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Phenological responses to climate in the Alberta native flora: Herbarium specimens reveal differential responsiveness between species in mesic and xeric habitats

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by

Cassiano Porto

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN BIOLOGICAL SCIENCES

CALGARY, ALBERTA

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Abstract

The field of phenology focuses on investigating the timing of biological events and phases of life cycles. The selection pressures involving phenological events are now rapidly changing in the face of projected and ongoing climate change and there is a growing interest in detecting the effects of climate change on plant phenology. The present research focuses on using herbarium specimens to examine whether 14 native plant species in Alberta have adjusted to changes in temperature and precipitation over the past century. I hypothesize that the soil moisture to which plants are exposed might affect their phenological responses and investigate: (1) if herbaria collections contain evidence of phenological responses to climate in species of the Alberta flora, and (2) if the responses are dependent on the typical moisture regime of their habitat. For this purpose, plants from mesic and xeric habitats are compared in terms of their phenological responses to air temperature and precipitation. In this study, the taxonomic relationships between the species were taken into account, by selecting a total of 14 species representing seven different angiosperm orders (one pair of species for each order). For each selected species, the preserved specimens available at three major Albertan herbaria collections were included in the study, if showing more than 50% of flower buds in anthesis (defined as peak flowering condition). The peak flowering date of each specimen, combined with the average temperature (or total precipitation) of the species' mean flowering month and the three months prior, allowed the use of a regression model for the estimation of a species' phenological responsiveness. The linear multiple regression analysis has revealed a significant responsiveness to temperature for six species from xeric habitats and one species from mesic habitats. My results suggest that species from xeric habitats have an average phenological responsiveness to temperature significantly more pronounced than species from mesic habitats. This tendency might be explained by the thermal properties of mesic habitats. The results indicate that for some species there is a set of environmental cues, as opposed to only one parameter, that governs flowering burst timing. The elucidation of the ecological implications involving plants phenological responses is key for the development of effective ecosystem conservation programs in the context of global climate change.

Preface

The present research has originated from discussions with my supervisor, Dr. Jana Vamosi, concerning a relatively recent method of studying plant phenology by using herbarium specimens. In its first days, these discussions were barely more than a curiosity largely inspired by Dr. Calinger's article published in a 2013 volume of *Ecology Letters*. Having access to a large collection in the University of Calgary's Herbarium, the idea of applying the method used by Dr. Calinger in the context of Alberta was a natural consequence of this initial curiosity. From these discussions, also was born the idea of comparing mesic and xeric species in terms of their phenological responses to climate. As soon as we started to consider more seriously this project, we encountered many questions concerning climate and the treatment of species distributions. Eventually, we realized that we would greatly benefit from having a strong support from a professor of the University's Geography Department. That's how Dr. David Goldblum has joined the team as my co-supervisor. This thesis is original, unpublished, independent work by the author, C. Porto.

Acknowledgements

Thanks to my supervisor Dr. Jana Vamosi and my co-supervisor Dr. David Goldblum for all the support, insights and persistence on this exciting (and often challenging) journey between the worlds of botany, climatology, geography and ecology. Thanks to Ms. Bonnie Smith, our herbarium technician and Alberta Flora oracle that has equally contributed to the existence of this thesis, by sharing her knowledge. Thanks to members of my supervisory committee, Dr. Jessica Theodor and Dr. Samuel Yeaman for their intellectual contributions.

I dedicate this work to my Love and eternal partner, Karina Oliveira

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“Spring is sooner recognized by plants than by men”

- Chinese proverb

Chapter 1 – Introduction

1.1 Phenological Responses to a Changing Climate

Phenology is defined as the study of the timing of recurring biological events (Lieth 1974). Living organisms have adapted to respond to environmental cues to start and end various life stages or reproductive phases (Cleland et al. 2007) in order to maximize their access to resources or mates, while avoiding exposure to unfavorable conditions.

The difference between the usual rates of change in the seasonal cycles and the generation timescale of organisms results in a landscape where the selection pressures have acted under a fairly regular and reliable “calendar” for many species (Davies et al. 2013). These selection pressures are now rapidly changing in the face of projected and ongoing rates of climate change. Because of these adaptations, it is widely acknowledged that many species will be negatively affected by climate change, yet our ability to predict which ones will be most affected and why is still very incomplete.

The succession of the seasons is often detected in the form of shifts in physical environmental parameters, like temperature, photoperiod and moisture availability (Davies et al. 2013). These parameter shifts need to be consistently effective as indicators of the passage of the seasons for the species that rely on them to adapt their life stages to the seasonal cycles. Many types of organisms, from marine zooplankton (Aberle et al. 2012) to grasses (Primack and Gallinat 2017), have adapted to respond to changes in its surrounding’s temperature or changes in the photoperiod as indicators of the passing seasons. Other environmental cues are also sometimes used for phenological

adjustments, as water availability (dry/rainy seasons) or even lunar cycles (Grant et al. 2009).

The recent alteration of climate patterns caused by a global warming processes has produced observable effects on many species that rely on physical environmental parameters (e.g. temperature) as indicators of seasonal changes (Parmesan and Yohe 2003). There is an emerging interest in the inherent value of phenology in providing insights into the impacts of climate change (Sparks and Carey 1995, IPCC 2001, Root et al. 2003, Parmesan and Yohe 2003, Parmesan 2007). This interest is in part because phenology datasets can be used as evidence for climate change, independent from instrumental weather station data (IPCC 2007). Additionally, as pointed by Beaubien (2013), phenology studies have a number of practical applications in fields like agriculture (e.g., to predict timing of seeding, pest control, and harvest), human health (e.g., to predict shifts in pollen seasons and allergies) and biodiversity conservation (e.g., to understand how the timing of rare plant flowering synchronizes with pollinators of open flowers).

Many recent studies focus on detecting climate change effects on plant phenology, particularly on flowering and budburst timing (Doi and Katano 2008). While studying *Solidago rugosa* Mill. phenology in North America, Miller-Rushing & Primack (2008) have identified that warmer average seasonal temperatures have produced flowering peaks that are ~ 11 days $^{\circ}\text{C}^{-1}$ earlier. Nevertheless, phenological responses to climate change can be highly variable even among species from the same genus (Calinger et al. 2013). Fitter and Fitter (2002) have identified that, in the United Kingdom, *Geranium rotundifolium* L. showed a 6 day delay in its first flowering date (FFD) between 1954 and 2000, while *Geranium pyrenaicum* Burm. F. advanced its FFD 3 days in the same period.

Because these two species of *Geranium* share the same distribution range in the UK, these results indicate that the phenological responses might vary due to allele or gene expression differences, even for closely related species.

The consequences of plant phenological responses to climate change may also be variable. Kudo et al. (2008) have identified that phenologically flexible species (i.e., species exhibiting pronounced phenological responses or sensitivity to changes in environmental cues) may benefit from greater early season productivity, which would produce an increased abundance of these particular species. Cleland et al (2012) suggests that phenological response to spatial variation in climate may correspond to the ability of a species to adapt to climate change, and greater sensitivities to climate have been associated with better species performance in the context of climate change. For example, earlier flowering species could be favored in temperate regions, where the growing season is limited to the warmer months of the year (Mungia-Rosas et al. 2011). On the other hand, earlier flowering may also have deleterious effects over phenologically flexible species. Having an earlier budburst can increase the risk of frost exposure (Inouye 2008) which, in turn, may lead to decreased local species abundance (Calinger et al. 2013).

Often, responses to climate change may lead to adverse community-level effects. By favouring some species, others might be facing competitive disadvantages. In some cases, pollinator mismatch may occur when the flowers become receptive before the emergence of pollinators (Kudo et al. 2008). This situation has the potential of adversely affecting both the fitness of plants and pollinators, with potential impacts to crops (Giannini et al. 2017). Additionally, it has been suggested that invasive plants may be

taking advantage of early warm temperatures, due to having a greater phenological flexibility when compared to native plant populations (Wolkovich and Cleland 2011), increasing the chance of biodiversity losses.

1.2 Different approaches in phenology studies

There are different approaches used in the investigation of plant phenological responses to climate. Some research has explored the problem with an experimental approach, by manipulating the environmental conditions to which plants are exposed. This approach, however, might underpredict advances in the timing of flowering and leafing by 8.5-fold and 4.0-fold, respectively, compared with long-term observations (Wolkovich *et al.* 2012). Long-term observations usually refer to using historical records of direct observation, which often comprise decades of data. The efforts in creating annual records of budburst and flowering time for selected species along long periods is, in many occasions, supported by citizen-science programs as the *Alberta PlantWatch*. This program relies on citizen reports concerning when certain plants bloom and leaf out in spring (Nature Alberta 2018).

There is, however, a scarce amount of data supplied by direct observation, which typically is limited in the number of species included, the timespan and the geographic range. This lack of data has led to information gaps on species' and functional groups' responses to climate (Calinger *et al.* 2013), which is an obstacle in our ability to identify and predict community-level patterns of responses to climate change. Therefore, there is a demand for methods in phenology investigation that can make use of different sources

of data, as an alternative to direct observation only, and increase the available information on species and functional groups.

In this thesis, I used herbarium specimens to exam whether 14 native plant species in Alberta have adjusted or adapted to changes in temperature and precipitation based on the records obtained in the past century. Rather than assessing a possible shift in the flowering peak date for different species through the century, the present research focus on determining the phenological responses of plants to fluctuations on climate parameters (temperature and precipitation) or location (latitude). As detailed in the following two chapters, it is important to account for taxonomical relationships and ecological context when using this approach.

Chapter 2 – Using Herbarium Specimens in the Investigation of Phenological Responses to Climate in Alberta

2.1 Introduction

The present investigation makes use of herbaria collections in order to determine potential phenological responses to climate in the Alberta flora. Using herbaria collection specimens to access historical phenological changes is a relatively recent method (Primack et al. 2004; Lavoie & Lachance 2006; Miller-Rushing et al. 2006; Robbirt et al. 2011; Panchen et al. 2012). The herbarium-based method greatly expands the amount of historical data available for long-term phenology shifts studies, when compared to the availability of direct observation records. The method has, however, its own limitations, largely stemming from the fact that usually herbarium-preserved specimens do not represent a spatially randomized sample (many specimens are collected from roadsides). Nevertheless, in order to address this type of potential issue, Robbirt et al. (2011) have tested and validated this herbarium-based method, finding a significant correlation between the phenological advancement in flowering date, as indicated by herbaria collection, and direct observation surveys.

Another limitation associated with previous herbarium-based phenological studies is that they usually require intensive historical sampling of a single study site as they do not allow for spatial variation in the temperatures paired with individual herbarium specimens, a level of sampling that is not characteristic of most locations (Calinger et al. 2013). This drawback is addressed in this study with the methods developed by Calinger et al. (2013), by associating the specimen's date of collection with spatially interpolated historical

weather data. This approach gives the ability to look for patterns of phenological responses to climate parameters, even when observed across individuals that are spatially and temporally separated.

As already evidenced by some researchers (Fitter and Fitter 2002, Miller-Rushing and Primack 2008, Calinger et al. 2013) different plant functional groups may have distinct phenological responses to climate changes. For example, Calinger et al. (2013) revealed significantly different phenological responses between between wind- and biotically pollinated species. Pronounced phenological responses to temperature change in wind-pollinated species is likely a consequence of the high benefit of releasing pollen before trees leaf out in the spring, allowing greater pollen dispersal through the leafless canopy (Rathcke & Lacey 1985; Whitehead 1969). Also, early flowering species tend to show stronger phenological responses to temperature than early- and late-summer flowering species (Calinger et al. 2013). This phenomenon is perhaps a consequence of the increased light availability, and energy accumulation opportunity, presented before canopy closure in the early season (Kudo et al. 2008). The less noticeable responses of early- and late-summer flowering species to temperature may suggest that these species respond more strongly to other environmental factors such as precipitation (Calinger et al. 2013).

I hypothesize that the soil moisture that characterizes the habitat of a given plant species might affect their phenological responses. A greater soil water content represents a higher soil heat capacity, requiring more heat accumulation during the spring and summer for increasing its temperature. In order to induce flowering, some plants respond to changes in its internal temperature, which might be more correlated with soil and root-

zone temperature than to air temperature (Greer et al. 2005). Therefore, plants from dry habitats might exhibit a higher phenological flexibility to changing air temperatures when compared to species occupying soils with greater water content, because the latter occupy habitats with higher thermal inertia.

In summary, the present research investigates (1) if herbaria collections contain evidence of phenological responses to climate in species of the Alberta flora, and (2) if the responses are dependent on the typical moisture regime of their habitat. For this purpose, plants from mesic and xeric habitats are compared in terms of their phenological responses to air temperature and precipitation (see Methods for the definition used for “mesic and xeric habitats”). In order to assess a possible pattern on the north-south axis of distribution in the province, the latitude of collection for each specimen was also considered in the model. Latitude was also used as a proxy for measuring possible responses to photoperiod in the selected species, as photoperiodic cues in a locality are essentially invariant from year to year (Lechowicz, 2002).

2.2 Methods

In order to achieve a representative temporal sample for statistical analysis, frequently and consistently collected vascular plant species native to Alberta (Canada) were selected for the study. A preliminary assessment of the number of specimens per species available in the herbaria collections revealed appropriate candidate species for this study.

Davies et al. (2013) have demonstrated the importance of considering evolutionary and taxonomical relationships in phylogeny studies by evaluating evidence for phylogenetic conservatism – the tendency for closely related species to share similar ecological and biological attributes – in phenological traits across flowering plants. In this study, the taxonomical relationships between the species were taken into account, by selecting a total of 14 species representing seven different orders (one pair of species for each order).

Within each order two species were selected: the first usually found in dry habitats (here referred to as “species from xeric habitats”) and the second commonly present in environments with higher moisture availability (here referred to as “species from mesic habitats”) as described for each species by Moss (2012) (Appendix – Table A).

In order to avoid terrains with potentially extreme temperature variation over small spatial scales (e.g. due to elevation or slope orientation), the specimens collected in the subalpine and alpine natural subregions (Natural Regions Committee 2006) were excluded from this study (Figure 1). Based on these criteria for species selection (being frequently and consistently represented in herbaria collections, representing a pair of species from mesic and xeric habitats for each order, and being collected in Alberta

excluding alpine and subalpine natural subregions) the species included in this study are listed in Table 1.

Table 1: Species included in the present study. Each order is represented by a pair of mesic/xeric-habitat species.

Order	Mesic-Habitat Species	n	Xeric-Habitat Species	n
Asparagales	<i>Sisyrinchium montanum</i> Greene	23	<i>Allium textile</i> Nelson & Macbride	37
Liliales	<i>Prosartes trachycarpa</i> S. Watson	24	<i>Lilium philadelphicum</i> L.	27
Poales	<i>Calamagrostis canadensis</i> (Michx.) P.Beauv.	20	<i>Koeleria macrantha</i> (Ledeb.) Schultes	31
Ranunculales	<i>Thalictrum venulosum</i> Trel.	32	<i>Pulsatilla patens</i> (L.) Mill.	31
Fabales	<i>Astragalus americanus</i> (W.J. Hooker) M.E. Jones	24	<i>Thermopsis rhombifolia</i> (Nutt.) Richards	44
Rosales	<i>Rosa acicularis</i> Lindl.	27	<i>Rosa arkansana</i> Porter	20
Asterales	<i>Petasites frigidus</i> (L.) Fr.	23	<i>Achillea millefolium</i> L.	67

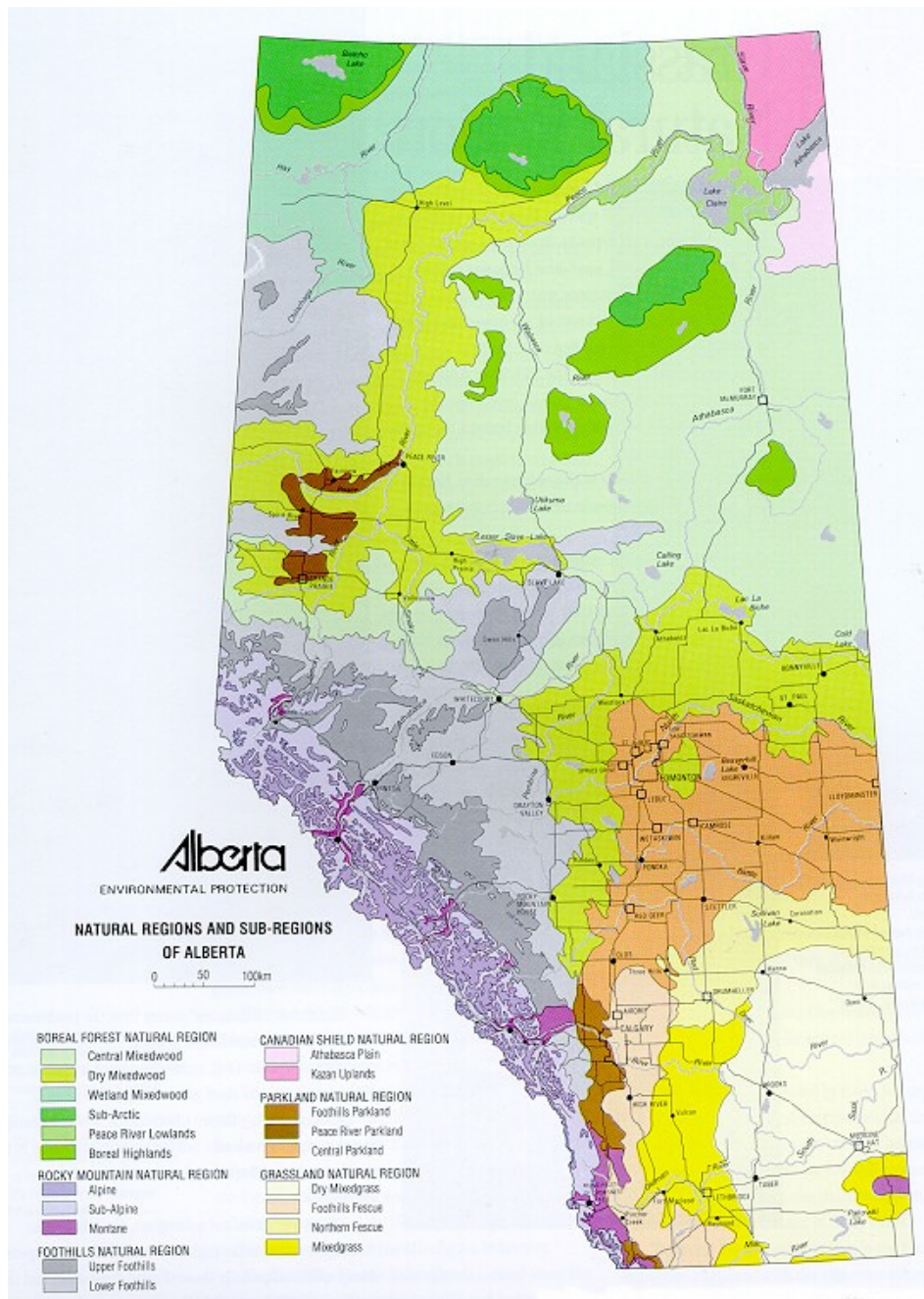


Figure 1: Natural regions and sub-regions of Alberta. The alpine and subalpine sub-regions were not included in this study (source: <http://www.uleth.ca/vft/cypresshills/map1.html>).

For each selected species, the preserved specimens available at the University of Calgary, University of Alberta and University of Lethbridge herbaria collections with more than 50% of flower buds in anthesis were included in the study (Figure 2). These

specimens were considered to be in peak flowering, as adopted by Calinger et al. (2013), and the location and date of collection was recorded. For every species, the specimens within the 1% latest peak flowering dates were excluded from the study in order to minimize the effects of a possible second flowering cycle in the same year. A total of 430 specimens were included in the study by observing the species and specimen selection criteria.



Figure 2: The anthesis phases can be evident in preserved specimens from certain species as *Rosa acicularis* Lindl. (top row). In other species, as in *Koeleria macrantha* (Ledeb.) Schultes (bottom row), the use of optical magnification is often necessary for the identification of their flowering cycle phase. The pre-anthesis is shown in pictures **a** and **d**. The anthesis phase (pictures **b** and **e**) is characterized by the exposure of the anthers (indicated by the arrows). The specimens shown in **b** and **e** are also considered in peak flower and they fit this criteria for being included in the study (because they show over 50% of the flower buds in anthesis). Pictures **c** and **f** represent specimens in a late phase (post-anthesis).

The historical climate data used for each specimen was obtained from geographically interpolated data using ClimateNA (Hamman 2017), from which the information of local average monthly temperatures and total monthly precipitation for the year of collection

was approximated. Besides year and month, the interpolated climate data takes into account latitude, longitude and elevation. Given that the ClimateNA data currently spans from 1901 to 2013, the present study focused on the specimens collected in this window of time.

The peak flowering date of each specimen, combined with the average temperature of the species' mean flowering month and the three months prior, allowed the use of a regression model for the estimation of a species' phenological responsiveness, using the combined observations for all the specimens included for a given species, as defined by Calinger et al. (2013):

$$D_{xi} = m_x(\bar{T}_{4i}) + b_x$$

Where:

D_{xi} is the flowering date of specimen i in species x (day of the year),

\bar{T}_{4i} is the average temperature of species' x mean flowering peak month and the three months prior, for the date and location of the specimen i collection ($^{\circ}\text{C}$),

m_x is the phenological responsiveness to temperature ($\text{day } ^{\circ}\text{C}^{-1}$)

Using a similar model, the precipitation data was regressed against the peak flowering date.

$$D_{xi} = m_x(\bar{P}_{4i}) + a_x$$

Where:

D_{xi} is the flowering date of specimen i in species x (day of the year),

P_{4i} is the average of the total precipitation of species' x mean flowering peak month and the three months prior, for the date and location of the specimen's i collection (mm),

m_x is the phenological responsiveness to precipitation (day °mm⁻¹)

The influence of the photoperiod duration over the peak flowering date was indirectly investigated by using the specimen latitude as a proxy. Between spring and fall equinoxes, higher latitudes represent longer periods of sunlight. A linear model was tested:

$$D_{xi} = m_x L + c_x$$

Where:

D_{xi} is the flowering date of specimen i in species x (day of the year),

L is the latitude of the specimen's i collection site (degrees),

m_x is the phenological responsiveness to latitude (day degrees⁻¹)

The temperature, precipitation and latitude parameters were tested via multiple regression in R, observing its assumptions of independence, normality, homoscedasticity, linearity and non-colinearity. A taxonomic sister-group test was conducted in R (R Core Team 2013) and a meta-analysis was run in OpenMEE (Wallace *et al.* 2012) to test possible differences in phenological responsiveness between mesic-habitat and xeric-habitat species.

2.3 Results

The average values of peak flowering date, T4i, P4i and latitude across all species included in the study is presented in Table B (Appendix 1). The multiple regression analysis has revealed a significant responsiveness to temperature for six species from xeric habitats (*Lilium philadelphicum* L., *Rosa arkansana* Porter, *Thermopsis rhombifolia* (Nutt.) Richards, *Koeleria macrantha* (Ledeb.) Schultes, *Achillea millefolium* L. and *Allium textile* Nelson & Macbride) and one species from mesic habitats (*Prosartes trachycarpa* S. Watson) (linear multiple regression, d.f.*L. philadelphicum*=23, d.f.*R. arkansana*=16, d.f.*T. rhombifolia*=40, d.f.*K. macrantha*=27, d.f.*A. millefolium*=63, d.f.*A. textile*=33, d.f.*P. trachycarpa*=20, $p < 0.05$). The species exhibiting significant phenological responsiveness to temperature show a negative slope relationship between flowering peak date and average temperatures. This means that for these seven species, either from mesic or xeric habitats, the flowering peak date is advanced with an increase in temperature (Table 2). On average, the budburst advanced 3.8 ± 0.8 days $^{\circ}\text{C}^{-1}$ across all studied species in Alberta between 1914 and 2013.

Three mesic-habitat species (*Rosa acicularis* Lindl., *Thalictrum venulosum* Trel. and *Astragalus americanus* (W.J. Hooker) M.E. Jones) have shown significant responsiveness to precipitation (linear multiple regression, d.f.*R. acicularis*=23, d.f.*T. venulosum*=28, d.f.*A. americanus*=20, $p < 0.05$). On two of these cases (*Rosa acicularis* Lindl. and *Astragalus americanus* (W.J. Hooker) M.E. Jones), an increase in average monthly total precipitation preceding the species' average peak flowering date leads to a delayed flowering timing. For one species, *Thalictrum venulosum* Trel., the opposite pattern was

detected, meaning that an increase in precipitation induces earlier flowering in this species (Table 2 and Figure 10). None of the xeric-habitat species responded to precipitation ($p>0.05$). Two species from mesic habitats (*Thalictrum venulosum* Trel. and *Calamagrostis canadensis* (Michx.) P.Beauv.) have demonstrated significant responsiveness to latitude (Table 2, Figures 10 and 6), producing earlier flowering peaks in higher latitudes (linear multiple regression, d.f.*T. venulosum*=28, d.f.*C. canadensis*=16, $p<0.05$).

Table 2: Phenological responsiveness (\pm SEM) to temperature, precipitation and latitude across all species considered in this study. The highlighted values represent statistically significant responsiveness (linear multiple regression, $p<0.05$).

Habitat	Species	d.f.	Temperature	Precipitation	Latitude
			Phenological responsiveness (day °C ⁻¹)	Phenological responsiveness (day mm ⁻¹)	Phenological responsiveness (day degree ⁻¹)
Mesic	<i>Prosartes trachycarpa</i>	20	-3.23 (\pm 1.06)	0.21 (\pm 0.12)	-0.16 (\pm 1.00)
	<i>Rosa acicularis</i>	23	-1.90 (\pm 1.50)	0.31 (\pm 0.15)	0.09 (\pm 1.35)
	<i>Thalictrum venulosum</i>	28	-1.92 (\pm 0.98)	-0.44 (\pm 0.10)	-1.77 (\pm 0.82)
	<i>Astragalus americanus</i>	20	-0.35 (\pm 1.60)	0.29 (\pm 0.12)	-0.20 (\pm 0.70)
	<i>Calamagrostis canadensis</i>	16	-1.34 (\pm 2.57)	-0.14 (\pm 0.18)	-5.87 (\pm 1.30)
	<i>Petasites frigidus</i>	19	-2.56 (\pm 1.26)	-0.04 (\pm 0.17)	-1.29 (\pm 1.37)
	<i>Sisyrinchium montanum</i>	19	-2.62 (\pm 1.97)	0.24 (\pm 0.26)	1.37 (\pm 1.62)
Xeric	<i>Lilium philadelphicum</i>	23	-3.69 (\pm 1.31)	-0.10 (\pm 0.10)	-0.38 (\pm 0.83)
	<i>Rosa arkansana</i>	16	-7.75 (\pm 2.82)	0.31 (\pm 0.22)	-1.25 (\pm 1.72)
	<i>Pulsatilla patens</i>	27	-2.15 (\pm 2.49)	0.09 (\pm 0.24)	-4.56 (\pm 4.27)
	<i>Thermopsis rhombifolia</i>	40	-3.27 (\pm 1.16)	0.31 (\pm 0.20)	-3.57 (\pm 2.75)
	<i>Koeleria macrantha</i>	27	-6.99 (\pm 1.83)	0.01 (\pm 0.12)	1.10 (\pm 0.71)
	<i>Achillea millefolium</i>	63	-11.61 (\pm 3.22)	0.46 (\pm 0.31)	-4.12 (\pm 2.27)
	<i>Allium textile</i>	33	-3.17 (\pm 1.32)	0.14 (\pm 0.12)	3.83 (\pm 2.42)

One objective of this thesis was to ensure a wide sampling of angiosperm clades from the angiosperm phylogenetic tree. A brief description of the phenological responsiveness organized in terms of their placement in angiosperm evolutionary history (monocots, basal eudicots, rosids, asterids) is provided in the following narrative.

2.3.1 Monocots

Both mesic-habitat and xeric-habitat species from the Liliales order responded significantly only to temperature (Figures 3 and 4), by exhibiting earlier flowering peaks with an increase in temperature (linear multiple regression, d.f.*L. philadelphicum*=23, d.f.*P. trachycarpa*=20, $p < 0.05$).

The xeric-habitat grass species (Order Poales – Figures 5 and 6) included in this study, *Koeleria macrantha* (Ledeb.) Schultes, presented a significant phenological responsiveness to temperature, by having earlier flowering peaks with an increase in temperature (linear multiple regression, d.f.*K. macrantha*=27, $p < 0.05$). The same effect was not observed in *Calamagrostis canadensis* (Michx.) P.Beauv., which responded only by flowering significantly earlier with an increase in latitude (linear multiple regression, d.f.*C. canadensis*=16, $p < 0.05$).

In the Asparagales order, only the xeric-habitat species, *Allium textile* Nelson & Macbride, showed a significant phenological responsiveness to the parameters used in the regression (Figures 7 and 8). The species *Allium textile* Nelson & Macbride exhibited earlier flowering with an increase in temperature (linear multiple regression, d.f.*A. textile*=33, $p < 0.05$).

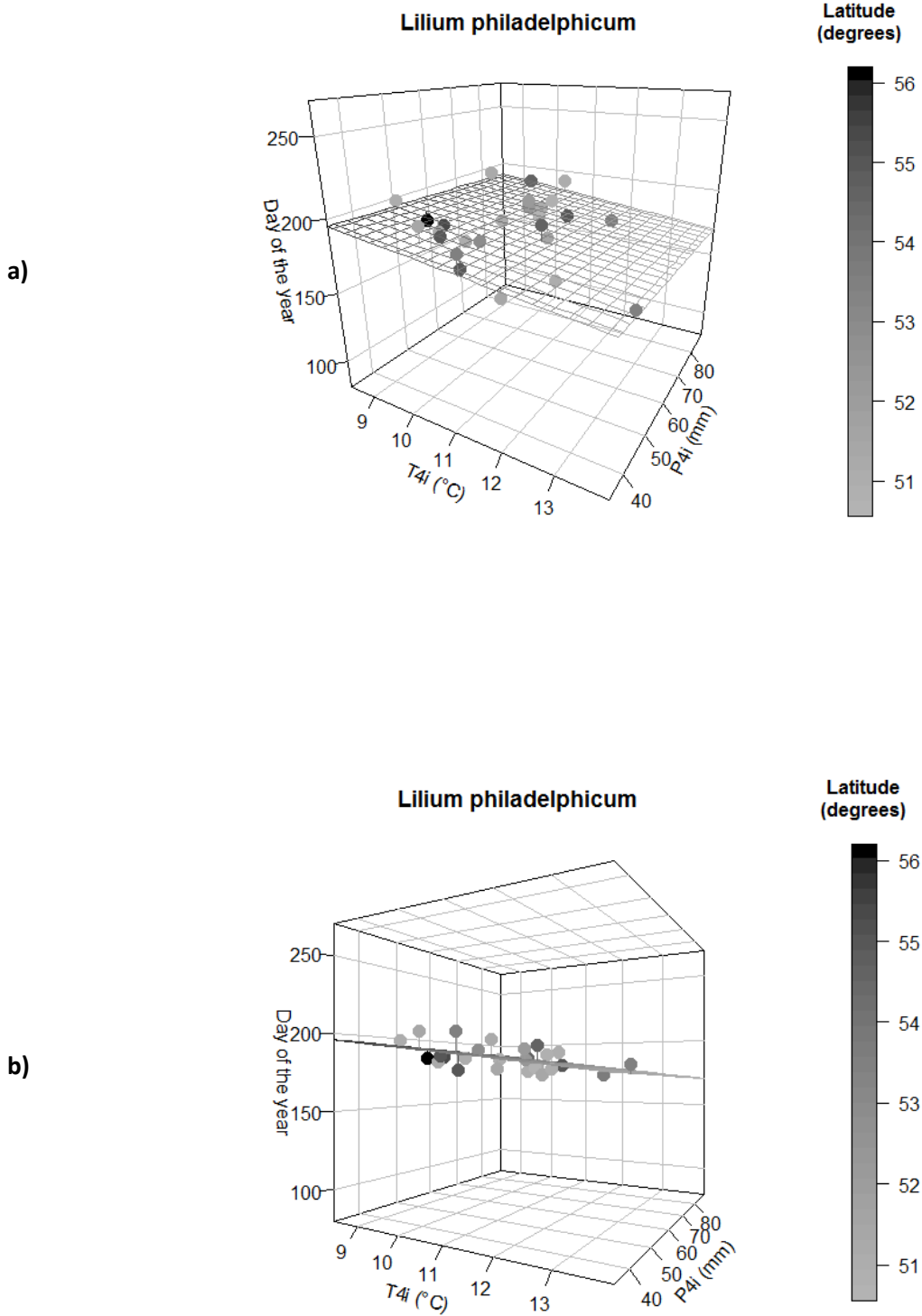


Figure 3: *Lilium philadelphicum* (Liliales - xeric) multiple regression visualisation showing significant responsiveness to temperature ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

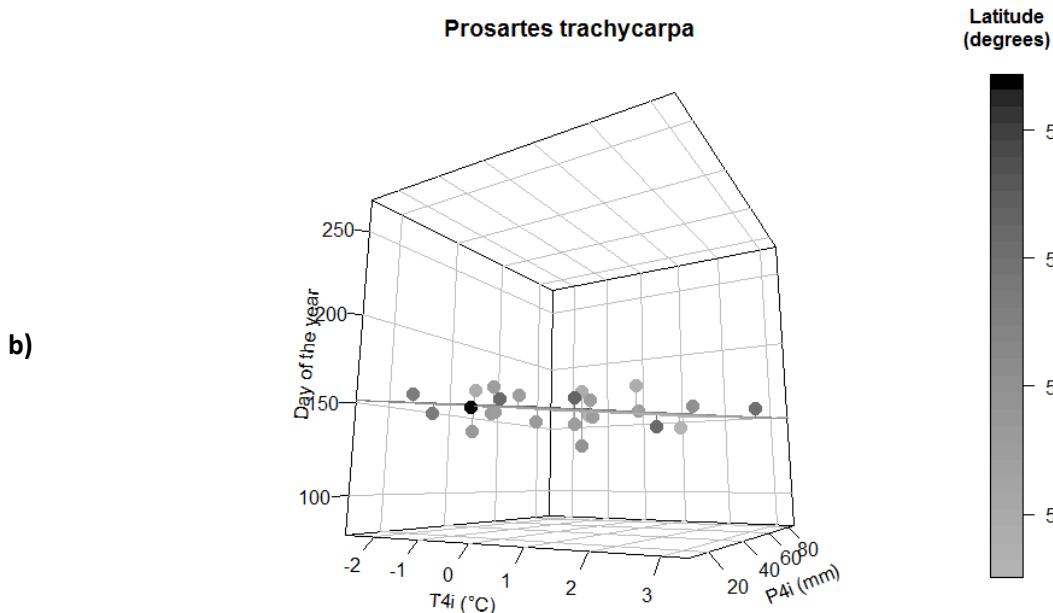
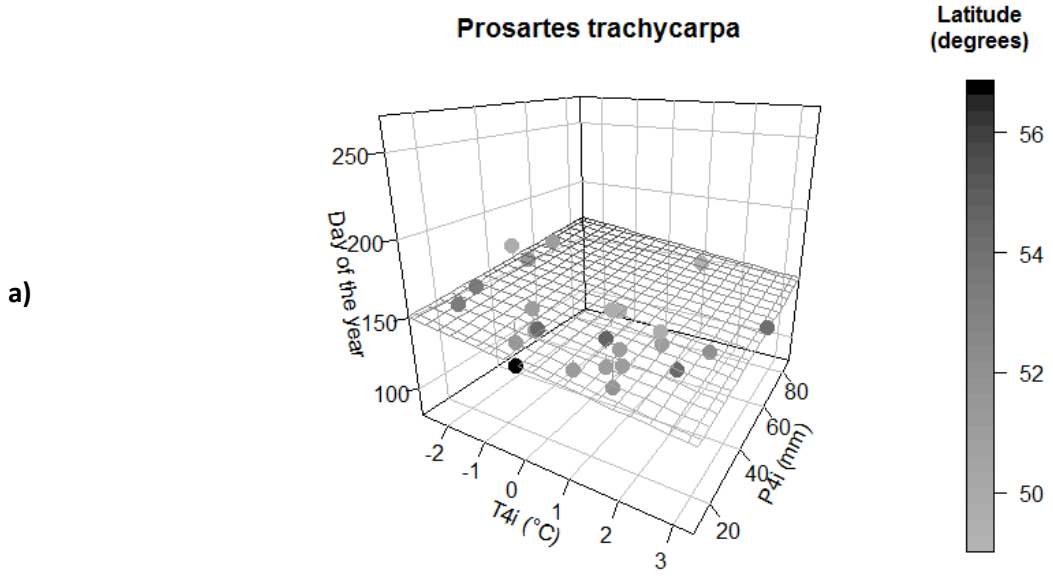


Figure 4: *Prosartes trachycarpa* (Liliales - mesic) multiple regression visualisation showing significant responsiveness to temperature ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T4i), precipitation (P4i) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

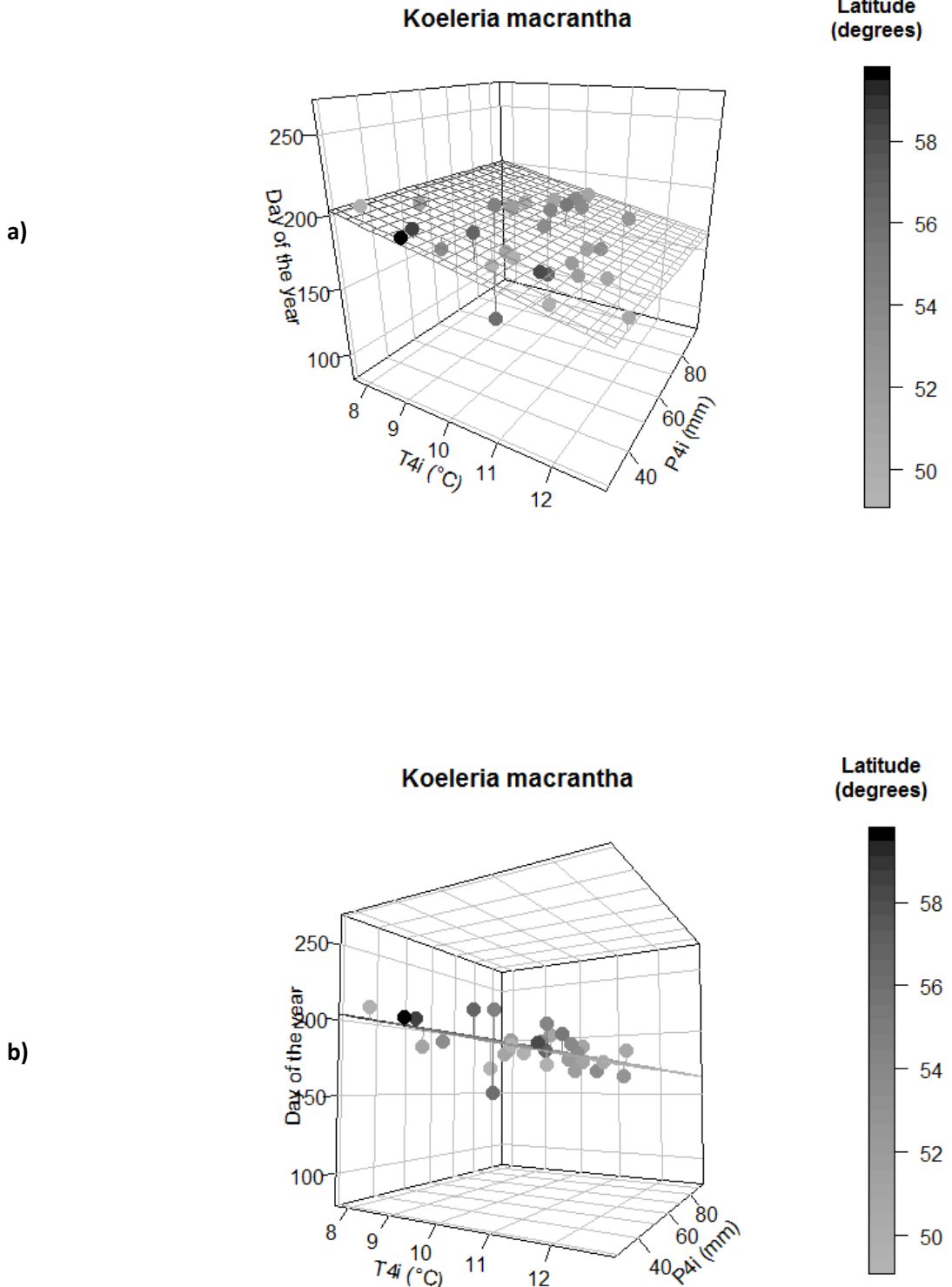


Figure 5: *Koeleria macrantha* (Poales - xeric) multiple regression visualisation showing significant responsiveness to temperature ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T4i), precipitation (P4i) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

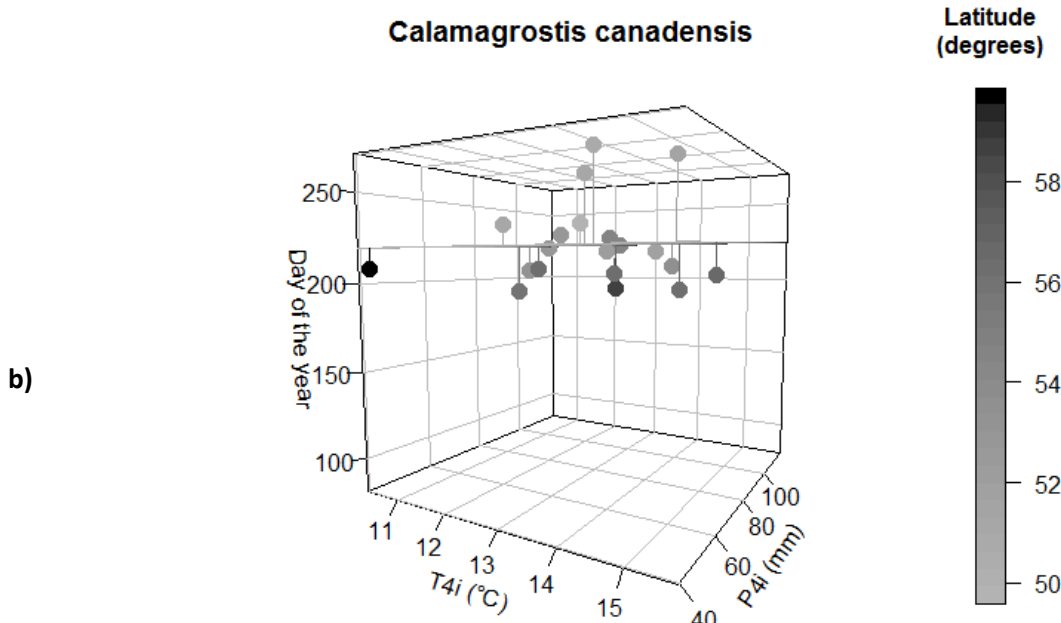
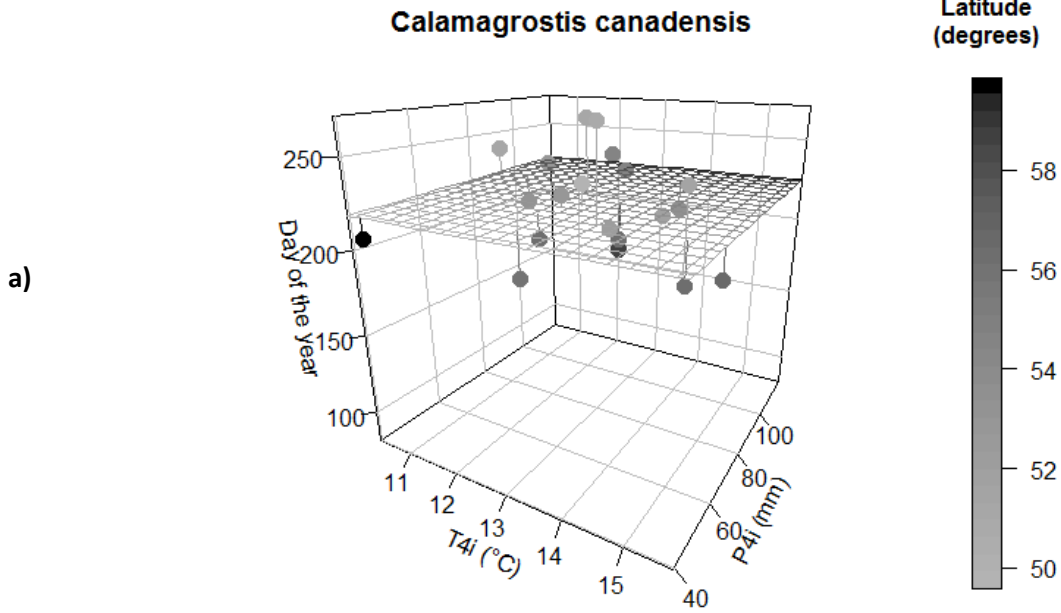


Figure 6: *Calamagrostis canadensis* (Poales - mesic) multiple regression visualisation showing significant responsiveness to latitude ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

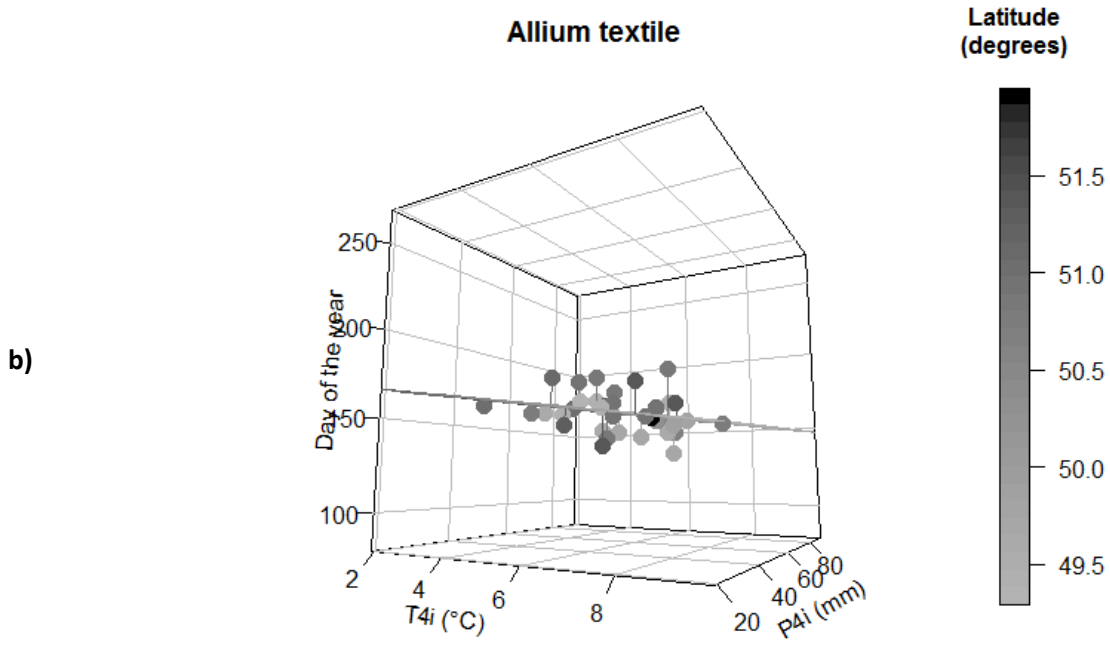
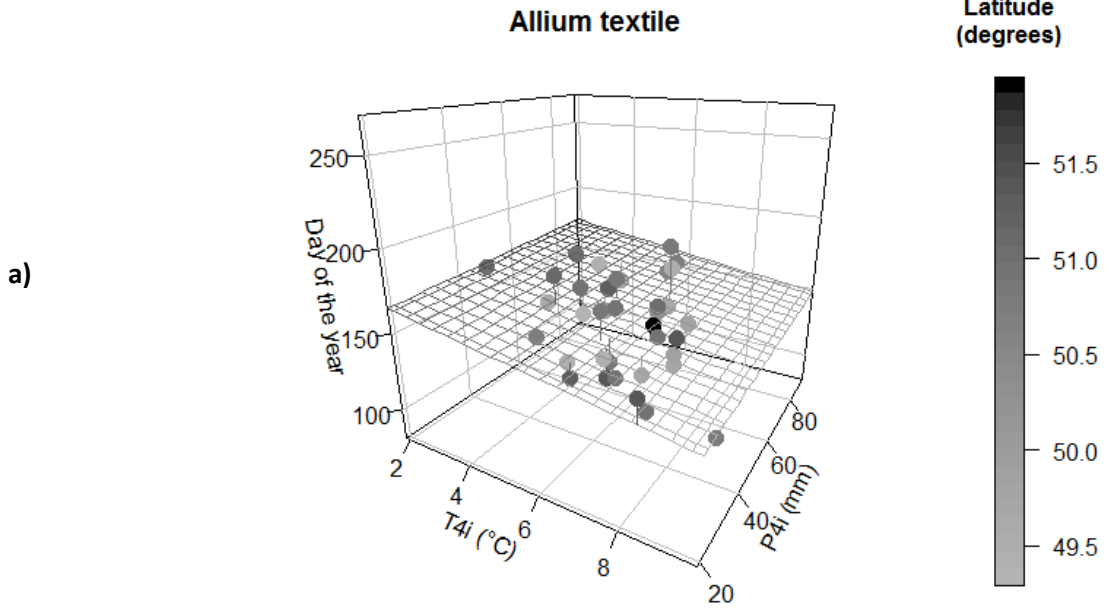


Figure 7: *Allium textile* (Asparagales - xeric) multiple regression visualisation showing significant responsiveness to temperature ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

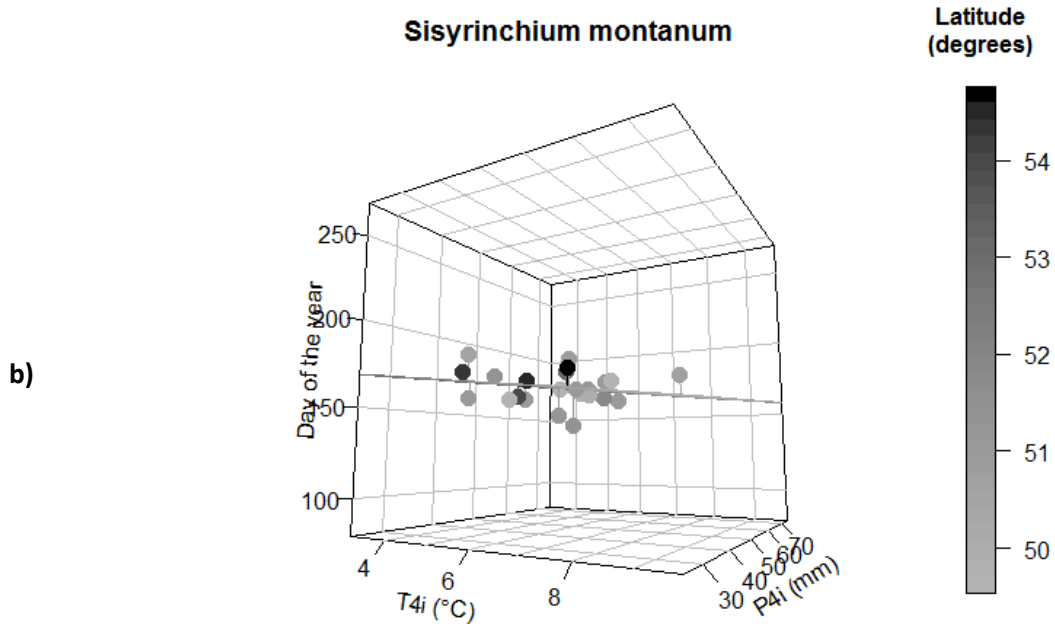
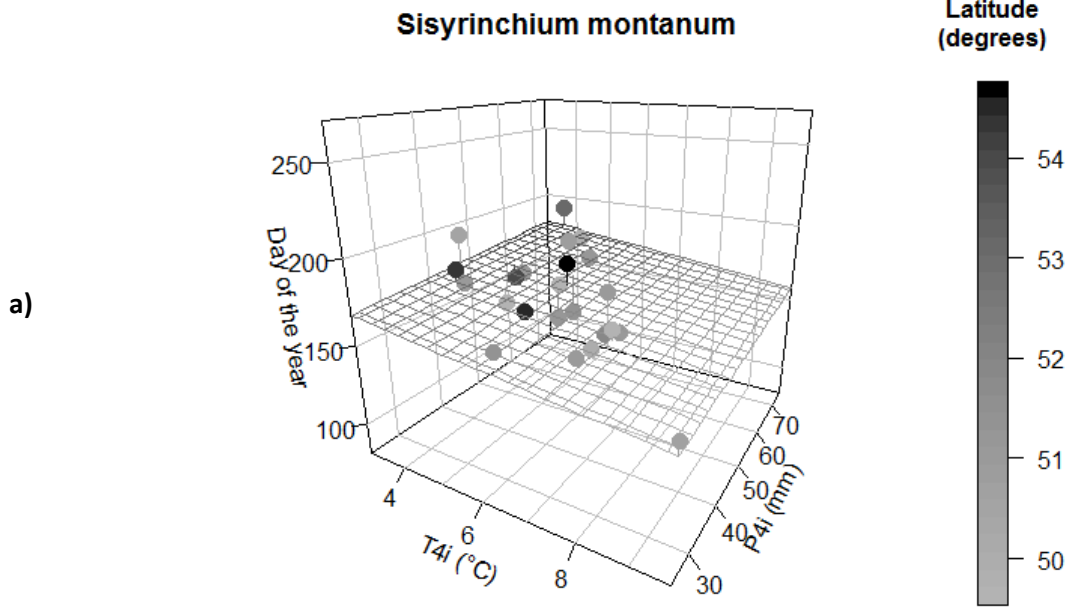


Figure 8: *Sisyrinchium montanum* (Asparagales - mesic) multiple regression visualisation. No significant responsiveness was detected. For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

2.3.2 Basal eudicots

The prairie crocus (*Pulsatilla patens* (L.) Mill.) represented a xeric-habitat species from the Ranunculales order and did not reveal any significant phenological responsiveness (Figure 9). The mesic species, *Thalictrum venulosum* Trel., did not show any response to temperature (Figure 10). However the results suggest that it flowers earlier with an increase in precipitation or latitude (linear multiple regression, d.f.*T. venulosum*=28, $p < 0.05$).

2.3.3 Rosids

In the order Fabales, species from mesic and xeric habitats responded to different environmental parameters (Figures 11 and 12). The xeric species *Thermopsis rhombifolia* (Nutt.) Richards have shown earlier flowering peaks with an increase in temperature (linear multiple regression, d.f.*T. rhombifolia*=40, $p < 0.05$), while the mesic species *Astragalus americanus* (W.J. Hooker) M.E. Jones has responded with a delayed flowering with an increase in precipitation (linear multiple regression, d.f.*A. americanus*=20, $p < 0.05$). In the Rosales order, the xeric prairie rose (*Rosa arkansana* Porter) advances its cycle by producing earlier flowering peaks with an increase in temperature (linear multiple regression, d.f.*R. arkansana*=16, $p < 0.05$), while the same was not observed in the mesic *Rosa acicularis* Lindl. (Figures 13 and 14). Alternatively, *Rosa acicularis* Lindl. has showed a significantly delayed blooming as a response to higher precipitation in the months preceding its flowering (linear multiple regression, d.f.*R. acicularis*=23, $p < 0.05$).

2.3.4 Asterids

In the Asterales order, only the xeric-habitat species *Achillea millefolium* L. responded significantly to temperature, by getting earlier flowering peaks with an increase in temperature (linear multiple regression, d.f.*A. millefolium*=63, $p < 0.05$) (Figures 15 and 16).

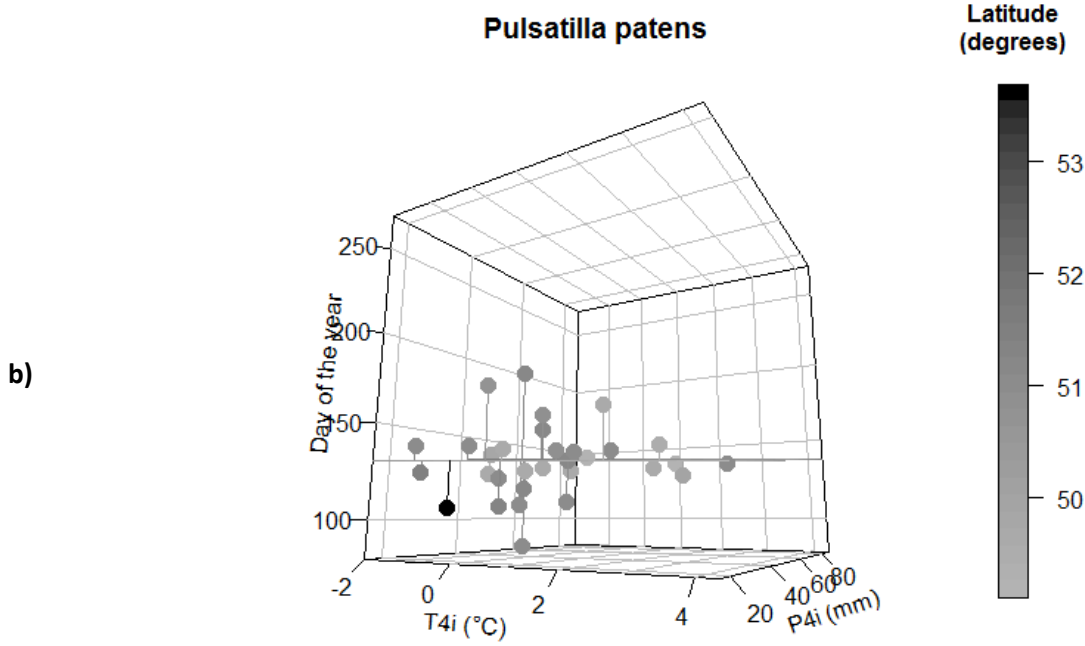
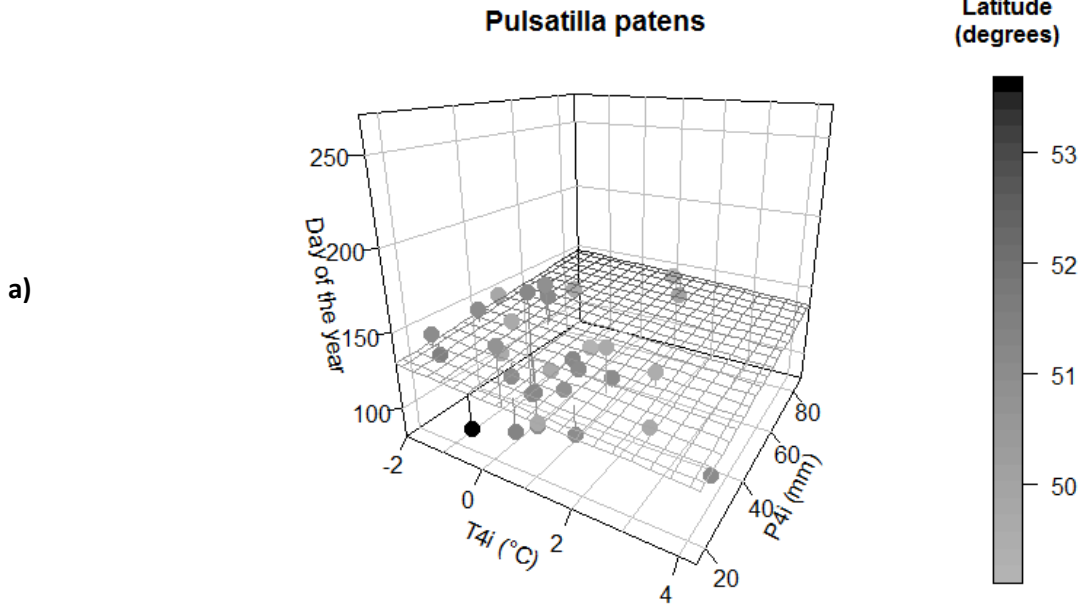


Figure 9: *Pulsatilla patens* (Ranunculales - xeric) multiple regression visualisation. No significant responsiveness was detected. For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

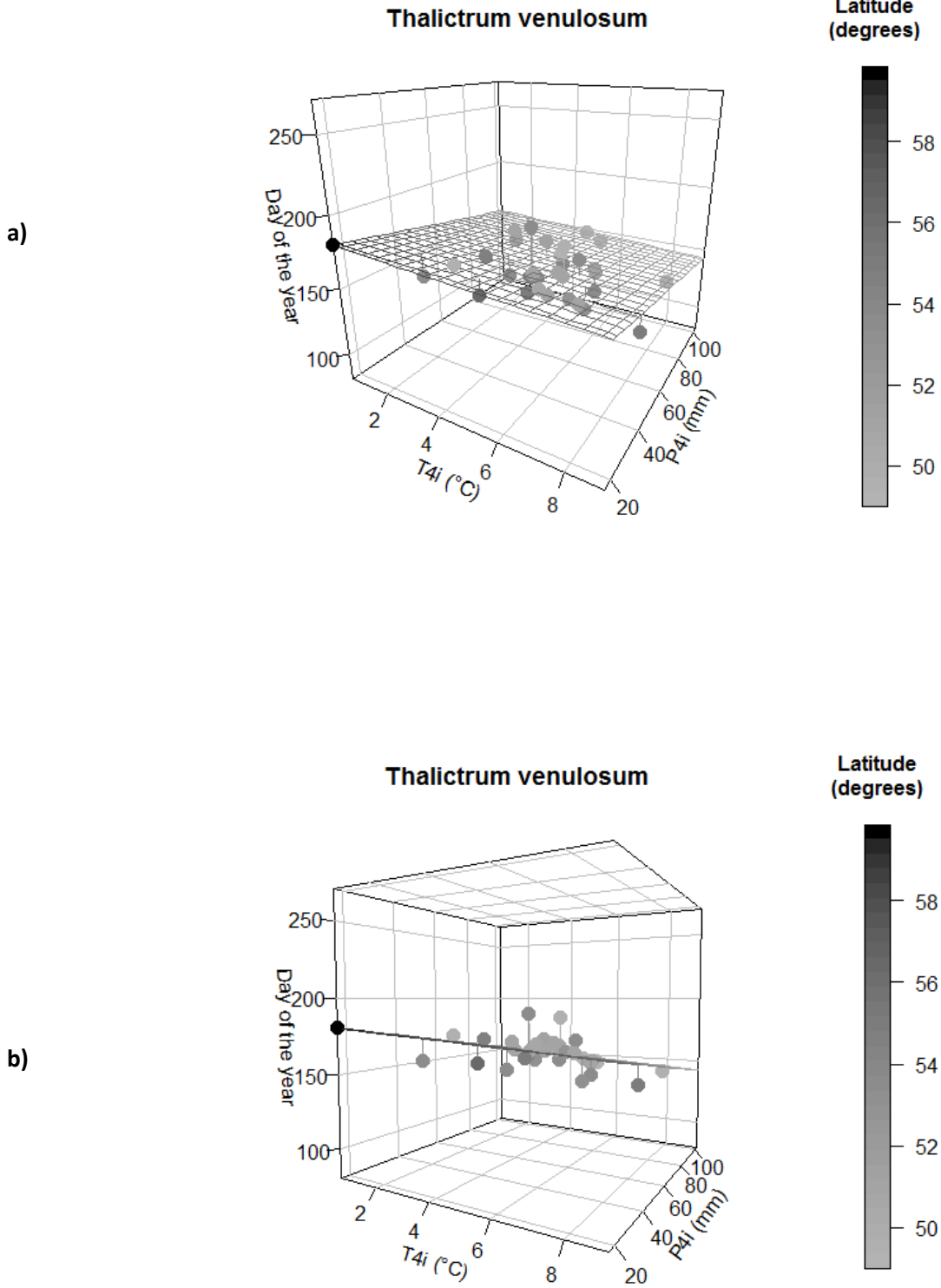


Figure 10: *Thalictrum venulosum* (Ranunculales - mesic) multiple regression visualisation showing significant responsiveness to precipitation and latitude ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature ($T4i$), precipitation ($P4i$) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

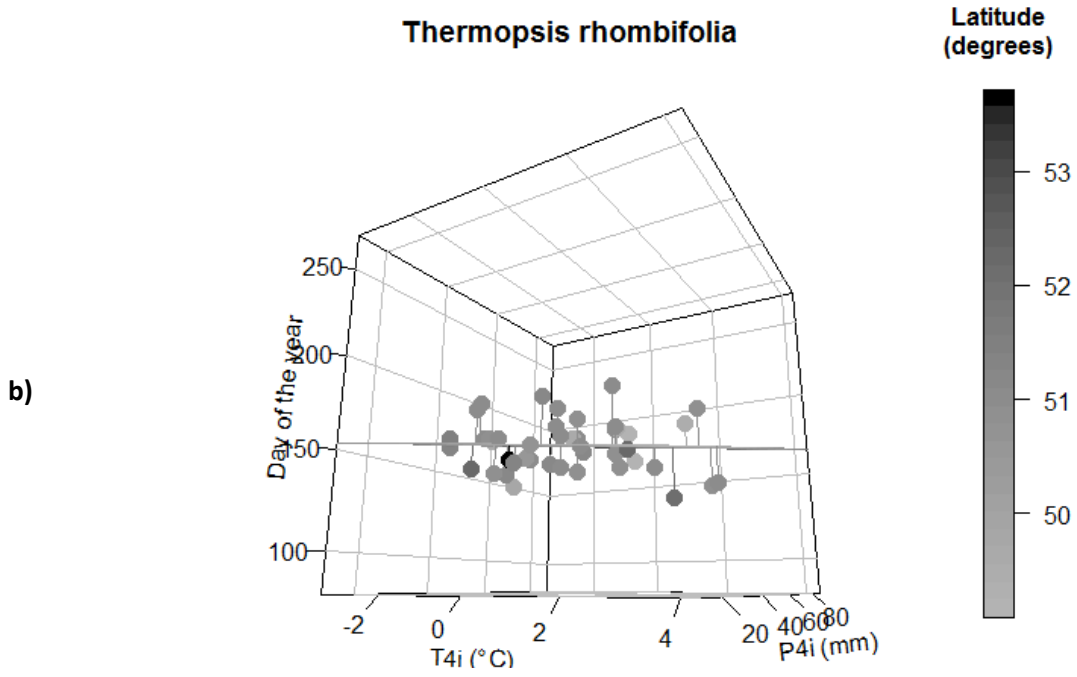
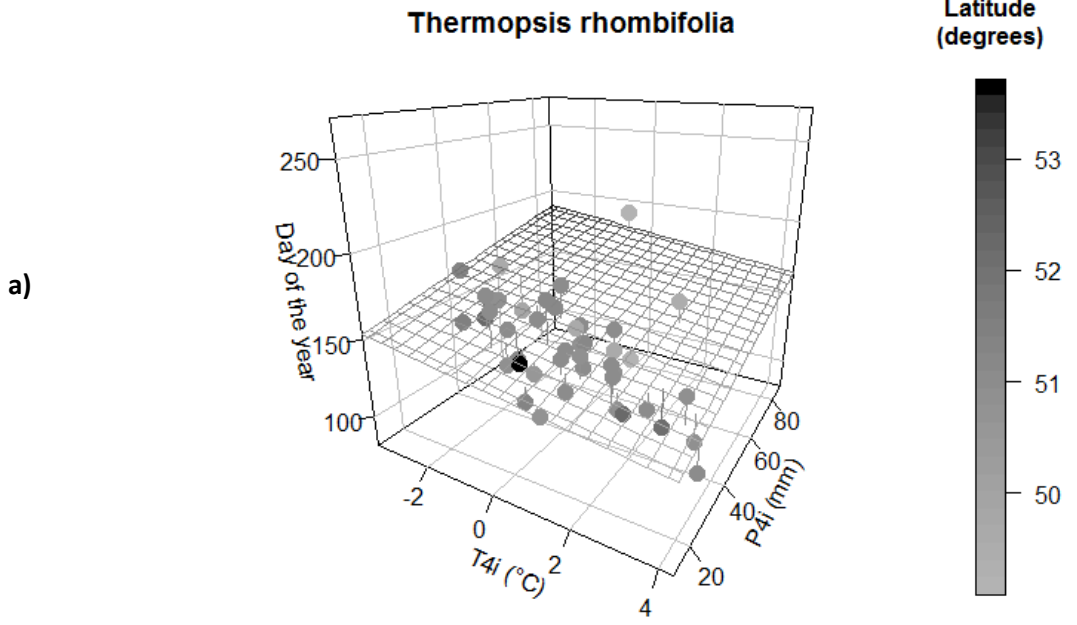


Figure 11: *Thermopsis rhombifolia* (Fabales - xeric) multiple regression visualisation showing significant responsiveness to temperature ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

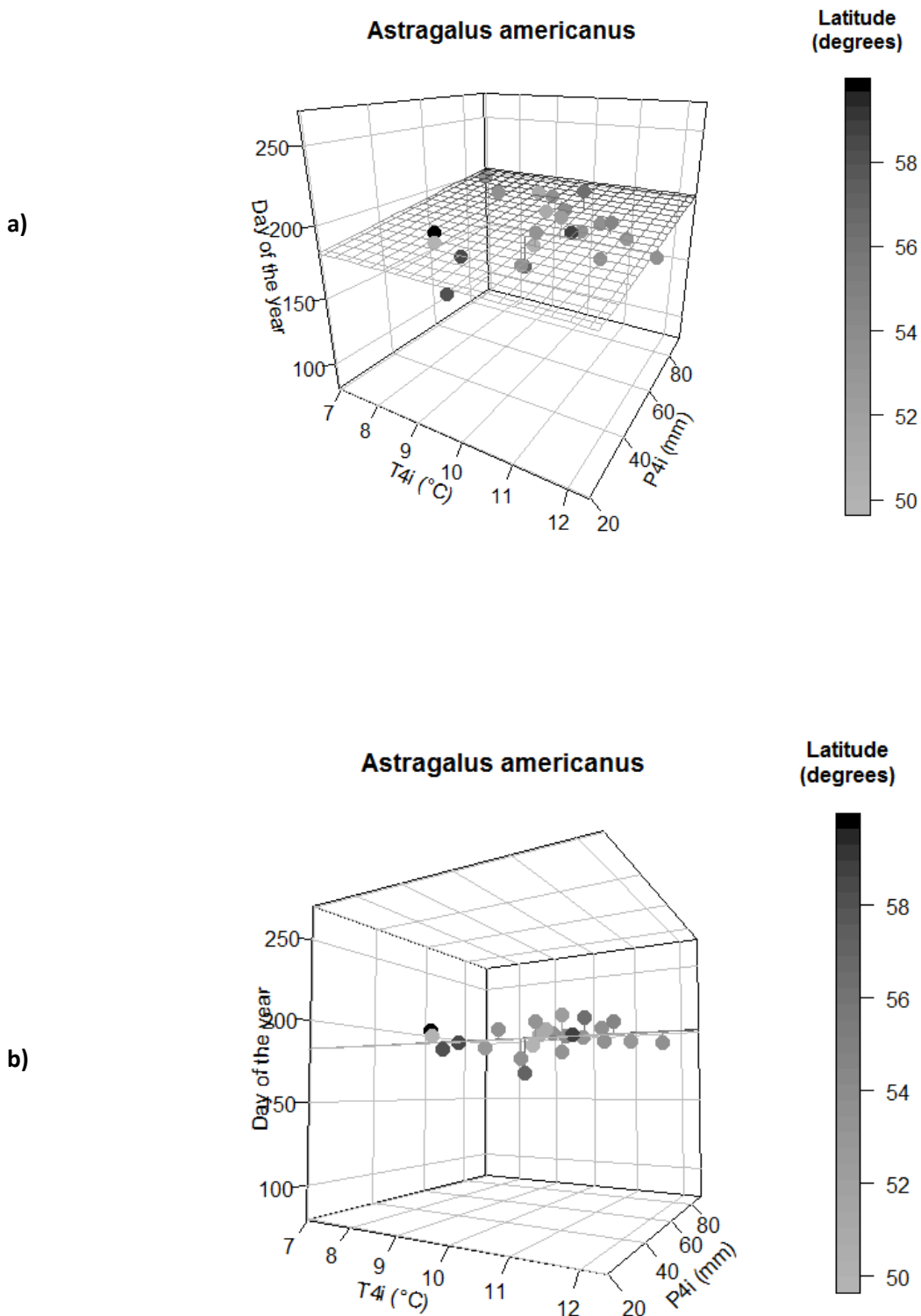


Figure 12: *Astragalus americanus* (Fabales - mesic) multiple regression visualisation showing significant responsiveness to precipitation ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

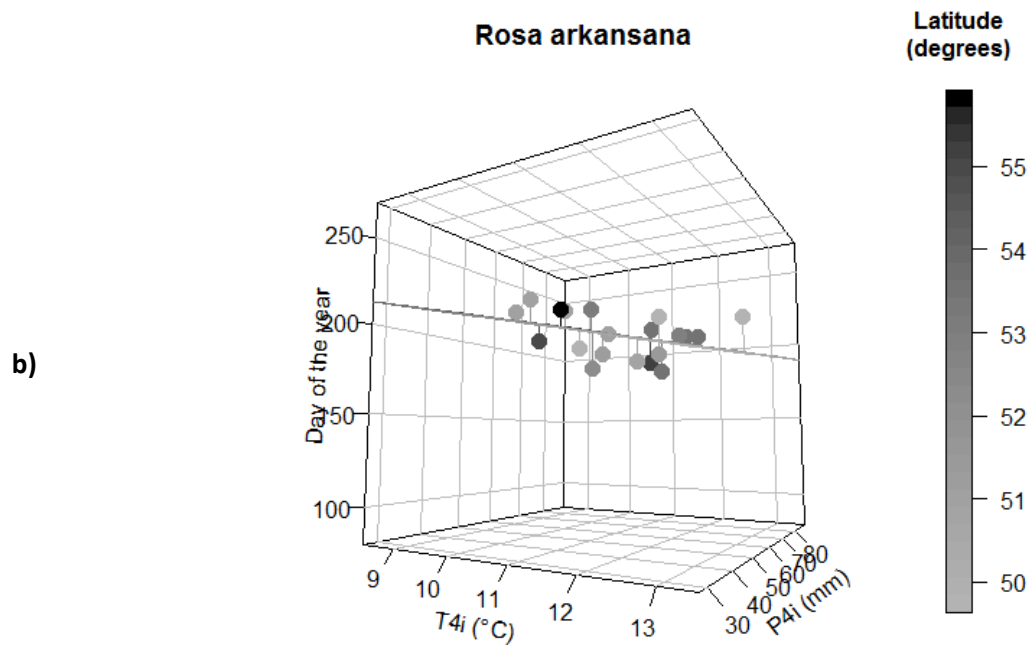
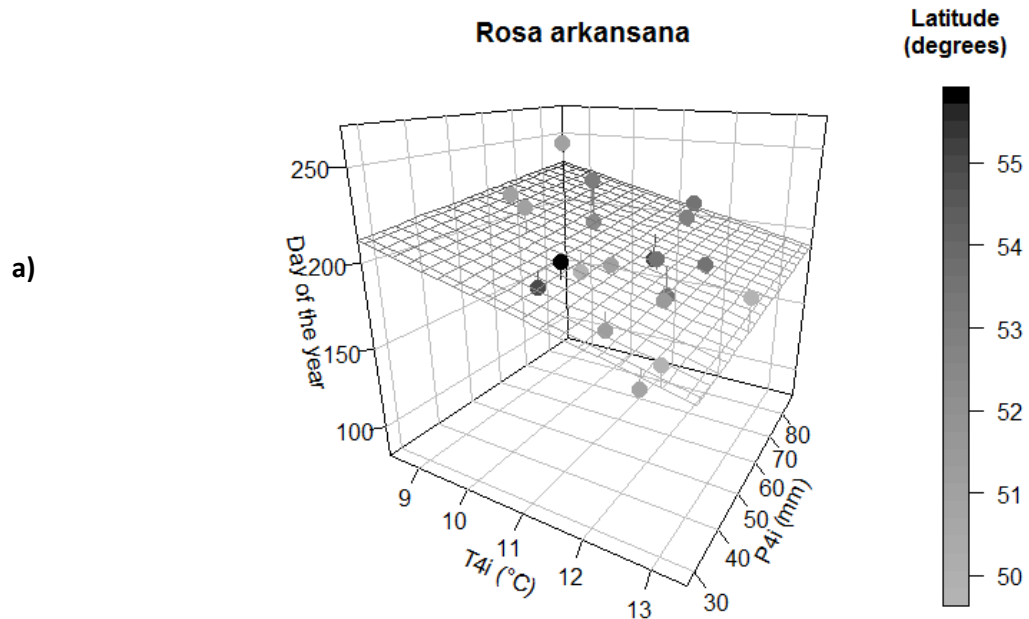


Figure 13: *Rosa arkansana* (Rosales - xeric) multiple regression visualisation showing significant responsiveness to temperature ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

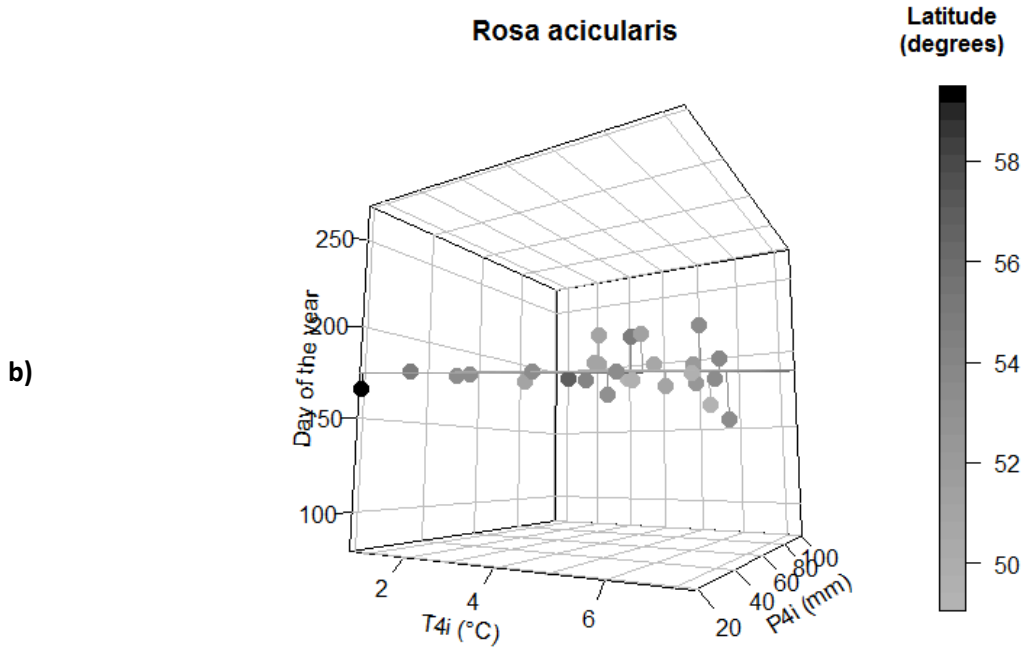
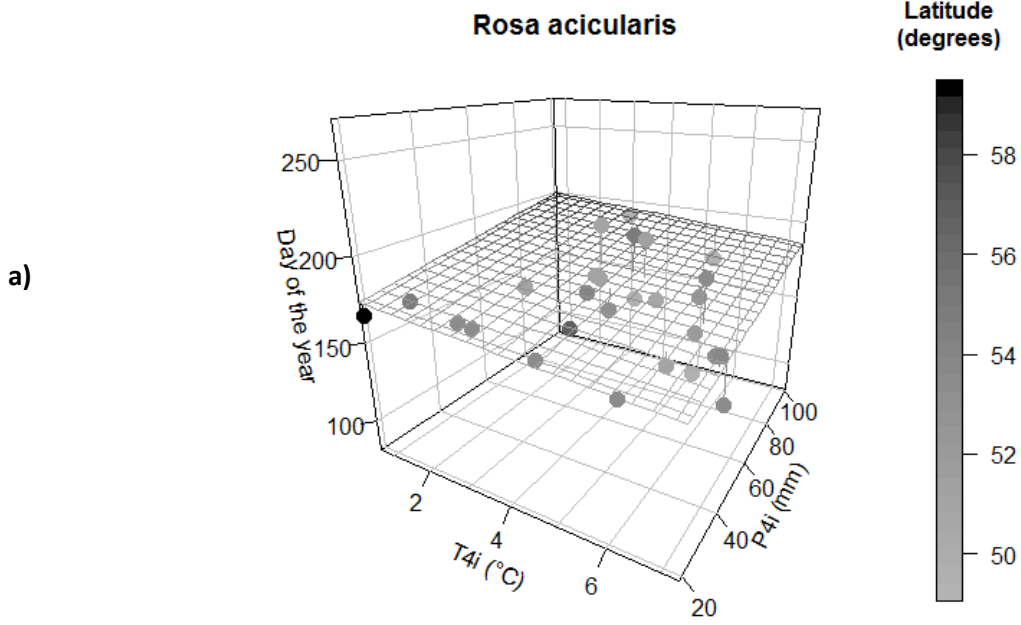


Figure 14: *Rosa acicularis* (Rosales - mesic) multiple regression visualisation showing significant responsiveness to precipitation ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

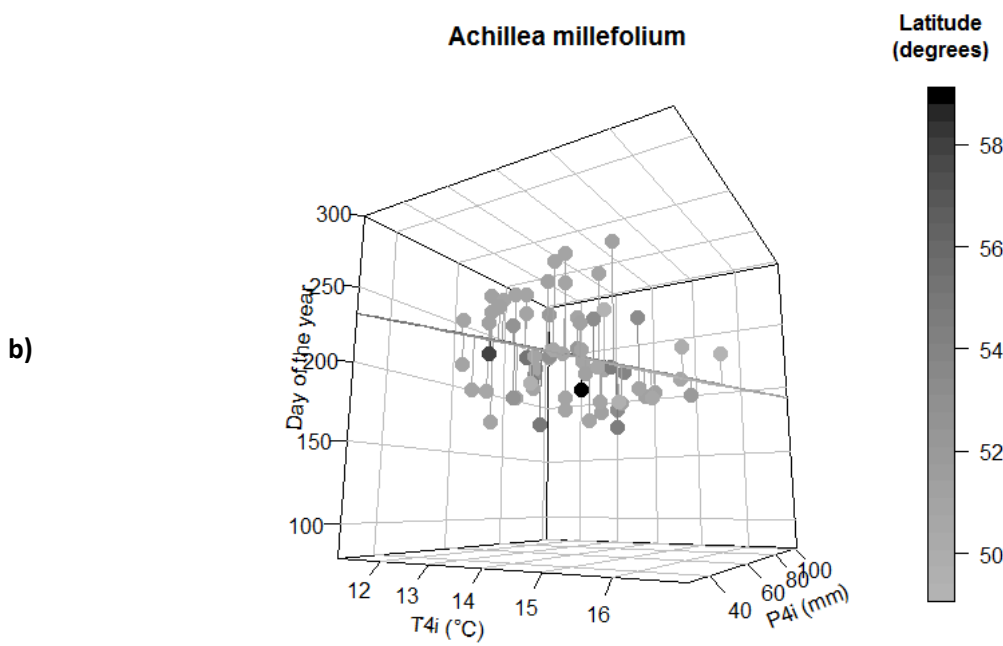
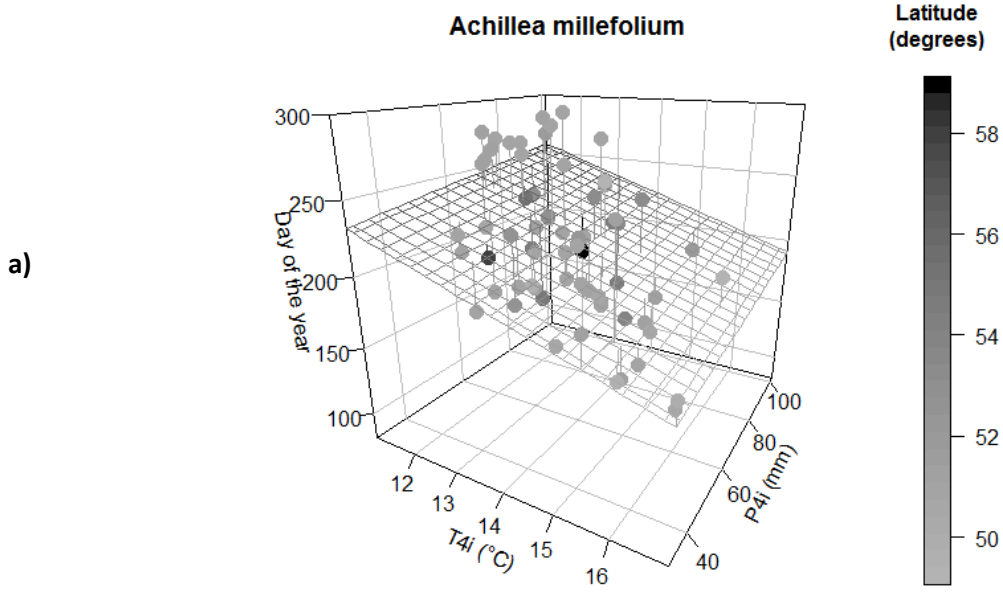


Figure 15: *Achillea millefolium* (Asterales - xeric) multiple regression visualisation showing significant responsiveness to temperature ($p < 0.05$). For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

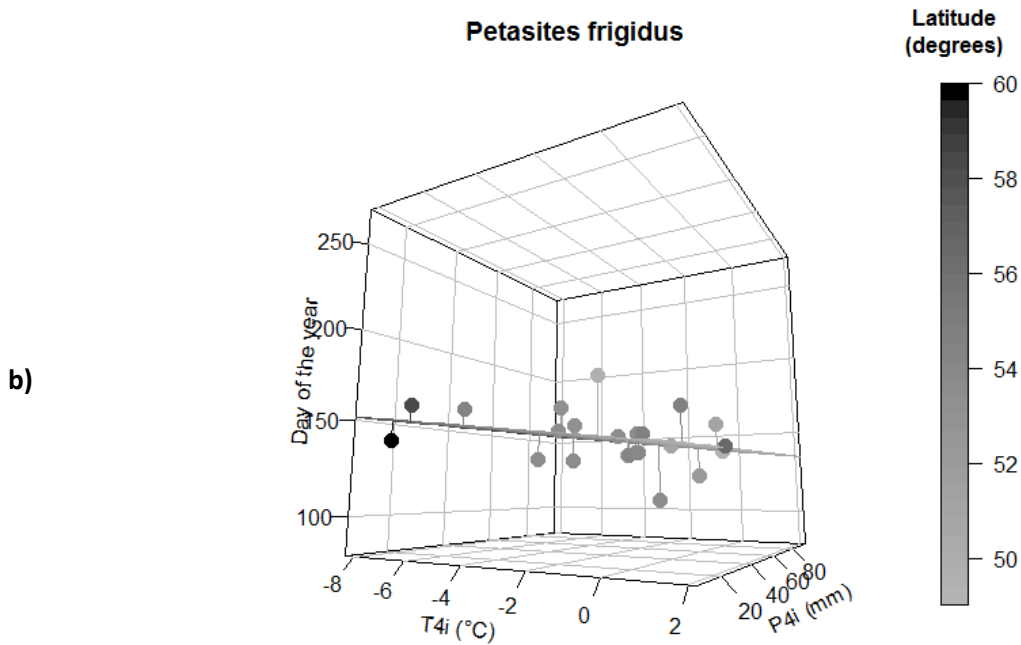
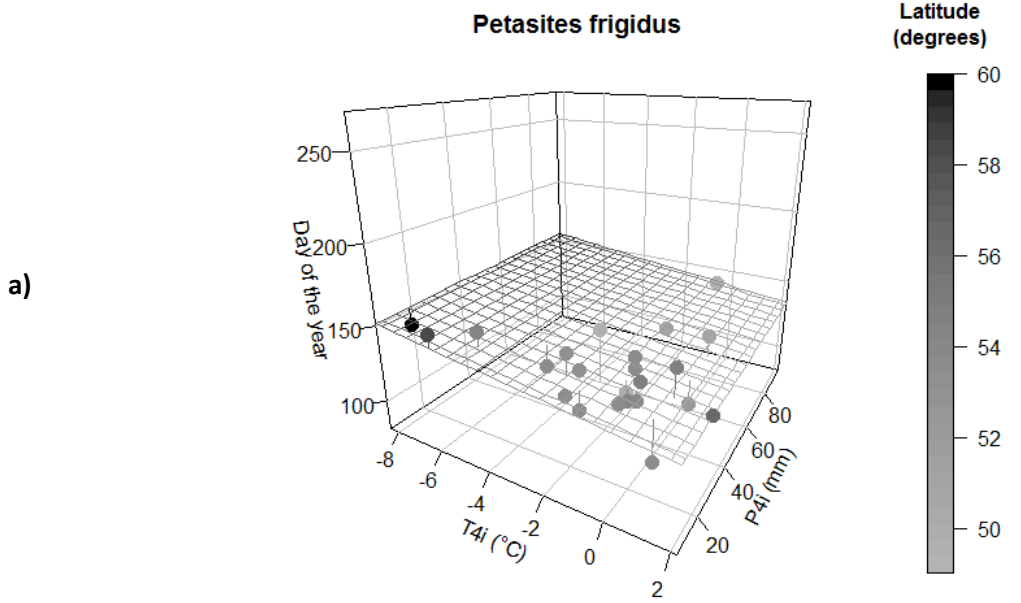


Figure 16: *Petasites frigidus* (Asterales - mesic) multiple regression visualisation. No significant responsiveness was detected. For each species, peak flowering date (day of the year) was regressed against temperature (T_{4i}), precipitation (P_{4i}) and latitude. The slope of the regression plane quantifies the phenological responsiveness (a) and the regression plane view (b) show the dispersion of the data points.

2.3.5 Taxonomic sister-group and meta-analyses

In the taxonomic sister-group test of species by order, a significant difference between mesic- and xeric-habitat species was observed for the phenological responsiveness to temperature (Table 3, Figures 17 and 18). No significant difference between xeric- and mesic-habitat species' phenological responsiveness to precipitation or latitude were found (dependent 2-group Wilcoxon Signed Rank Test, $p > 0.05$). On average, species from xeric habitats had a significantly more pronounced phenological responsiveness to shifts in temperature than species from mesic habitats (dependent 2-group Wilcoxon Signed Rank Test, $p < 0.05$).

Table 3: Paired difference in phenological responsiveness per order. The value highlighted represent a mean difference significantly different from zero ($p < 0.05$).

Order	Difference in phenological responsiveness per order (mesic – xeric)		
	Temperature (days/°C)	Precipitation (days/mm)	Latitude (days/degree)
Rosales	5.85	0.01	1.34
Ranunculales	0.24	-0.53	2.78
Poales	5.65	-0.16	-6.97
Asparagales	0.55	0.10	-2.46
Asterales	9.05	-0.50	2.83
Fabales	2.92	-0.03	3.37
Liliales	0.46	0.31	0.22
Mean difference (±SEM)	3.53 (±1.29)	-0.11 (±0.12)	0.16 (±1.40)

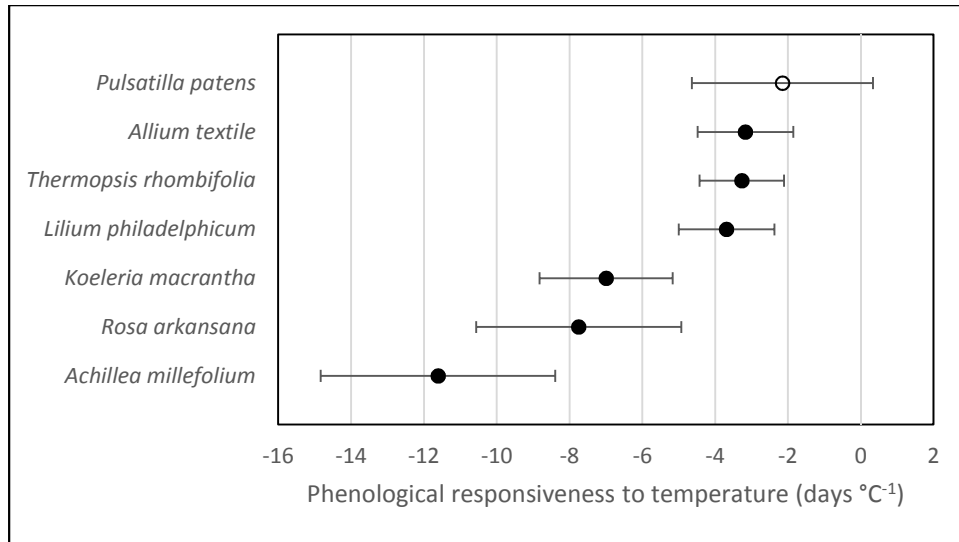


Figure 17: Phenological responsiveness to temperature within xeric-habitat species. Mean±SE represented by marker and horizontal bars. Closed points show a significant ($p < 0.05$) phenological response to temperature, while open points indicate species which the flowering date was not dependent on the temperature ($p > 0.05$). Orders from top down: Ranunculales (*P. patens*), Asparagales (*A. textile*), Fabales (*T. rhombifolia*), Liliales (*L. philadelphicum*), Poales (*K. macrantha*), Rosales (*R. arkansana*) and Asterales (*A. millefolium*).

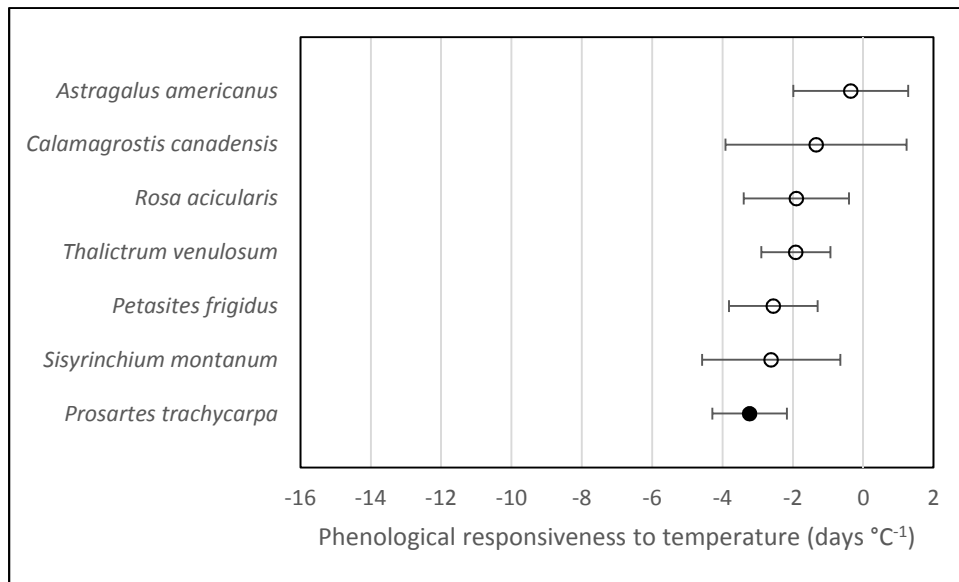


Figure 18: Phenological responsiveness to temperature within mesic-habitat species. Mean±SE represented by marker and horizontal bars. Closed points show a significant ($P < 0.05$) phenological response to temperature, while open points indicate species which the flowering date was not dependent on the temperature ($p > 0.05$). Orders from top down: Fabales (*A. americanus*), Poales (*C. canadensis*), Rosales (*R. acicularis*), Ranunculales (*T. venulosum*), Asterales (*P. frigidus*), Asparagales (*S. montanum*) and Liliales (*P. trachycarpa*).

The meta-analysis on the difference of phenological responsiveness also indicates that xeric-habitat species produced significantly earlier flowering with an increase in temperature, when compared to mesic-habitat species (standard meta-analysis on raw mean difference, $df=6$, $p<0.05$) (Figure 19). The meta-analysis did not reveal any significant differences between xeric- and mesic-habitat species for the phenological responsiveness to precipitation (Figure 20) or latitude (Figure 21).

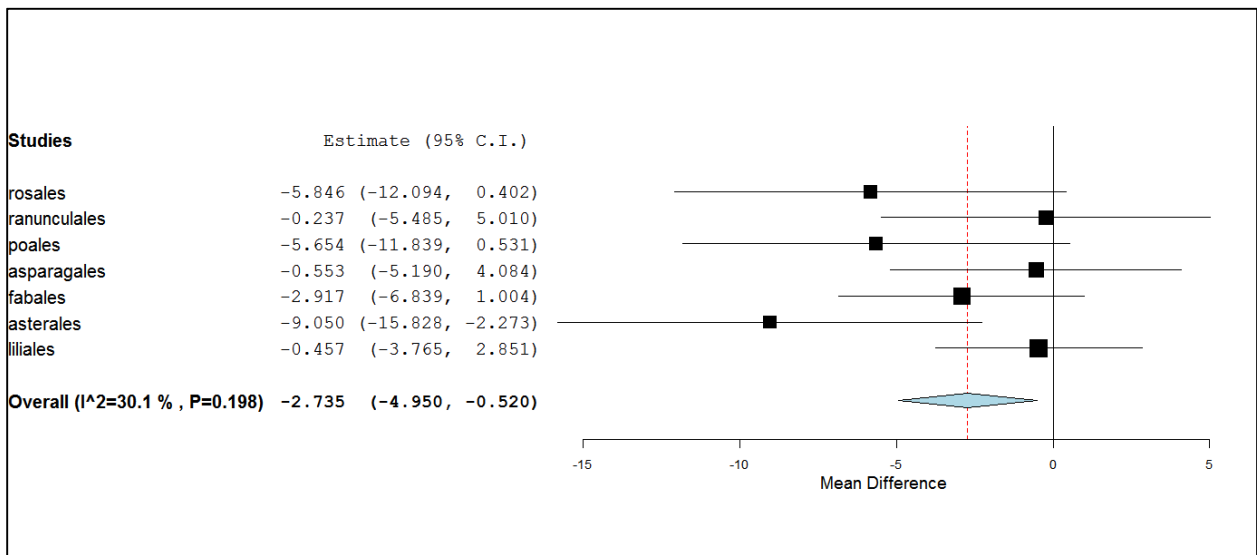


Figure 19: Meta-analysis forest plot of differences between mesic and xeric-habitat species in phenological responsiveness to temperature (days. $^{\circ}\text{C}^{-1}$) for each order. There is a significant mean overall difference ($p<0.05$).

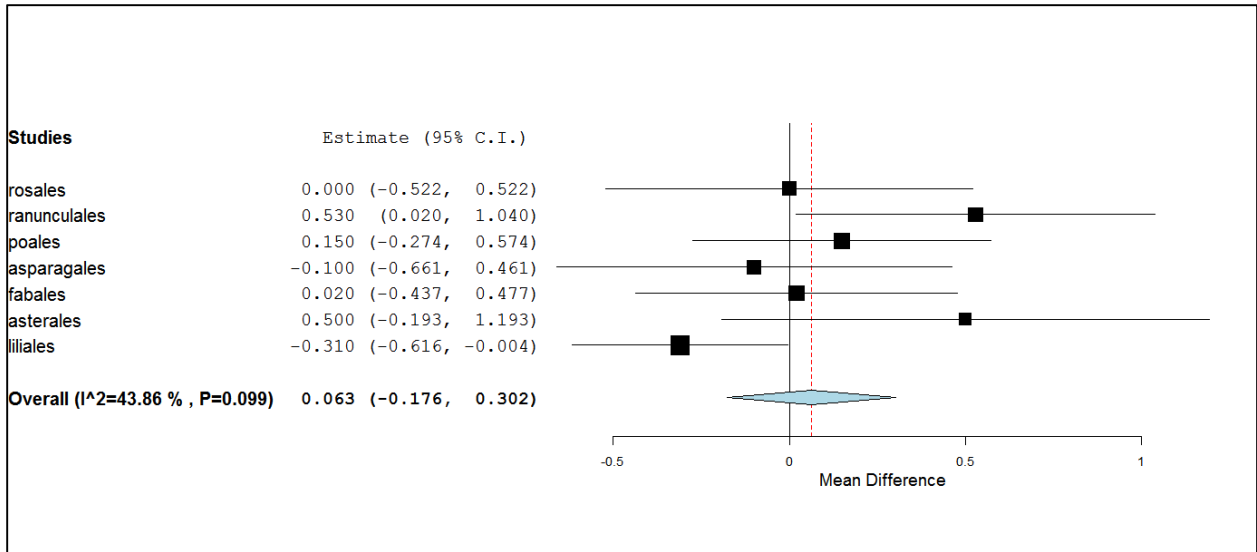


Figure 20: Meta-analysis forest plot of differences between mesic and xeric-habitat species in phenological responsiveness to precipitation (days. mm⁻¹) for each order. There is no significant mean overall difference ($p>0.05$).

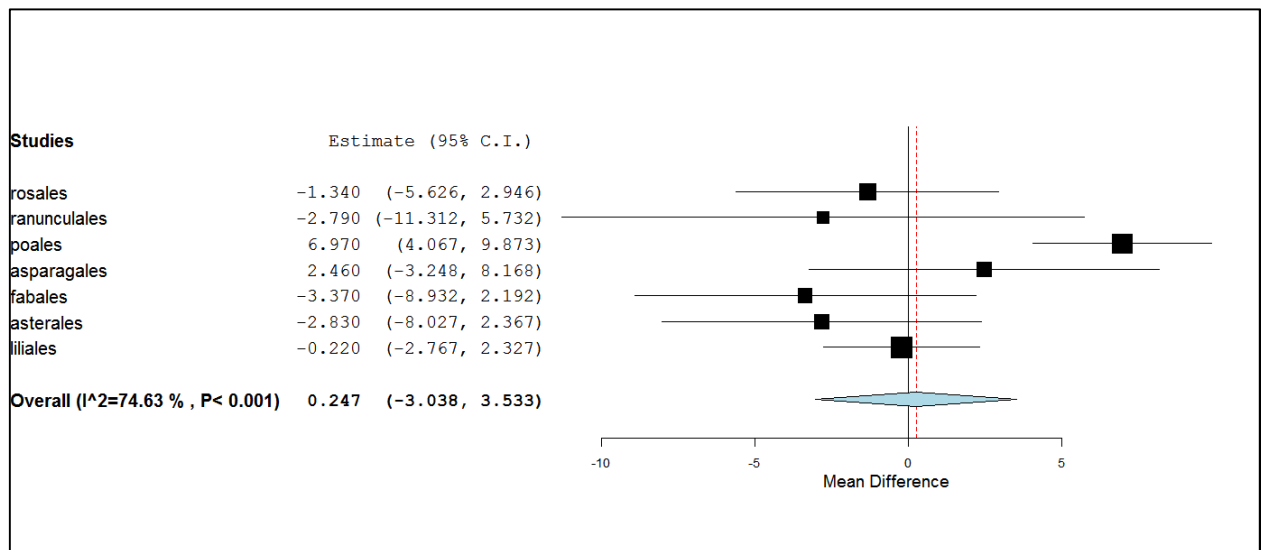


Figure 21: Meta-analysis forest plot of differences between mesic- and xeric-habitat species in phenological responsiveness to latitude (days. degree⁻¹) for each order. There is no significant mean overall difference ($p>0.05$).

2.4 Discussions

2.4.1 Phenological responses to temperature

The herbarium specimens revealed that some plant species in Alberta are regulating their life cycle timing as a response to temperature shifts. The induction of earlier flowering burst with an increase in the average temperature was observed in 7 out of a total of 14 species studied. On average, the budburst advanced 3.8 ± 0.8 days $^{\circ}\text{C}^{-1}$ across all species. This result from Alberta species is not far from the observations made by other researchers. For example, Callinger et al. (2013) have found that angiosperm species in the U.S. had an overall average phenological responsiveness of 2.4 days $^{\circ}\text{C}^{-1}$ and while studies done in Europe and China that have found a range of 2.6 to 6 days advancement in spring phenological events per $^{\circ}\text{C}$ increase (Wang et al 2015). The range of phenological responses to temperature varied from -0.35 ± 1.60 days $^{\circ}\text{C}^{-1}$ (*Astragalus americanus* (W.J. Hooker) M.E. Jones) to -11.61 ± 3.22 days $^{\circ}\text{C}^{-1}$ (*Achillea millefolium* L.).

Callinger et al. (2013) have found that spring flowering species show higher phenological responsiveness to temperature than early- and late-summer flowering species. However, I found no evidence that the phenological responsiveness to temperature is dependent on the species' average date of peak flowering (linear regression, d.f. =12, $p > 0.05$). When compared to other similar phenology investigations conducted in North America (Abu-Asab 2001, Callinger et al. 2013, Miller-Rushing & Primack 2008, Panchen, et al. 2012), the present study focused in relatively high latitudes, comprising specimens collected between latitudes 49° and 60° north. The variability in geographic distribution and species composition among phenology studies

may play a key role in explaining some discrepancies in the results found by different studies.

The earliest flowering species included in the study were the prairie crocus (*Pulsatilla patens* (L.) Mill.) and the sweet coltsfoot (*Petasites frigidus* (L.) Fr.). These two species did not show any clear signs of responsiveness to temperature. However, Beaubien (2013) reports that *Pulsatilla patens* (L.) Mill. has revealed an advancement of two weeks in its flowering date between 1936 and 2006 in Alberta. These two species (*P. patens* and *P. frigidus*) tend to bloom from early- to mid-May, when frozen soil conditions, snow cover and frost are still common in the Albertan spring. These factors likely affect how temperature changes are experienced by plants, producing a complex set of interacting environmental cues to which early flowering species might be responding. Corroborating this idea, *Petasites frigidus* (L.) Fr shows one of the two examples of significant interaction amongst variables in this study. The effect of temperature over the peak-flowering date is dependent on the precipitation in this species (linear multiple regression, d.f.=16, $p < 0.05$). In other words, using air temperature or precipitation data alone might be too simplistic to explain the phenological responsiveness of very early flowering species in Alberta, and other variables should be incorporated in the model (e.g. snow depth and solar radiation).

2.4.2 Phylogenetic signals in phenological responses

The responsiveness to temperature, precipitation and latitude showed a high degree of variability among orders. The order Asterales (representing the Asterids clade in the study) have showed the highest average phenological responsiveness among the orders

included in the study (-7.1 ± 4.5 days $^{\circ}\text{C}^{-1}$). In its turn, the basal eudicots from the order Ranunculales produced unusual results, indicating that this group might use alternative cues in regulating its phenological responses. In one hand, *Pulsatilla patens* (L.) Mill. (Ranunculales) seems to be unresponsive to the parameters tested in this study. In the other hand *Thalictrum venulosum* Trel. (Ranunculales) was the only species in this study to respond by producing significantly earlier flowering with an increase in precipitation. Additionally, *T. venulosum*, along with *Calamagrostis canadensis* (Michx.) P.Beauv. (in the Poales order from the group of Monocots) seem to produce earlier flowering peaks with an increase in latitude. Contrary to what might be expected, plants at a more northerly location in Canada tend to flower earlier than conspecifics at a more southerly location (Panchen 2016).

Besides exhibiting the highest average responsiveness to temperature, the order Asterales has also showed the greatest contrast between mesic and xeric responsiveness to temperature, while neither of the two selected species in the order Ranunculales showed significant responsiveness to temperature. This suggests that the phenological strategies adopted by different angiosperm clades can be highly diverse and influenced by their evolutionary history. Davies *et al.* (2013) have shown the importance of taking into account the taxonomical relationship between the species in phenology studies by illustrating that it is not the time of year that is conserved within closely related species, but rather the phenological responses to a common set of abiotic cues.

Mazer *et al.* (2013) suggests that predictions could be made for species not yet studied based on results from related species. In this study, however, two species very closely related under the same genus (*Rosa acicularis* Lindl. and *Rosa arkansana* Porter) have

showed significantly different phenological responsiveness to both temperature and precipitation, and only *R. acicularis* has revealed a significant interaction effect between precipitation and latitude (linear multiple regression, d.f.=20, $p < 0.05$). It appears that taxonomical relationships alone are insufficient in some cases to predict a species' phenological responses and it must be combined with ecological or functional group considerations.

2.4.3 Ecological signals in phenological responses

My results suggest that species from xeric habitats have an average phenological responsiveness to temperature significantly more pronounced than species from mesic habitats. In this study, species from xeric habitats have advanced their flowering in average 5.5 ± 1.3 days $^{\circ}\text{C}^{-1}$, while species from mesic habitats have advanced their flowering peak in average 2.0 ± 0.4 days $^{\circ}\text{C}^{-1}$. This tendency might be explained by the mesic habitat thermal properties. A greater soil water content represents a higher soil heat capacity, requiring more heat accumulation during the spring and summer in order to increase its temperature. In order to induce flowering, some plants respond to changes in tissue temperature, which might be more correlated with soil temperature than to air temperature. Therefore, it is likely that xeric-habitats species exhibit a higher phenological responsiveness to changing air temperatures when compared to species from mesic habitats, because the latter occupy habitats with higher thermal inertia. It is suggested that this habitat property affects the plant's meristematic temperature, leading to a relatively delayed response to air temperature in the case of mesic-habitat species. This hypothesis, however, is yet to be tested by future investigations.

Hypothetically, xeric-habitat species might take advantage of an earlier (and possibly longer) flowering season, while mesic-habitat species might face competitive challenges. On the other hand, the phenological flexibility of species from xeric habitats might put them under greater risk of frost exposure. Iler et al. (2019) have found, however, that frost damage to flower buds, a consequence of climate change-induced earlier flowering, does not contribute strongly to population declines.

The differential response to temperature between xeric- and mesic-habitats species suggests that there is a risk of pollination mismatch for one of these groups. Pollinators also use cues in their environment to commence events in their life cycle, and these processes are similarly subjected to climate change (Bartomeus *et al* 2011). If the pollinator's phenological responsiveness is not aligned with the target plant's phenological responsiveness, mismatch may occur where the plant is available for pollination but there is no available pollinators (or the opposite). This study has shown that xeric- and mesic-habitats species respond differently to air temperature shifts. Therefore, one of these groups might be under greater risk of pollination mismatch, depending on which group is misaligned with its pollinator phenological responsiveness and the environmental cues they are using. The determination of which group is more vulnerable to pollination mismatch (i.e. mesic or xeric) depends on elucidating what are the environmental cues used by their respective pollinators and if their phenological response is better aligned with mesic or xeric patterns of response.

For some species, other environmental factors might be more important than air temperature in order to regulate flowering timing. Precipitation was a key factor for three species flowering timing (out of 14). Latitude was the only strong predictor of flowering

date peak for *Calamagrostis canadensis* (Michx.) P.Beauv. Between spring and fall equinoxes, the day is longer at higher latitudes, therefore this result suggests that, for this species, the flowering burst timing is more dependent on photoperiod rather than temperature or precipitation.

The observed difference in phenological responsiveness between xeric- and mesic-habitats species is consistent with the expected outcome from considering the effects of the thermal properties of the soil over the temperatures experienced by the individual plants. However, the causation relationship between soil temperature fluctuations based on moisture regime and plant phenological responses remains to be experimentally tested. The use of quantitative models of energy conservation for the prediction of soil temperatures might produce promising results in predicting flowering peak dates. These models should incorporate data of radiation and conduction of thermal energy, as well as consider the soil type and moisture as well as precipitation effects over thermal inertia or albedo (when there is a formation of a snow cover).

Chapter 3 - Conclusions and future directions

3.1 The herbarium-based method in phenology studies

The herbarium-based method employed has the potential to increase the available data for phenology studies when combined to direct observation approaches. By including information acquired in herbarium collections, phenology research might expand to incorporate a larger array of species and geographic regions, which otherwise would be limited to the selected species and areas considered in a direct observation phenology study. Also the herbarium-based method does not require the existence of a decades-old phenological monitoring program previously in place. The herbarium-based method, however, suffers from being limited by the number of specimens collected, as well as on geographic and temporal range. This characteristic poses an immediate challenge towards applying a herbarium-based method for arctic regions, from where available preserved specimens from a wide historical window might be scarce. Additionally, by choosing to work only with specimens in peak flower, a significant portion of the collection is not included. Depending on the species the proportions of rejected specimens, based on these selection criteria, might range roughly from 50% to 90%. Ultimately that leads to the limitation that often only the most frequently collected species can be included in these sorts of phenology studies, which might leave many rare and threatened species excluded.

Another limitation of the herbarium method is related to the uncertainty inherent to the attempt of estimating precisely the location of collected herbarium that lack GPS coordinates. This is typically the case of specimens collected pre-1990's. In these circumstances, it might be impossible to pinpoint the precise geographical location of a

collected specimen based on municipality the descriptions provided about how some landscape features appeared many decades in the past. A similar situation was identified for the reliability of the climate data associated with each specimen. Older specimens were associated to interpolated climate data originating from fewer weather stations than what is currently in operation. This introduce an extra source of imprecision for old specimens. Although not likely to bias the conclusions about the xeric and mesic habitats species differences, a greater margin of error for location and climate data is indeed expected for older specimens, and it is likely to increase the noise in the statistical analysis. A large number of replicates is suggested for any attempts of replicating the method used in this study, in order to capture the phenological signal.

When combined in a single group (Figure A – Appendix A), the species included in this study did not revealed a significant association between the dates of flowering peak and the year of collection in Alberta between 1914 and 2013 (linear multiple regression, $d.f.=426$, $p>0.05$). This multiple regression analysis also included latitude and elevation as variables. Therefore, the present study was incapable of detecting any general temporal trends in the date of flowering peak, when all specimens of every species studied where analysed together. Similarly, when grouped by species of mesic and xeric habitats, the species did not revealed any significant temporal trends in their flowering peak dates (linear multiple regression, $d.f._{xeric}=253$, $d.f._{mesic}=169$, $p>0.05$). These results are not aligned with the observations made by Beaubien (2013) that evidenced an advancement in flowering dates for early blooming species in Alberta between 1936 and 2006.

The herbarium method might be limited in detecting temporal trends over a large spatial range. This is likely due to the relative difficulty in obtaining a large number of replicates in herbaria and in association to a high volume of environmental confounding factors affecting phenological responses, such as latitude, elevation, moisture availability, soil type and terrain slope orientation. The use of historical weather data combined to peak flowering dates (and the estimation of phenological responsiveness) seems to be more successful in detecting signals of phenological responses when adopting a herbarium-based method.

I suggest that the field of plant phenology in general can benefit from associating peak flowering date information (or any other phenological event) with historical climate data. This technique offers the possibility to assess the phenological responsiveness of a particular species to a particular change in its environment. Beyond simply measuring the plant's phenological adjustments to climate shifts that happened in the past, the phenological responsiveness allows us to predict possible responses based on climate projections.

3.2 Phylogenetic and ecological signals: implications of our findings

When comparing plants from mesic and xeric habitats, we observed some significant differences in responsiveness between closely related species (e.g. between *Rosa acicularis* Lindl. and *Rosa arkansana* Porter), in disagreement with the strong phylogenetic signal in phenological responses reported in other studies (Mazer *et al.* 2013). I propose that this disagreement might be an artifact of the physical properties of the plant's environment and the parameters chosen for monitoring, rather than an

expression of a radically different phenological strategy among closely related species. I suggest that the phenological response of related species might be truly similar, but the plants are not responding to air temperature. Therefore, in some cases, using air temperature for assessing phenological responsiveness might create an impression of differential responses under apparently similar environmental conditions. Xeric and mesic habitats can create dissimilar soil temperature regimes even when exposed to identical air temperature fluctuations, as a consequence of their unequal thermal properties. Air temperature likely contributes significantly to soil temperature (therefore the observed correlation between T_{4i} and phenological responsiveness across all species) but it does not explain it entirely, being influenced also by other factors, as soil moisture regime.

Lakshimi *et al.* (2003) have observed that, during desiccation, surface temperature shows an increase that corresponds to a decrease in the soil moisture. Closely related species might be responding similarly to temperature in our study, but more strongly to soil temperature than to air temperature, producing the observed differences between xeric and mesic species.

It is also important to note that the present study did not have the statistical power that was used by Mazer *et al.* (2013) to detect phylogenetic similarities, in which they studied over 1000 species. This indicates that the phylogenetic signals in phenological responses might become more evident in studies incorporating a large number of species, where some of the ecological attributes of certain species (e.g. differences between species from mesic and xeric habitats) could be filtered out of the conclusions as background noise. Likely, both phylogenetic and ecological considerations must be made in attempting to predict phenological responses in plants.

The potential for pollinator mismatch also deserves a special attention. Studies concerned with this question should consider investigating the environmental cues that are used by pollinators in their life cycle timing (e.g. dormancy break, eggs eclosion, pupation period). Orton (1989) suggest that in temperate zones, both plants and insects develop in spring in response to heat accumulation similarly, inferring that plant phenology can help predict the timing of emergence of insect pests. However, if the predictions offered by this study are considered valid, this statement might not always be true.

Under certain circumstances, plant and pollinators phenology might not respond to environmental cues in the same way. Our observations suggest that the heat accumulation along the spring have different effects on plant species from mesic and xeric habitats. It is proposed that the thermal properties of an individual's environment determines the experienced soil temperature fluctuations and that soil temperature has a relevant role in regulating plant phenology (Greer 2005). If this is assumed to be true, then a hypothetical pollinator that responds to spring heat accumulation would have to develop in contact with the same type of soil, with similar moisture content, where its target plant species is found. This way both plant and pollinator would be exposed to the same soil thermal properties and temperature fluctuations. In all other cases, a pollinator mismatch could occur during climate shifts. In the case of the bumblebee (genus *Bombus*), some species nest underground and some above ground. If the nest is established above ground, it is expected that the pollinator phenology might be more correlated with air temperature than with soil temperature, which might create a larger

risk of pollinator mismatch for plants from mesic habitats (that would be exposed to an environment better insulated from the air temperature).

This study suggests that different plant species respond differently to shifts in climate, which is not necessarily aligned with their pollinator's phenological response. The determination of which environmental cues are used by pollinators will help to clarify which species might be under greater risk of pollination mismatch. Given that the environmental variability might be behind the differential phenological responses to shifts in climate, it is expected that plants could be adapted to coexist away from equilibrium with intermittent periods of pollination mismatch, which might even promote species diversity in some circumstances (Chesson & Warner 1981). It is unclear, however, how far this dynamic system can be extrapolated for certain species under the current rates of climate change. Climate-change-induced phenological shifts might also affect some species and communities adversely, exposing them to competitive disadvantages or higher frost risk, as described by Augspurger (2013).

3.3 Future directions

Further investigations are recommended in order to clarify some questions raised by this study. The hypothesis of soil thermal properties as a mechanism for explaining the observed differences between the phenological responses of plants from mesic and xeric habitats needs to be tested with models that incorporate the specimens' soil temperature fluctuations as a function of soil type, moisture regime and the environmental energy transfers in place during spring and summer. Also, experimental methods that directly

measure soil temperature and phenological responses under different moisture regimes could be used to test this hypothesis.

The results indicate that for some species there is a set of environmental cues, as opposed to only one parameter, that is behind the flowering burst timing. Even for the species and parameters considered in this study, it is important to consider that the limitations in number of specimen replicates, or in their geographic range or timespan, might restrict the statistical power and the ability to recognize true significant phenological responses. Future research might also consider including the analysis of genetic material from voucher specimens in order to help clarify the phenological responses of plant species, the mechanisms involved in the processes and their evolutionary history. The elucidation of the ecological implications involving plants phenological responses is key for the development of effective ecosystem conservation programs in a context of global climate change.

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Appendix

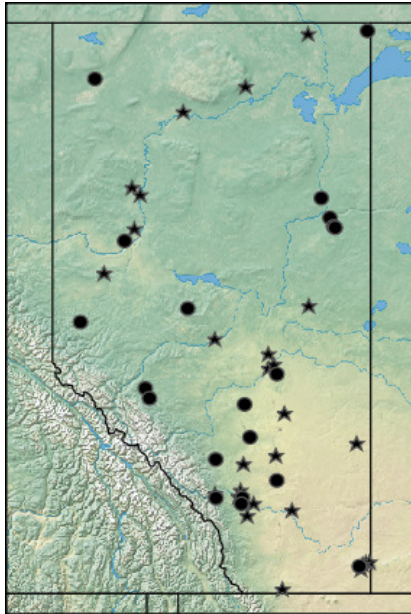
Table A: Habitat description in Alberta by Moss (2012) for the species selected in this study.

Order	Habitat description in Alberta	
	Mesic	Xeric
Lilales	<i>Prosartes trachycarpa</i> "Moist woods and thickets"	<i>Lilium philadelphicum</i> "Open woodlands and prairie"
Rosales	<i>Rosa acicularis</i> "Woods, banks roadsides"	<i>Rosa arkansana</i> "Plains and prairies"
Ranunculales	<i>Thalictrum venulosum</i> "Woods, thickets and moist prairie"	<i>Pulsatilla patens</i> "Prairies and dry open woods"
Fabales	<i>Astragalus americanus</i> "Stream banks and moist woods"	<i>Thermopsis rhombifolia</i> "Dry grassland"
Poales	<i>Calamagrostis Canadensis</i> "Marshes and moist woodland"	<i>Koeleria macrantha</i> "Common in prairie grasslands"
Asterales	<i>Petasites frigidus</i> "Moist woods and swamps"	<i>Achillea millefolium</i> "Prairie, clearings, roadsides, waste ground"
Asparagales	<i>Sisyrinchium montanum</i> "Moist open areas"	<i>Allium textile</i> "Common on dry plains and hills"

Table B: Average (\pm SEM) values of peak flowering date, T4i, P4i and latitude found for the species include in the study.

Habitat	Species	n	Average peak flowering date	Average T4i	Average P4i	Average Latitude
			Day-of-the-year (\pm SEM)	Mean \pm SEM ($^{\circ}$ C)	Mean \pm SEM (mm)	Degrees \pm SEM
Mesic	<i>Prosartes trachycarpa</i>	24	146.9 (\pm 2.2)	0.3 (\pm 0.4)	34.6 (\pm 3.4)	51.9 (\pm 0.4)
	<i>Rosa acicularis</i>	27	174.7 (\pm 3.9)	4.8 (\pm 0.3)	46.8 (\pm 4.1)	52.7 (\pm 0.5)
	<i>Thalictrum venulosum</i>	32	164.6 (\pm 2.0)	5.7 (\pm 0.3)	47.2 (\pm 3.6)	52.4 (\pm 0.4)
	<i>Astragalus americanus</i>	24	191.9 (\pm 1.8)	10.1 (\pm 0.2)	55.1 (\pm 3.3)	54.2 (\pm 0.6)
	<i>Calamagrostis canadensis</i>	20	221.9 (\pm 4.9)	13.1 (\pm 0.4)	74.3 (\pm 5.3)	54.1 (\pm 0.6)
	<i>Petasites frigidus</i>	23	138.2 (\pm 2.7)	-1.2 (\pm 0.5)	28.3 (\pm 3.9)	53.6 (\pm 0.5)
	<i>Sisyrinchium montanum</i>	23	165.6 (\pm 2.9)	5.6 (\pm 0.3)	48.2 (\pm 2.5)	51.5 (\pm 0.3)
Xeric	<i>Lilium philadelphicum</i>	27	185.4 (\pm 1.6)	10.5 (\pm 0.2)	59.5 (\pm 3.2)	52.6 (\pm 0.3)
	<i>Rosa arkansana</i>	20	198.3 (\pm 4.3)	11.2 (\pm 0.3)	56.6 (\pm 4.0)	52.3 (\pm 0.4)
	<i>Pulsatilla patens</i>	31	130.9 (\pm 3.4)	0.5 (\pm 0.3)	35.8 (\pm 3.1)	50.6 (\pm 0.2)
	<i>Thermopsis rhombifolia</i>	44	150.7 (\pm 2.3)	0.6 (\pm 0.3)	32.9 (\pm 1.9)	51.0 (\pm 0.1)
	<i>Koeleria macrantha</i>	31	183.1 (\pm 2.4)	10.4 (\pm 0.2)	54.0 (\pm 3.2)	53.1 (\pm 0.5)
	<i>Achillea millefolium</i>	67	214.4 (\pm 4.7)	13.6 (\pm 0.2)	62.5 (\pm 1.9)	51.8 (\pm 0.2)
	<i>Allium textile</i>	37	154.4 (\pm 2.0)	6.3 (\pm 0.2)	48.4 (\pm 2.8)	50.5 (\pm 0.1)

Poales



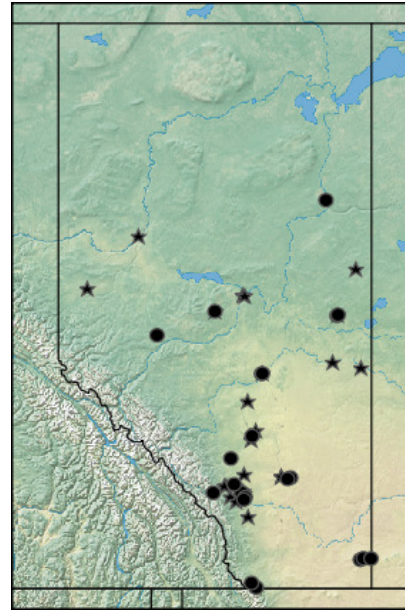
- Calamagrostis_canadensis
- ★ Koeleria_macrantha

Sample timespan

C. canadensis: 88 years (1925-2013)

K. macrantha: 83 years (1927-2010)

Liliales



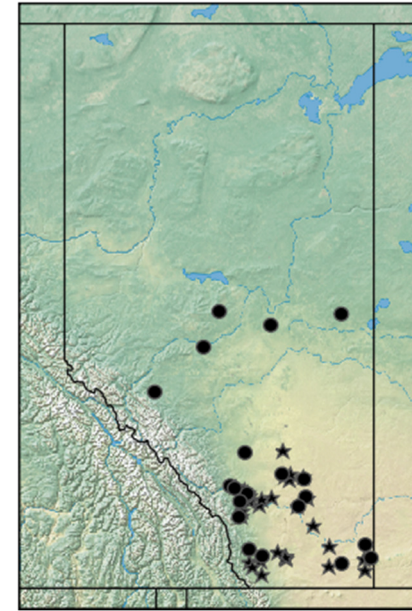
- Prosartes_trachycarpa
- ★ Lilium_philadelphicum

Sample timespan

P. trachycarpa: 51 years (1955-2006)

L. philadelphicum: 76 years (1934-2012)

Asparagales



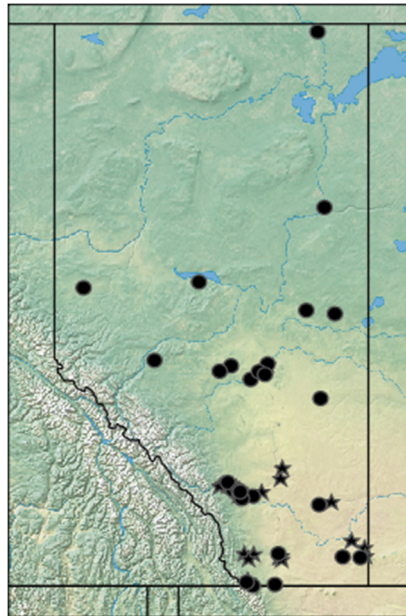
- Sieyrrinchium_montanum
- ★ Allium_textile

Sample timespan

S. montanum: 56 years (1953-2009)

A. textile: 98 years (1915-2013)

Ranunculales

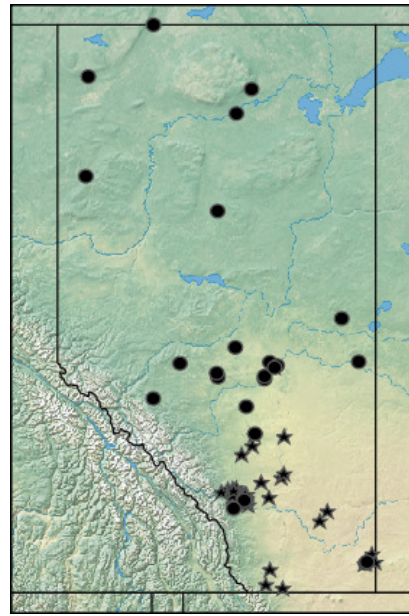


- *Thalicttrum_venulosum*
- ★ *Pulsatilla_patens*

Sample timespan

T. venulosum: 91 years (1915-2006)
P. patens: 66 years (1946-2012)

Fabales

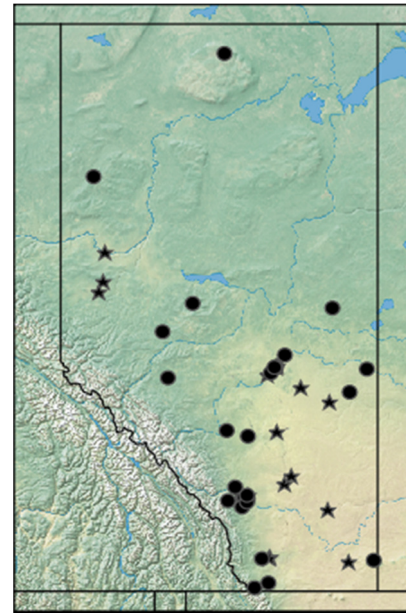


- *Astragalus_americanus*
- ★ *Thermopsis_rhombifolia*

Sample timespan

A. americanus: 95 years (1914-2009)
T. rhombifolia: 84 years (1925-2009)

Rosales

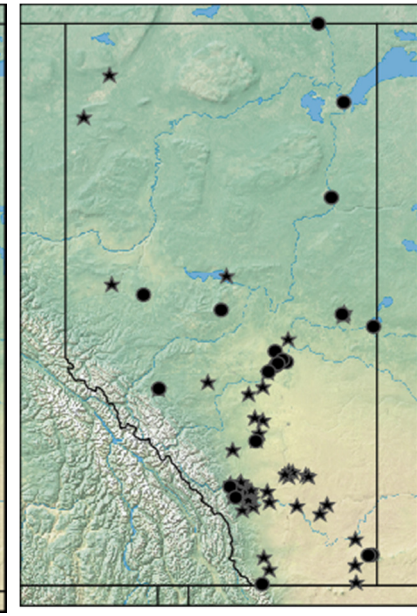


- *Rosa_acicularis*
- ★ *Rosa_arkansana*

Sample timespan

R. acicularis: 75 years (1936-2011)
R. arkansana: 82 years (1927-2009)

Asterales



- *Petasites_frigidus*
- ★ *Achillea_millefolium*

Sample timespan

P. frigidus: 79 years (1920-1999)
A. millefolium: 86 years (1925-2011)

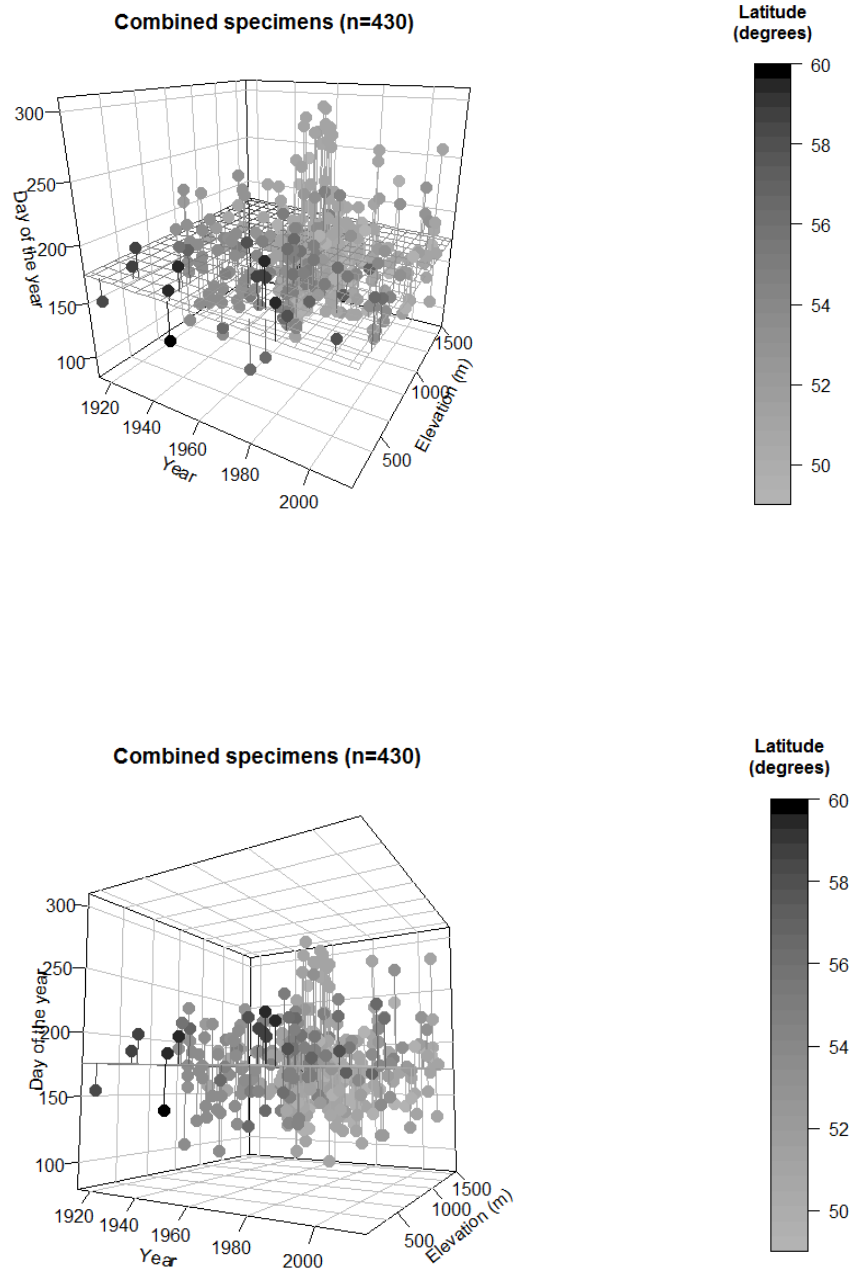


Figure A: Multiple regression visualisation for all combined specimens included in this study. No significant responsiveness was detected ($p > 0.05$). The peak flowering date (day of the year) was regressed against year, elevation (m) and latitude.