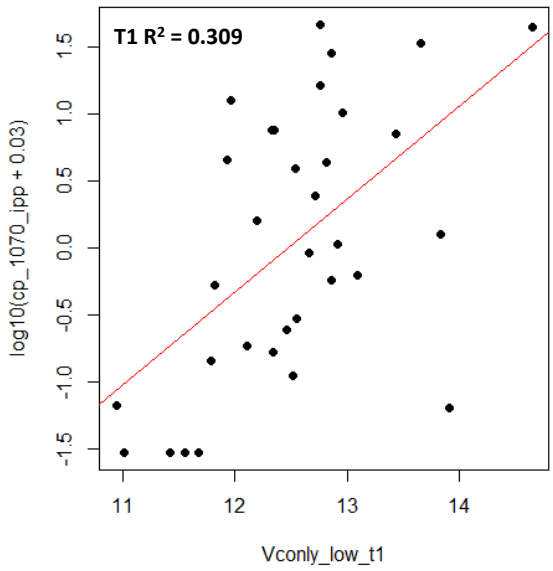


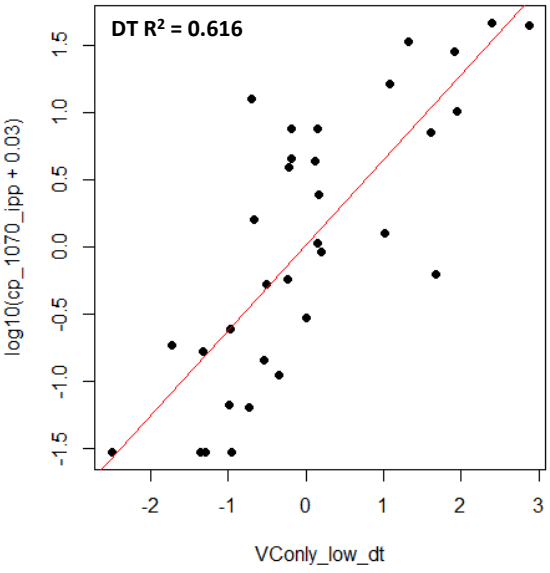
1070 m – VCSN for missing temperatures



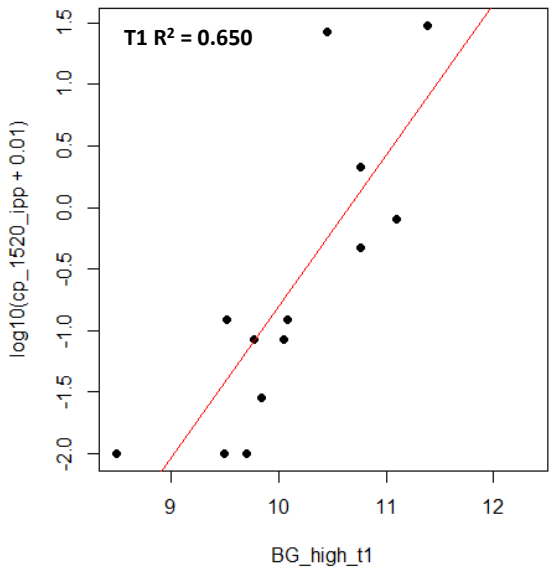


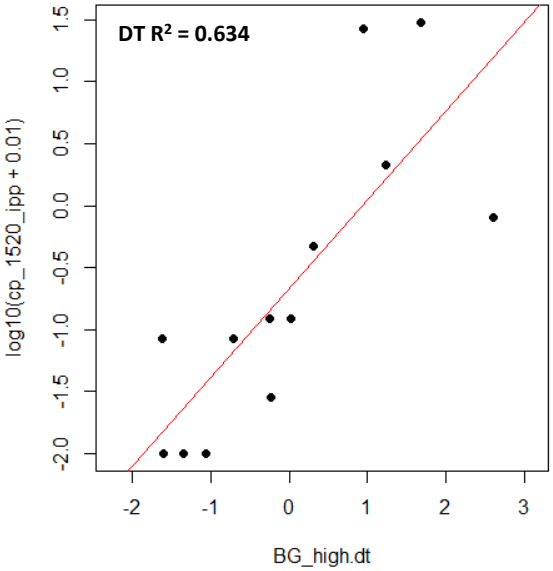
1070 m – VCSN for all temperatures





1520 m – On-site weather station as no missing temperatures





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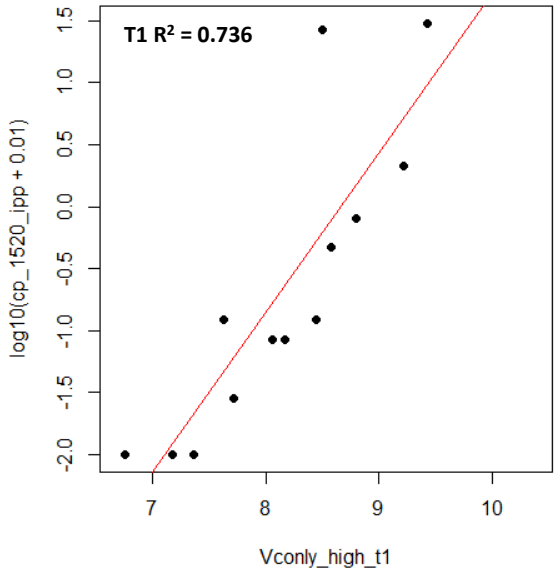
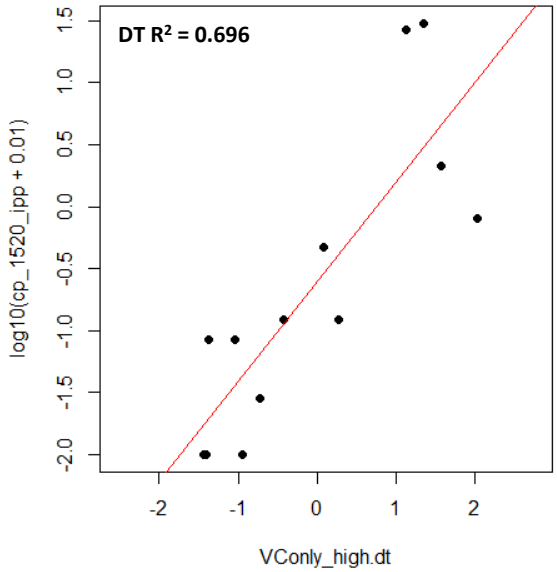
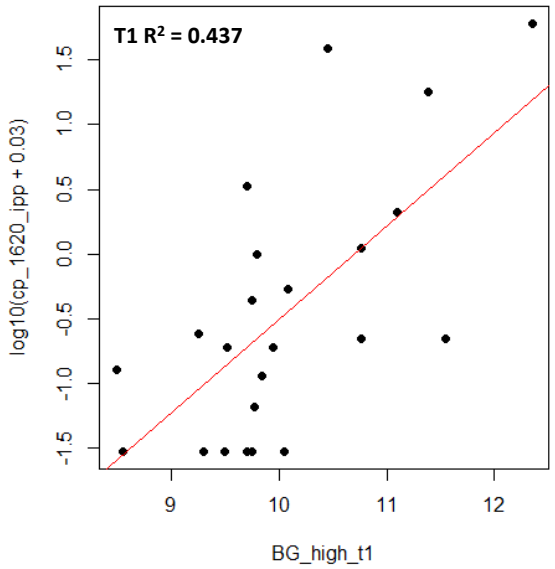
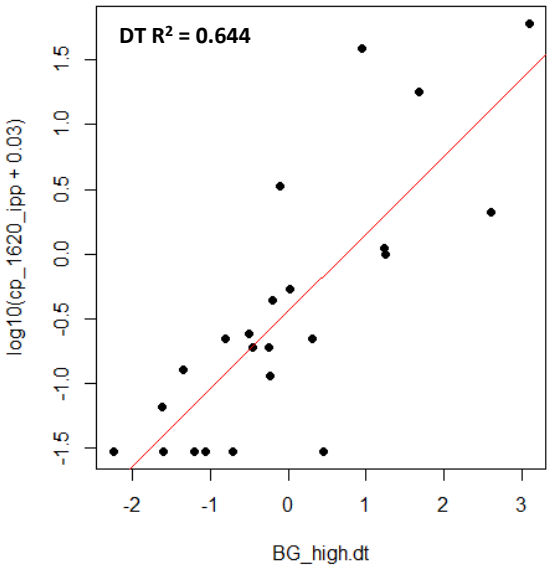


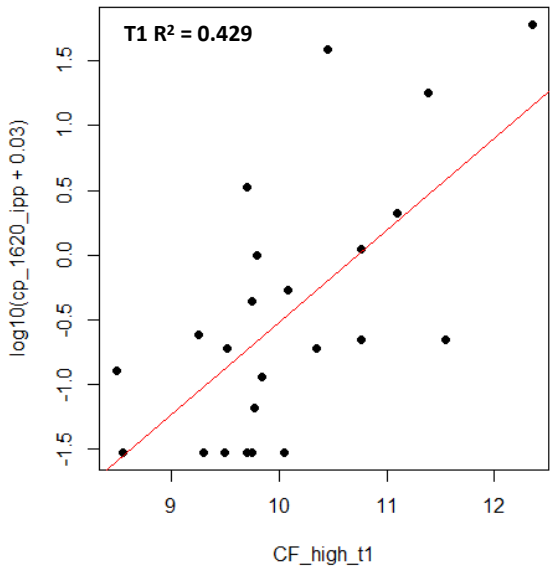


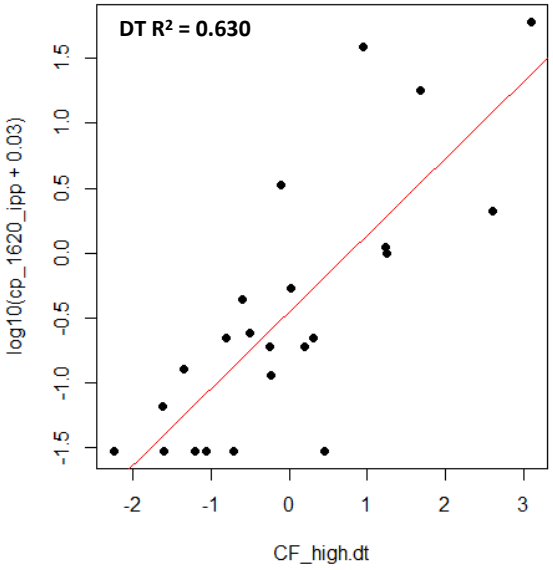
1620 m – CliFlo Botanic Gardens for missing temperatures



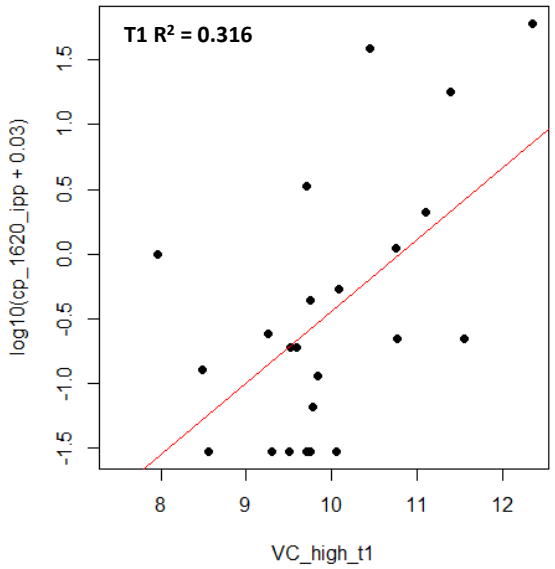


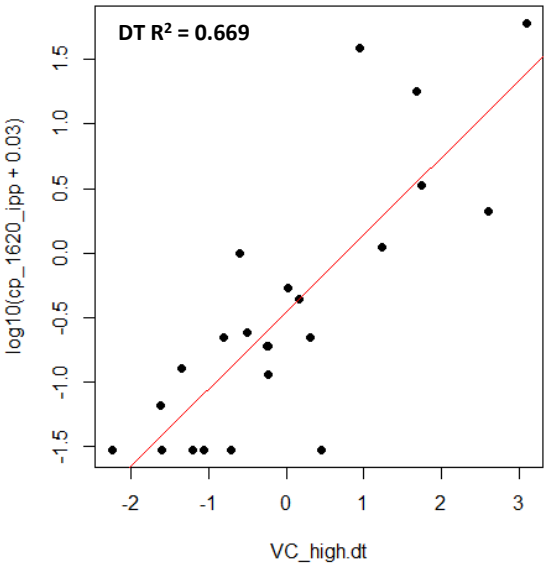
1620 m – CliFlo Darfield for missing temperatures



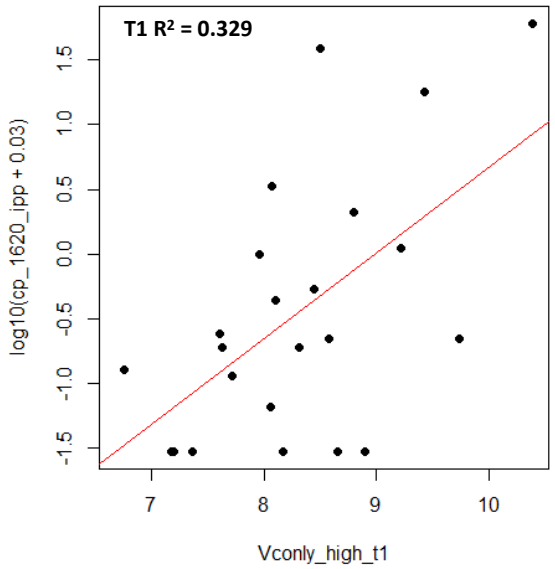


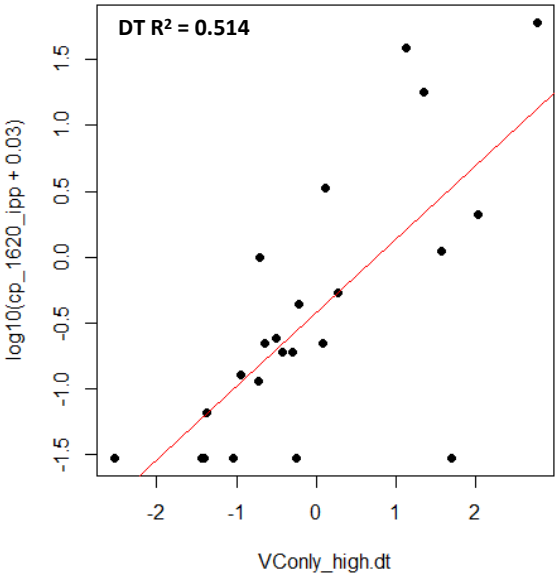
1620 m – VCSN for missing temperatures





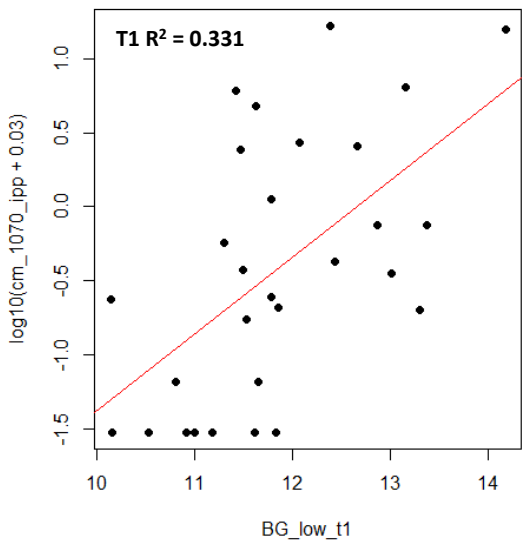
1620 m – VCSN for all temperatures

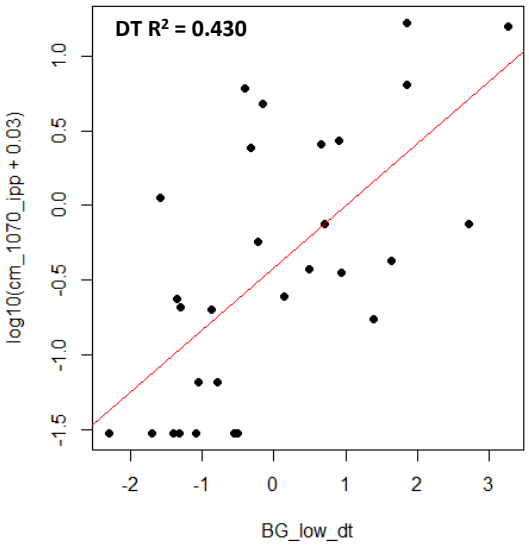




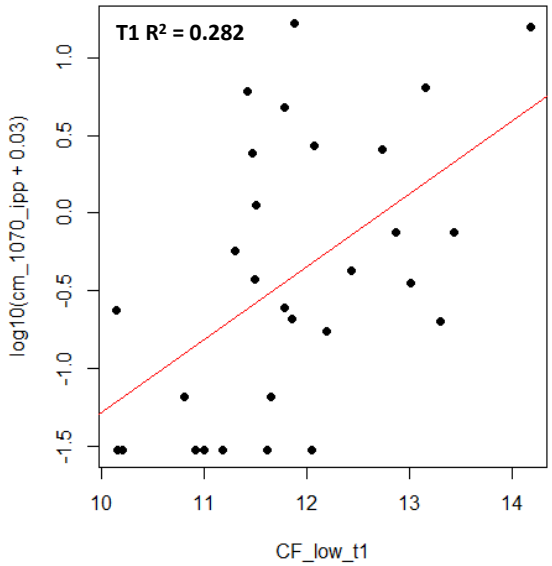
Chionochloa macra

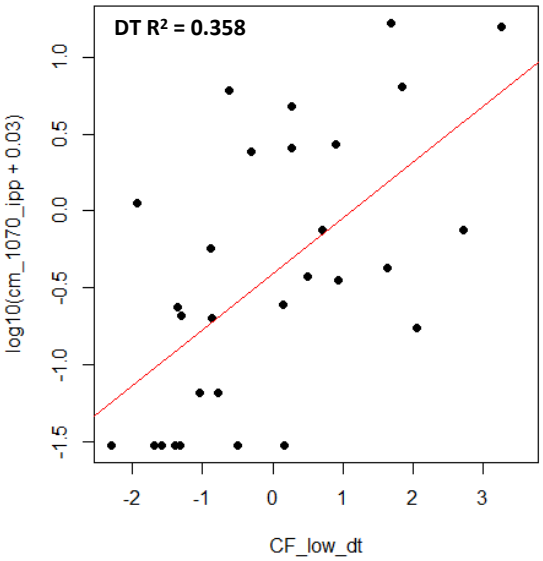
1070 m – CliFlio Botanic Gardens for missing temperatures



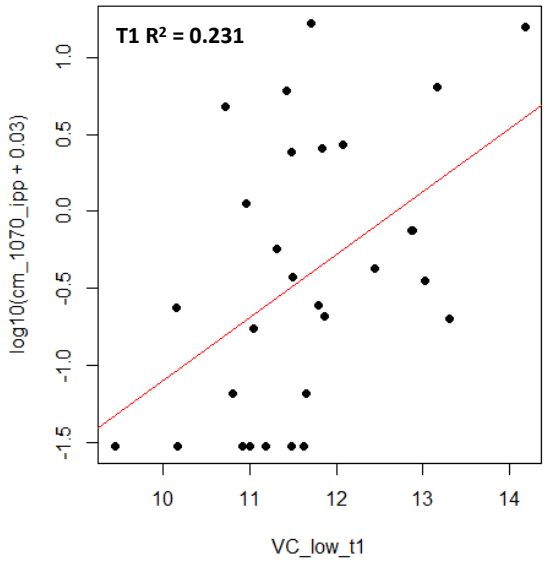


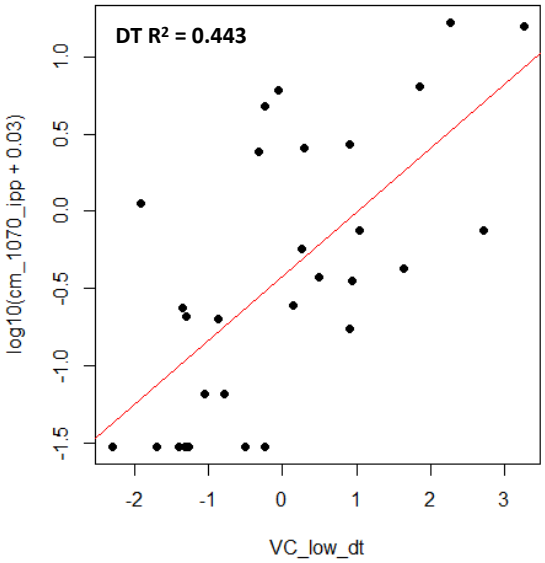
1070 m – CliFlo Darfield for missing temperatures



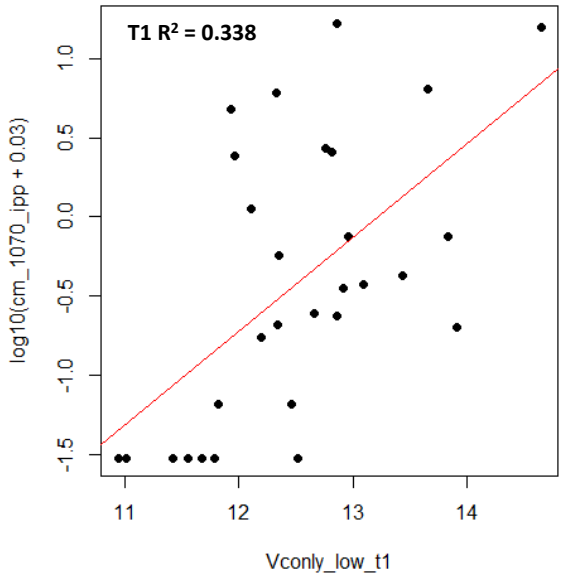


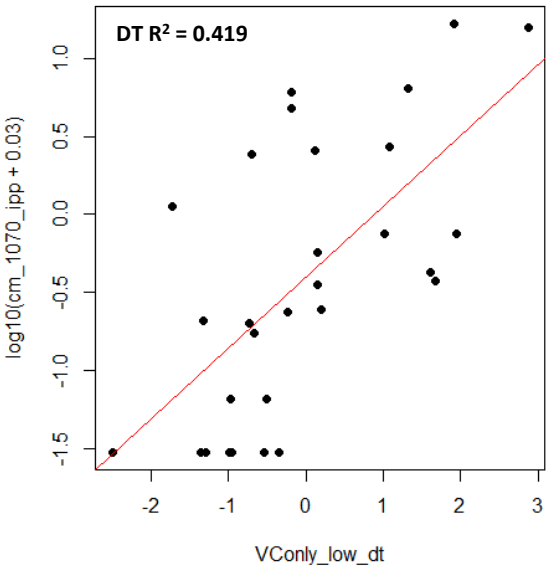
1070 m – VCSN for missing temperatures



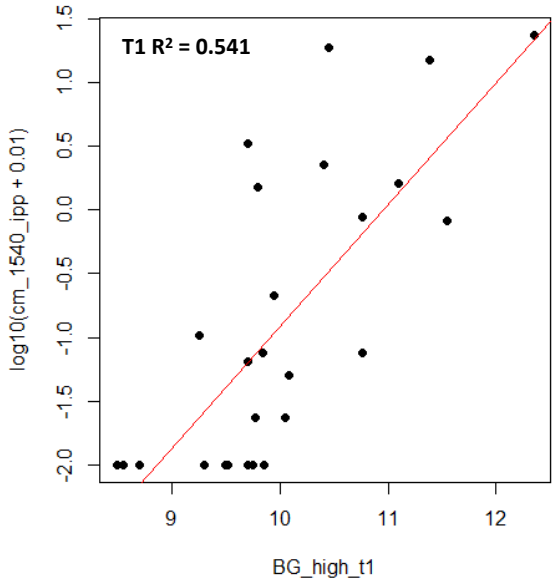


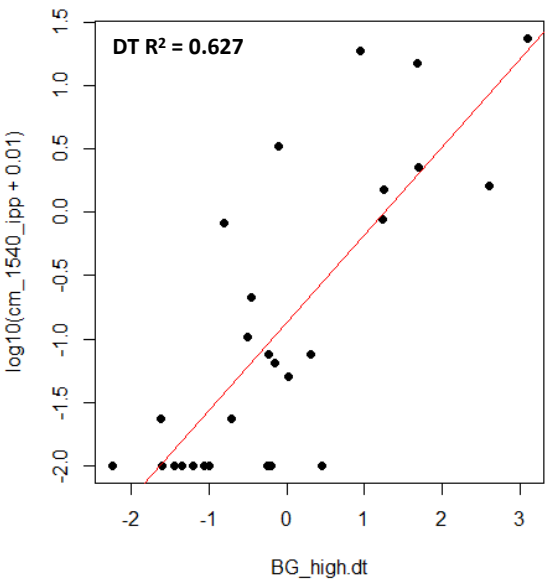
1070 m – VCSN for all temperatures



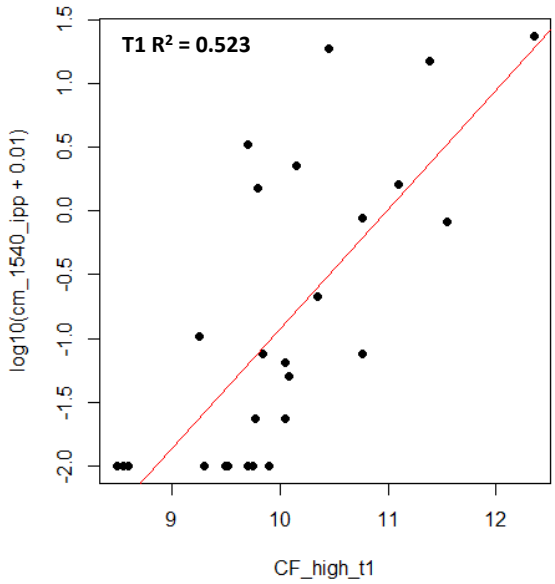


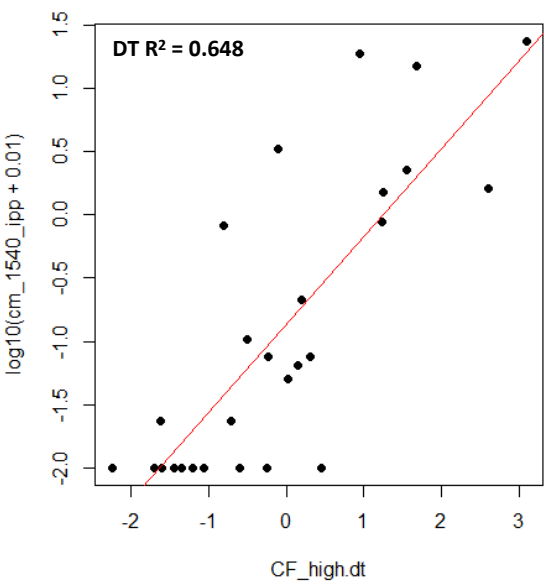
1540 m – CliFlo Botanic Gardens for missing temperatures



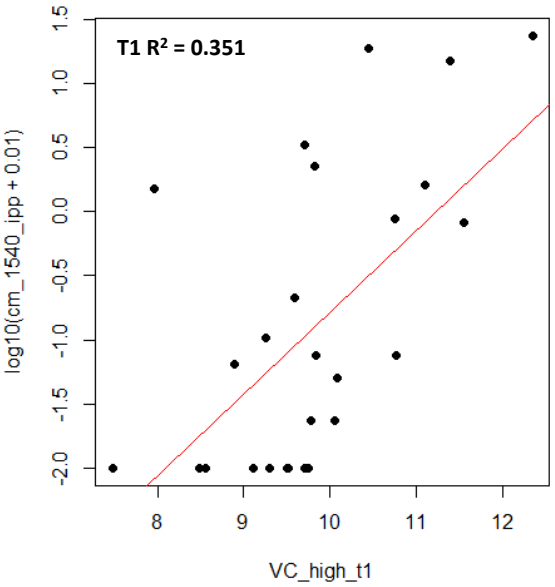


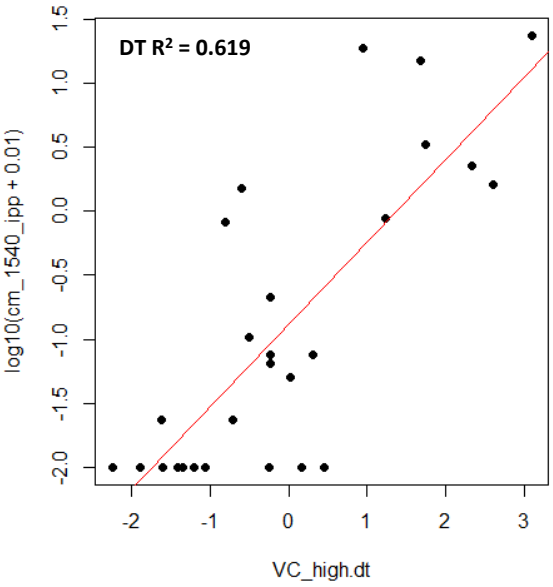
1540 m – CliFlo Darfield for missing temperatures



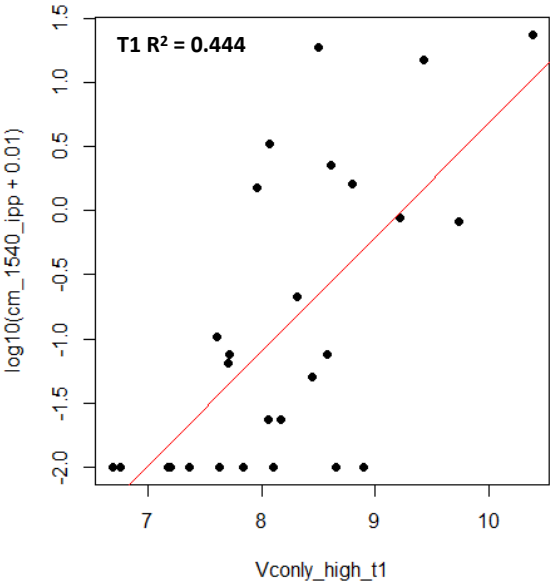


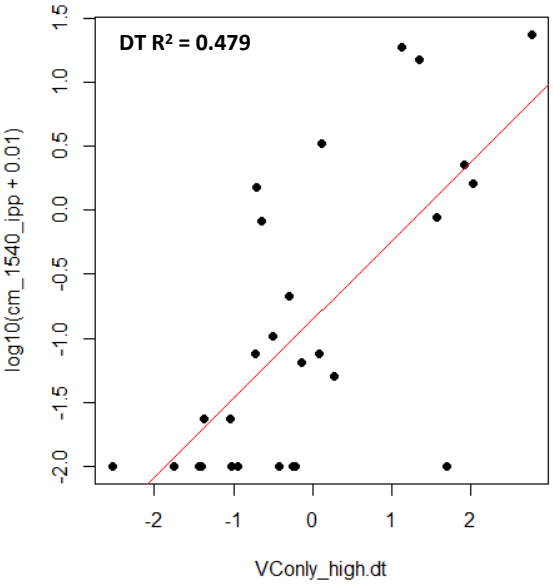
1540 m – VCSN for missing temperatures





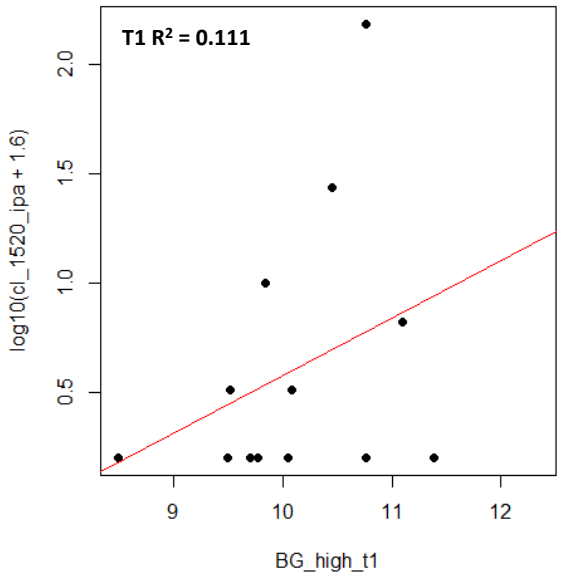
1540 m – VCSN for all temperatures

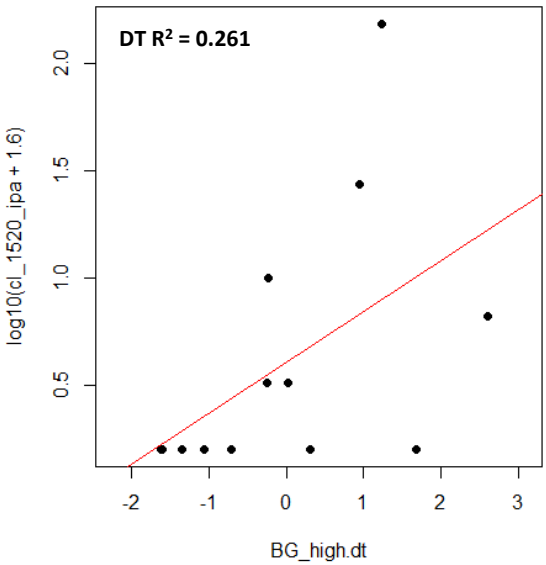




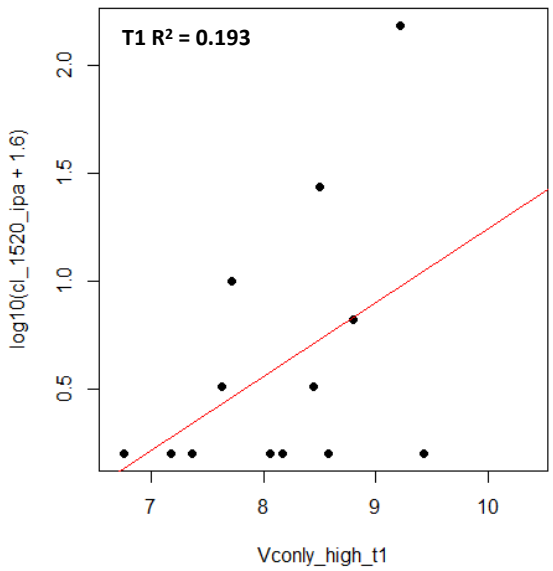
Celmisia lyallii

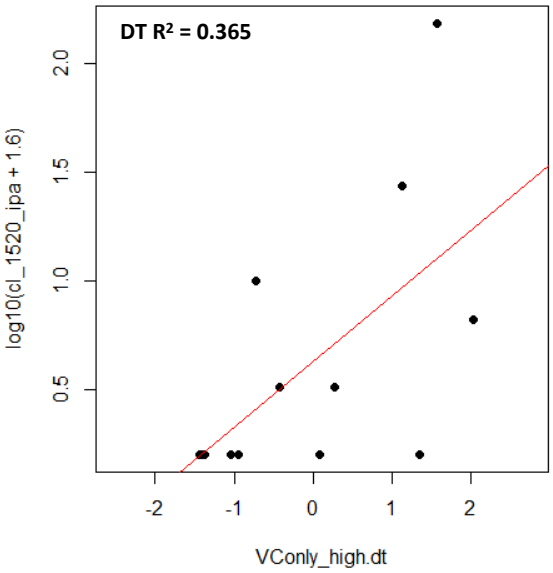
1520 m – On-site weather station as no missing temperatures



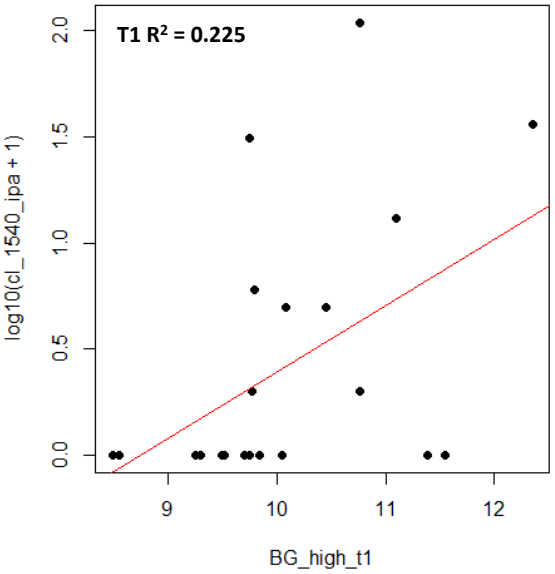


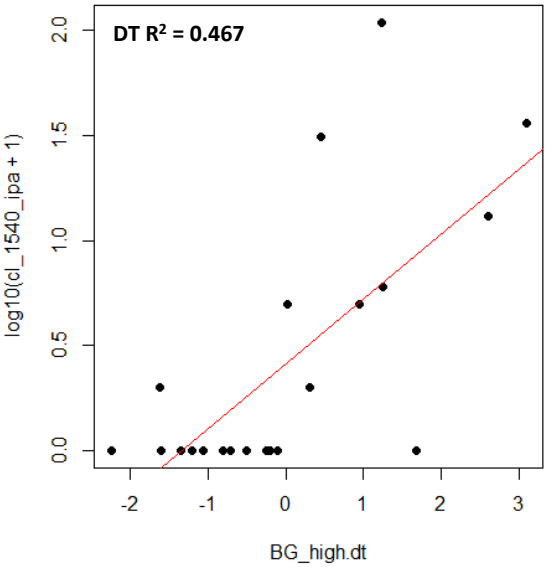
1520 m – VCSN for all tempertures



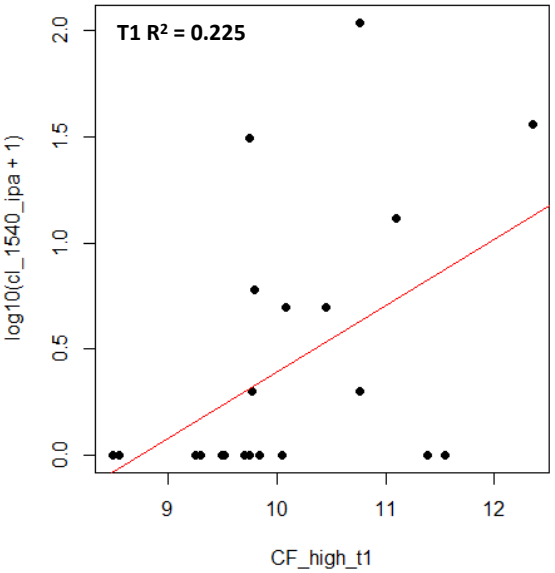


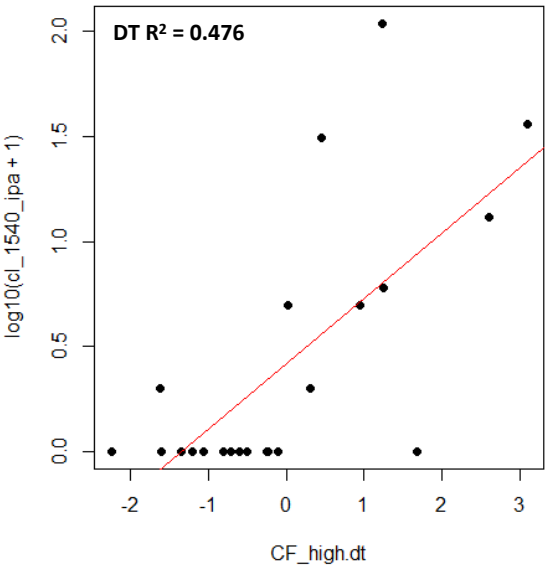
1540 m – CliFlo Botanic Gardens for missing temperatures



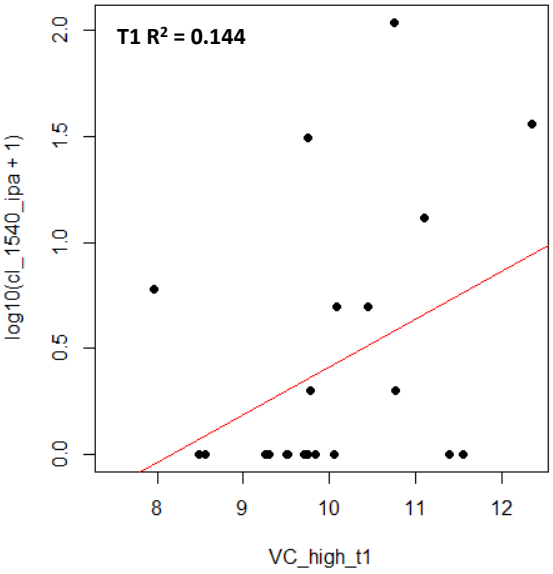


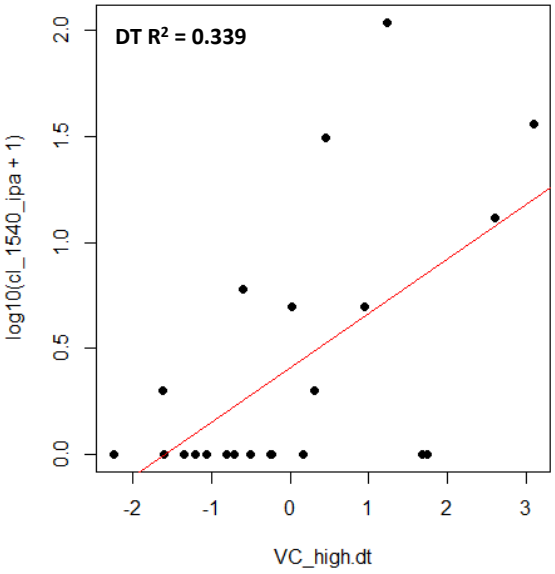
1540 m – CliFlo Darfield for missing temperatures



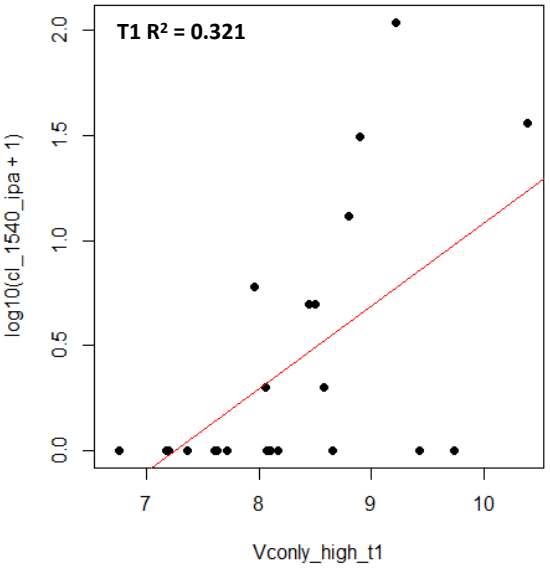


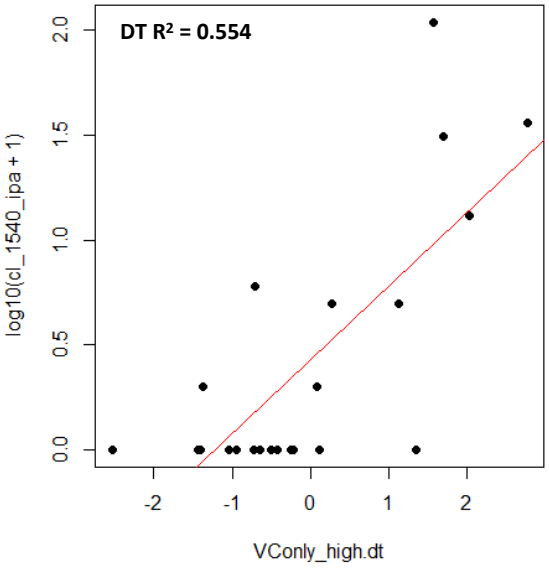
1540 m – VCSN for missing temperatures



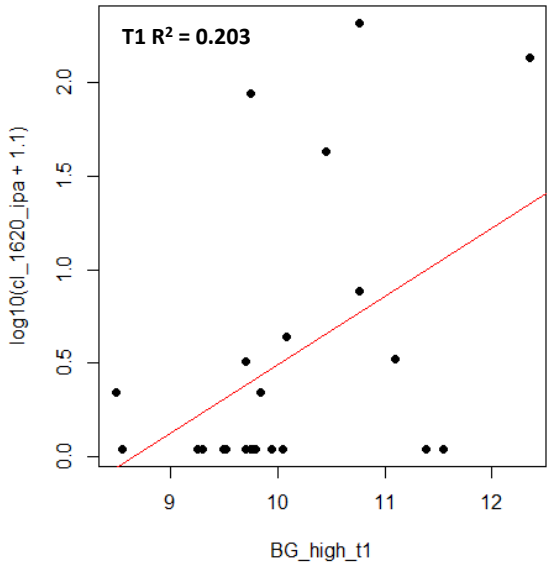


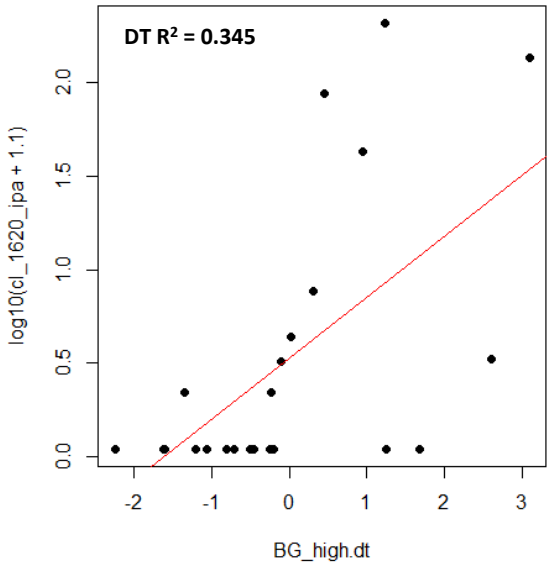
1540 m – VCSN for all temperatures



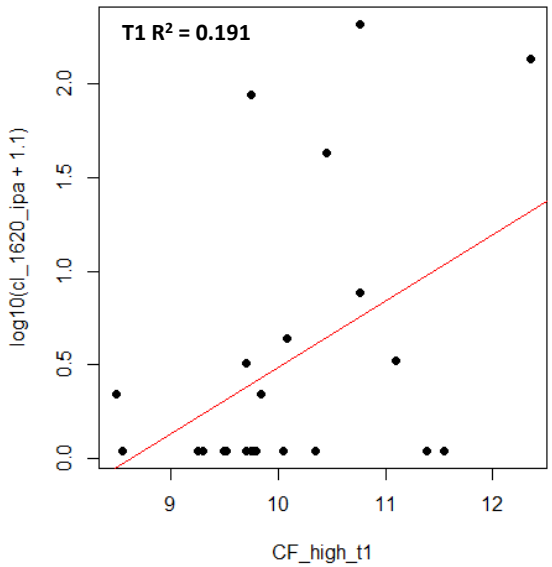


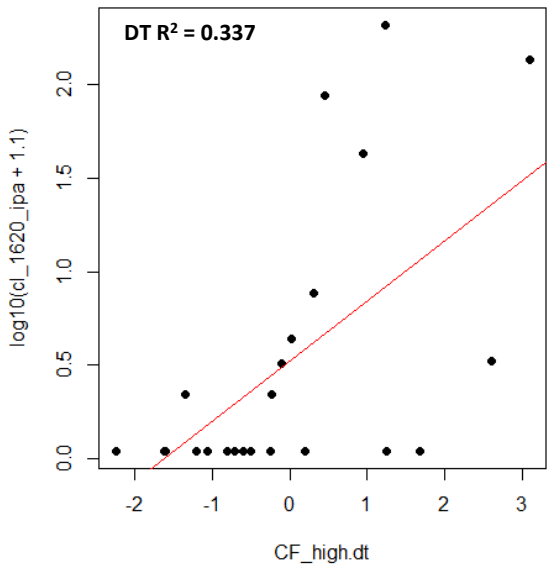
1620 m – CliFlo Botanic Gardens for missing temperatures



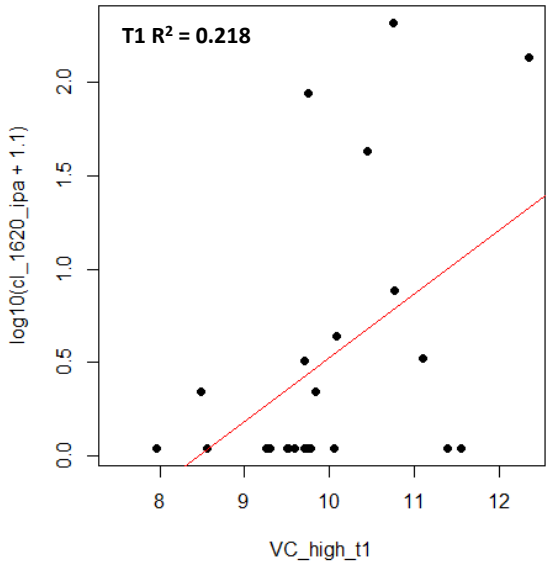


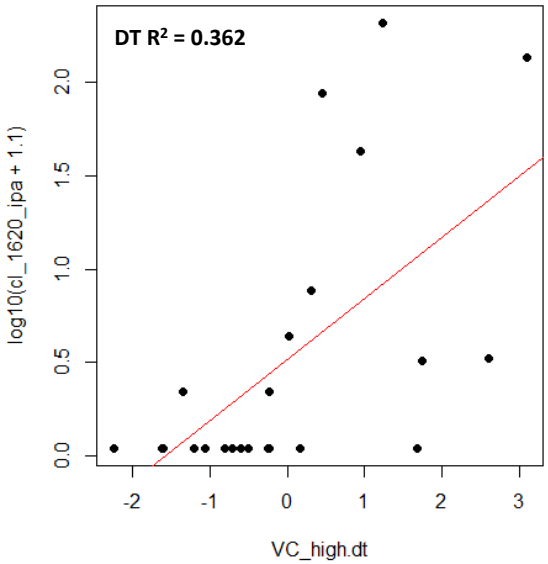
1620 m – CliFlo Darfield for missing temperatures



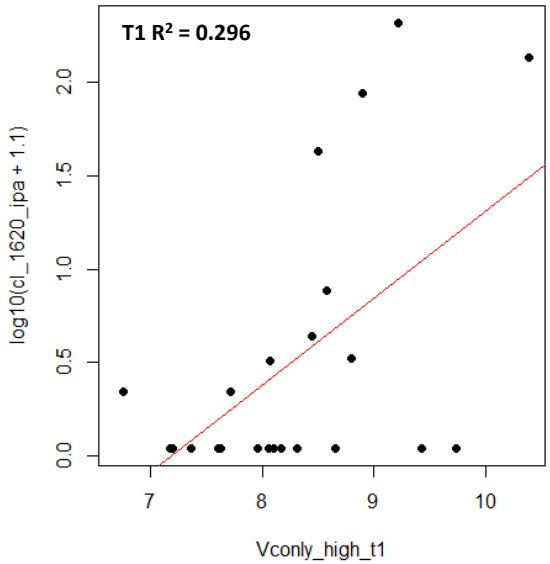


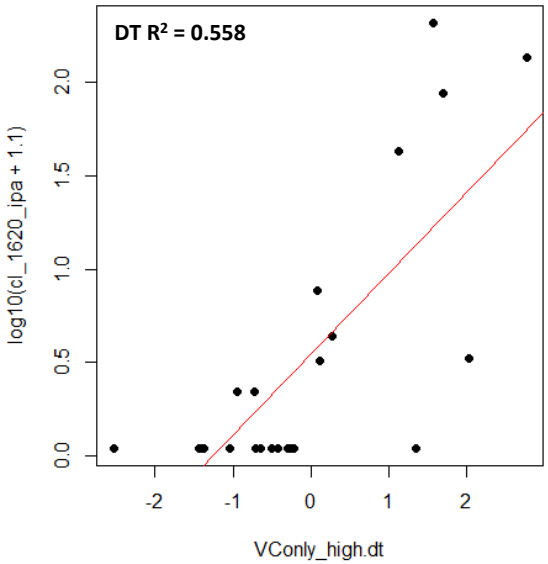
1620 m – VCSN for missing temperatures





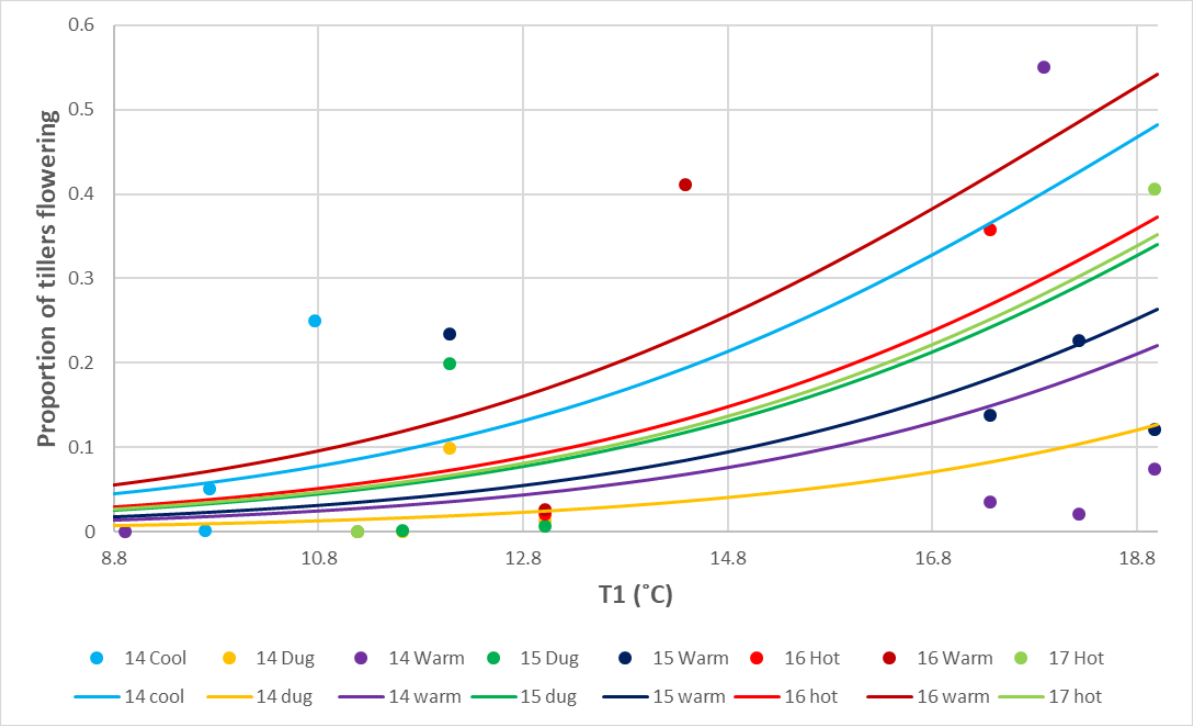
1620 m – VCSN for all temperatures



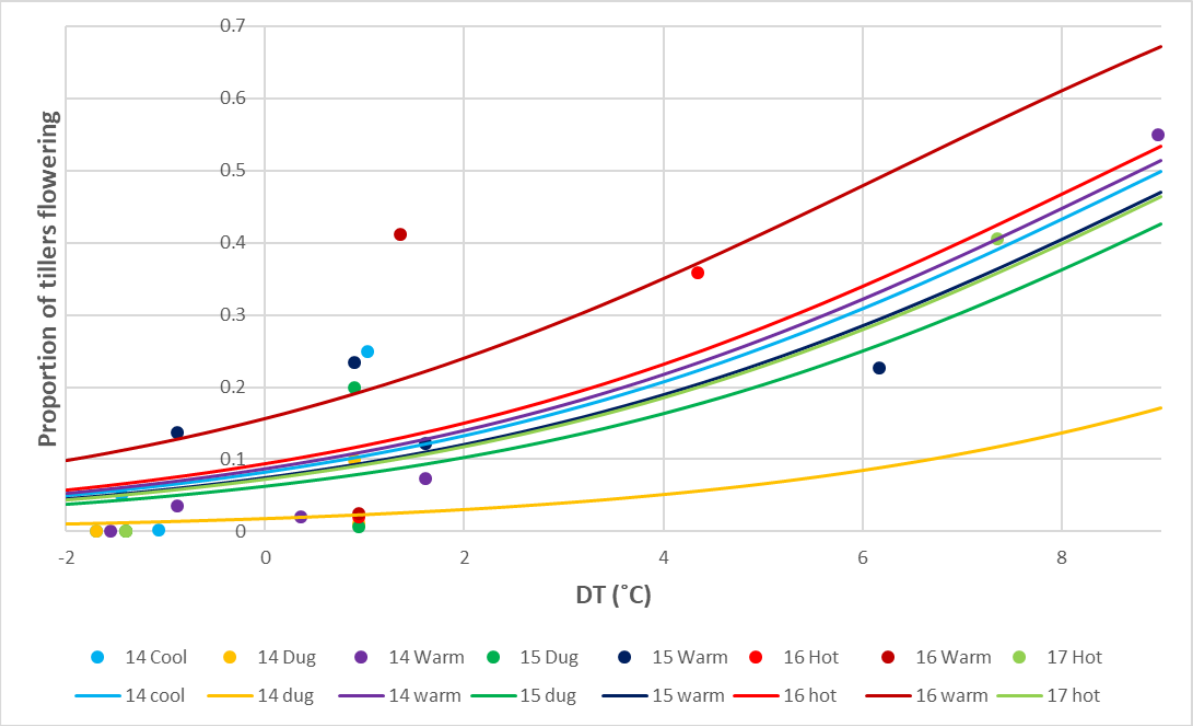


Appendix C: Relationship between the proportion of flowering tillers in *Chionochloa pallens* transplants and the T1 and DT models, showing back-transformed predicted fitted mean trend lines for each group of plants.

T1 model



DT model



Note: coloured dots are actual data points. Back-transformed fitted mean trend lines are predicted from low sample numbers for each group where some only include two flowering seasons.