MONASH University

Impacts of Phenology and Environmental Variation on the Reproductive Success of Invasive Willows

Thesis submitted for the degree of Doctor of Philosophy

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Abstract

Invasive plant prevalence is growing at a global-scale. This is a problem for native ecosystems due to the ability of invasive plants to replace native vegetation, act as disease vectors, and alter ecosystem nutrient cycling, hydrology, and disturbance regimes. In order to minimize the negative impacts of invasive plants, it is important to understand how those species are spreading through space and time. The ability of an invasive plant to spread is largely facilitated by its surrounding environment, particularly the weather and climate. Climate, for example, can affect the timing of reproductive events, or phenology, which can alter a plant's demography. One way to observe the effects of climate in-situ is by quantifying effects across elevational and latitudinal gradients.

In order to better understand the spread of invasive species, I focussed on capturing key information regarding the reproductive ecology and phenology of a highly invasive plant, *Salix cinerea*, across the elevation gradient inhabited in its invaded range. Specifically, I aimed to: 1) quantify the phenology of *S. cinerea* across low and high elevation, 2) investigate the effects of intraspecific phenology on reproductive output, 3) compare the germinability of seeds matured at two elevations and germinated at two temperatures, and 4) identify global trends in fecundity with respect to climate and geography for Salix at the genus-level. To answer these questions, I quantified phenology and seed output at low and high elevation in north-eastern Victoria, Australia, where *S. cinerea* is arguably the most widespread. Germination ability of seeds were tested experimentally to identify their viability across elevation, depending on where they were matured or their germination conditions. Global scale trends were also assessed using herbarium records collected from around the world.

The phenology of *S. cinerea* was successfully quantified, which had not previously been empirically recorded in the literature. I also identified differences in heat accumulation patterns for *S. cinerea* between low and high elevation, which may help describe the species phenology under climate warming. The phenology of pollen release and stigma receptivity overlap was indicative of seed output between low and high elevation. Seed output was 63% higher per individual at low elevation, but high elevation individuals produce seeds with about 15% higher germination rates than at low elevation overall. These results are significant because they describe where major seed sources might exist across the landscape of north-eastern Victoria, Australia.

Finally, when considering 17 Salix species globally, I found that fecundity varies more in response to environmental effects between species than across the genus.

Further, when accounting for climatic and geographic effects, three out of five species studied showed range (invasive or native) had a significant effect on fecundity. This thesis establishes novel insights to classic ecological concepts as they are related to invasive, wind-dispersed plants. I also have provided a greater understanding of how invasive plants may respond to a changing climate. This important ecological information can be used by land managers to assess how to best manage invasive plant species under future environmental changes.

General Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper currently under review in a peer-reviewed journal and three unpublished publications. The core theme of the thesis is ecology of invasive plants. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Biological Sciences under the supervision of Dr. Joslin Moore.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapters 2, 3, 4, and 5, my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
2	Duration, but not timing, of flowering phenology changes with elevation in an invasive shrub willow (*S. cinerea*)	Submitted	Design, data collection, data analysis, and writing of manuscript 80%	-Dr. Joslin Moore: design, analysis, contributions to writing, 10% -Dr. Cat Mills: analysis, contributions to writing, 6% -Dr. Richard Duncan: design, analysis, contributions to writing, 4%	No
3	New methods reveal incompatibility of flowering phenology across elevation causes lowered seed output in an invasive,	Not submitted	Design, data collection, data analysis, and writing of manuscript 80%	-Dr. Joslin Moore: design, analysis, contributions to writing, 10% -Dr. Martin Burd: analysis, contributions to writing, 7% -Dr. Richard Duncan: design, analysis, contributions to writing, 3%	No

	dioecious shrub				
4	Effects of elevation and seed size on the competition-colonization trade off in a small-seeded invasive species (*S. cinerea*)	Not submitted	Design, data collection, data analysis, and writing of manuscript 80%	-Dr. Joslin Moore: design, analysis, contributions to writing, 10% -Dr. Martin Burd: analysis, contributions to writing, 5% -Dr. Cat Mills: design, data collection, contributions to writing, 5%	No
5	Variation in fecundity across geographic and climatic gradients in the willow (*Salix*) genus	Not submitted	Design, data collection, data analysis, and writing of manuscript 80%	-Dr. Martin Burd: analysis, contributions to writing, 10% -Dr. Joslin Moore: design, contributions to writing, 7% -Dr. Richard Duncan: design, 3%	No

I have renumbered sections of my submitted paper in order to generate a consistent presentation within the thesis.

Student signature: [redacted] **Date:** 29 November 2018

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: [redacted] **Date:** 29 November 2018

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Table of Contents

Chapter 1

General Introduction

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Invasive plants in a changing world

Invasive species prevalence has been increasing over time largely due to human activities, with invasive plants making up the greatest proportion of total invasive species (Seebens et al., 2017). The global increase of invasive plants is problematic due to their ability to replace native vegetation and subsequently influence biodiversity loss (Vitousek et al., 1997, Strayer et al., 2006, Litt et al., 2014). Invasive plants can also act as vectors of new diseases, and are capable of altering ecosystem processes such as primary productivity, hydrology, and nutrient cycling (Vitousek et al., 1996). They have the potential to substantially modify existing disturbance regimes, or introduce novel disturbances, such as fire or erosion, which result in changes to community structure and ecosystem function (Mack and D'Antonio, 1998). Even invasive plant management itself can cause a 'weed-shaped hole' in ecosystems by increasing disturbances imposed by weed managers, and therefore increasing the likelihood of re-invasion by the target or another invasive plant (Buckley et al., 2007). As such, invasive plants have been widely shown by researchers to have lasting effects on their invaded ecosystems (Strayer et al., 2006).

In order to minimize the negative impacts of invasive plants, it is important to understand how those species are spreading through space and time. The successful spread of invasive species is the result of a number of different factors related to the attributes of the plant and its surrounding environment (Blackburn et al., 2011). Many of these attributes are nearly impossible to forecast, making predicting species spread inherently difficult (Clark et al., 2003). We can think of the spread of any plant species as the result of pre-dispersal, dispersal, and post-dispersal events (Travis et al., 2012). There are a number of pre-dispersal factors, including environmental conditions where an individual is grown, that can influence seed production up to the time of seed release. Conditions before dispersal events can influence the amount and quality of seed produced (Valencia-Diaz and Montana, 2005) as well as the timing of seed release (Jongejans et al., 2007). Dispersal includes the movement of a seed from release to the arrival site (Nathan et al., 2011) and is thought to affect spread more dramatically than a species' demographic traits (Coutts et al., 2011). Once the seed has dispersed and is at its germination site, it is in the post-dispersal phase. The environmental conditions at the arrival site influence

the ability of a species to establish, and vary substantially across a landscape. This implies that seeds which are capable of traveling long distances before germination may have arrival site conditions different from where they were developed and dispersed (Hampe, 2011).

The surrounding environment, climate and weather in particular, plays an important role in regulating all three phases of spread (Primack, 1987). Weather conditions in which an individual resides influence both the pre-dispersal and dispersal phases, as well as the success of arriving seeds post-dispersal to new locations. Pre-dispersal events such as seed abscission generally require warming temperatures and lowered humidity (Jongejans et al., 2007). The dispersal phase, particularly of wind-dispersed seeds, is highly influenced by wind speed (Nathan et al., 2011) and topographic position (Heydel et al., 2015). Weather conditions also affect post-dispersal events, including germination and establishment. Seed germination requires varying levels of light (Dlugos et al., 2015) and cumulatively warming temperatures (Jarvis and Moore, 2008, Hou et al., 2014). Similarly, growth to reproductive maturity and establishment can only occur if a germinated seed has enough of the required water, sun, and soil nutrients to support its maturation (Kozlowski and Pallardy, 1997).

Weather conditions also affect the timing of seasonal events, or the phenology, of plant species (Walther et al., 2002). Phenological events, like bud break, seed release, and foliation, often initiate in response to weather changes, usually in the form of changing temperatures (Gordienko and Sokolov, 2009) and precipitation levels, including rainfall, snowmelt, and relative humidity (Kudo and Ida, 2013, Schwartz, 2013). In particular, seed release initiation in deciduous trees is generally initiated by weather-related factors including ambient air temperature (Gordienko and Sokolov, 2009, Fernandez-Martinez et al., 2012) and precipitation (Borchert et al., 2002, Santinelo Pereira et al., 2007, Kushwaha et al., 2011), as well as individual flush development (Lechowicz, 1995, Olesen, 2005, Wilkie et al., 2008) and day length (Rivera and Borchert, 2001, Kushwaha et al., 2011). Fine-scale reproductive and foliation phenology data is crucial to predicting how the species may spread across a landscape (Cleland et al., 2012). Studies have shown that phenology influences reproductive output and is thereby a determinant of fitness (Lediuk et al., 2014, Wheeler et al., 2015). More specifically, the timing of flowering

affects the success of fruit maturation and progeny quality, the success of pollination, and the level of seed and fruit herbivory (Chuine, 2010). Reproductive phenology of plants indirectly affects the likelihood of seed survival post-dispersal as well; depending on the timing of seed release, the weather conditions in which the seed arrives at a new site may or may not be conducive to successful germination and establishment of the seed (Skov and Svenning, 2004). Plant species are more capable of establishment in a new habitat if their major reproductive phenology events occur at a time when resources are abundant and not utilized by other native species (Wolkovich and Cleland, 2011).

Since key species traits are so strongly affected by surrounding weather conditions, it is unsurprising and widely reported that climate change can affect species invasions (Dukes and Mooney, 1999, Sherry et al., 2007). There is evidence that climate change can lead to invasive species widening their distributions (Walther et al., 2009). Alternatively, temperature and precipitation changes as a result of climate change can have negative effects on species distributions by increasing pollinator mismatch in the invasive plant (Kudo and Ida, 2013). Previous research has shown the effect of phenological mismatches as a result of seasonal weather changes (Wheeler et al., 2015). In addition to temperature and precipitation climate change effects, there is substantial evidence that an increase in the frequency of extreme weather events associated with climate change will impact ecosystems by altering species distributions, species interactions, and other ecosystem processes (Coumou and Rahmstorf, 2012). Similarly, extreme weather events have potential to disrupt the timing and duration of major phenological events (Reyer et al., 2013). In response to these environmental changes, there is expected to be considerable variation among species in their invasibility as a result of climate change (Diez et al., 2012, Orsenigo et al., 2014).

As the globe continues to warm, there is evidence that the abundance of many invasive plants will increase and thus require even more strategic management intervention than before (Merow et al., 2017). However, management of invasive species is difficult because there are a multitude of factors to consider in determining how the distribution of a species will shift across a landscape (Buckley et al., 2007). Thus, despite their pervasive existence across natural landscapes, there is still considerable uncertainty on how to best manage invasive species.

Managers often focus on limiting the spread of invasive species across landscapes in order to contain the distribution of the species (Moody and Mack, 1988). To make the management of limiting species spread more practicable, it is vital that we understand what is significantly affecting the spread of individual invasive species across a wide range of space (Coutts et al., 2011, Glen et al., 2013). In particular, if we can predict the spread of invasive plants, managers will be able to target particularly threatening seed source locations for control, as well as focus surveillance efforts to regions where new populations are most likely to establish.

Why willows? The Salix *genus*

In this thesis, the willows genus, *Salix,* will be the taxon of interest as it includes many successful invasive species (Cremer, 2003) and requires additional information regarding its reproductive ecology in order to be properly managed. There are between 330 and 500 shrub and tree species of *Salix* growing worldwide and native on every continent except Australia (Isebrands and Richardson, 2014). The genus is important culturally, medicinally, and ecologically. Some species are used in cricket bat production or as the base for furniture, and others appear in children's books, novels and plays, from Kenneth Grahame to Shakespeare (Carleton, 1949). *Salix* species have also been used by aboriginal cultures for thousands of years as an analgesic. The pain-relieving salicylic acid has in the last century been isolated and manufactured as the primary active ingredient in aspirin (Vlachojannis et al., 2011). The genus also has broad ecological significance. Rows of willows are frequently planted by farmers as shade trees for cattle and to slow riverbank erosion (Ladson, 1997). Willows can also affect below-ground communities, altering arbuscular mycorrhizal fungi colonization (Becklin et al., 2012). Additionally, the genus is associated with a number of fungal and insect pests (Cha et al., 2009, Caron, 2011). In recent decades, the *Salix* genus has also become a prominent invasive plant across temperate Australia and New Zealand.

Over 80 species of *Salix* have been introduced in Australia from Europe and N. America, and the entire plant genus has been listed as a Weed of National Significance, giving it high government priority for control efforts (2003). The genus is almost exclusively riparian and were introduced to Australia for the protection of riverbanks and to provide shade to cattle (Cremer, 2003). However, over time *Salix*

spp. have actually been worsening the erosion of the river banks by widening and shallowing the stream beds (Cremer, 2003). Due to a multitude of unique demographic traits, the genus is exceedingly difficult to eradicate. For example, *Salix* species are capable of vegetative reproduction, in addition to reproduction by seed, which allows them to expand their geographic distributions via broken branches travelling downstream the rivers they have invaded (Isebrands and Richardson, 2014). This is significant because the genus is also dioecious, with many of the species having only male or female plants present in their invaded countries but not both (Cremer, 1999). Similarly, the many species within the genus hybridize readily and often, allowing hybrid crosses to often reproduce by seed regardless of the presence of both sexes of any given species (Cremer, 2003). Further, the genus produces tiny, pappus-bearing seeds capable of dispersing long distances by wind, enabling an ease of establishment to distant sites (Gage and Cooper, 2005).

The first three of the upcoming four data chapters aim to better understand the reproductive ecology of a particularly invasive *Salix* species, *Salix cinerea,* in order to inform managers how to intelligently and effectively manage their removal. Specifically, they examine how surrounding environmental conditions regulate the fecundity and potential spread of *S. cinerea*. *S. cinerea* is a widely invasive deciduous shrub willow native to northern Europe and now found across south-eastern Australia, Tasmania, and New Zealand (Holland Clift and Davies, 2007). This species, like other members of the genus, causes severe erosion in riparian zones, as well as overshading and nutrient overload in surrounding rivers during autumn defoliation (Cremer, 2003) (Figure 1.1). *S. cinerea* is a particularly threatening weed in Australia because it can invade a wider range of habitats than all other members of its genus, moving out of riparian zones and into fragile, nationally protected alpine wetlands and *Sphagnum* bogs (Carr, 1996). The species also grows across nearly the entire elevation gradient found in Australia, from approximately 200-2000 meters. Additionally, *S. cinerea* is one of the few *Salix* species that produce seed in Australia, as a result of both male and female individuals having been introduced in the 1950s (Cremer, 2003) (Figure 1.2). Maternal and paternal genetic assignment analysis has identified that *S. cinerea* seeds frequently disperse downstream, upstream, and between catchments in south-eastern Australia, with 25% of seeds travelling between 25 and 50 kilometres (Hopley, 2011).

Thesis objectives and organisation

This thesis uses *Salix* to better understand several ecological concepts and answer important land management questions. The research presented here was developed as part of a larger multi-institutional project aiming to understand the spread of *S. cinerea* across the landscape of south-eastern Australia. My thesis will fit into this project by providing key information about the reproductive ecology of the species, as well as phenological data that is currently being incorporated into a landscape-scale seed dispersal model. Overall, this project aims to identify current primary seed sources of *S. cinerea* across south-eastern Australia and identify where new populations are likely to become problematic next, based on where they are currently the fastest-spreading.

In this thesis, I attempt to better understand several elements of each of the pre-dispersal, dispersal, and post-dispersal phases of spread in *S. cinerea.* My thesis is organised into the following chapters, each of which acknowledge different aspects of the three identified phases of spread (Figure 1.3):

- **Chapter 2** focuses on *S. cinerea* phenology, and the effect of surrounding temperature on populations growing at low and high elevation. In this chapter we discuss how the phenology of an invasive plant in varying climatic conditions can be descriptive of the species' success under a changing climate. This chapter primarily creates new knowledge about the species pre-dispersal spread phase by quantifying the phenological timing of key reproductive and foliation events.
- **Chapter 3** delves further into *S. cinerea* pre-dispersal by investigating the influence of intraspecific phenological overlap on seed output across low and high elevation.
- **Chapter 4** investigates elements of all three phases of spread by considering the germinability of *S. cinerea* seeds produced between low and high elevation, and under average low and high elevation temperature conditions. This chapter is discussed in the context of the competition-colonisation trade-off hypothesis (Turnbull et al., 2004) and its applicability for wind-dispersed seeds.
- **Chapter 5** looks broadly at the influence of climatic and geographic parameters on fecundity at a global scale. This chapter provides novel

insights about the pre-dispersal environmental effects on the reproductive output of both *S. cinerea* and 16 other *Salix* species.

This thesis is presented as a “thesis by published works”, plus a general introduction (Chapter 1) and general discussion (Chapter 6). Chapter 2 has been submitted to Plant Ecology and was returned with a ‘reject and resubmit’ outcome, and we aim to resubmit this chapter shortly after this thesis is submitted. I was responsible for the planning and execution of all field, lab, and herbarium data collection, data analysis, and manuscript preparations as described in this thesis. However, I use the first-person plural in each chapter to reflect the collaborative nature of the research, except in the general discussion (Chapter 6), where I will discuss my personal reflections. Because these chapters were written in the style of free-standing, independent research articles, there is some repeating content between Chapters 2-5, specifically information describing the reproductive ecology of the *Salix* genus and *S. cinerea.*

Figures

Figure 1.1. *Salix cinerea* growing along the highly disturbed Yarrabula Creek in south-eastern Australia. *S. cinerea* are the light green shrubs hugging the river on the right, as well as in the river "island" on the left side of the image. The surrounding forest is dominated by *Eucalyptus* and *Acacia* species. Photograph was taken in late October, 2016. Image credit: Emily De Stigter.

Figure 1.2. Maturing *Salix cinerea* female catkins (top left), female catkins in seed release (top right), and male catkins in pollen release (bottom). All images taken at low elevation Australia in the spring of 2016. Image credit: Emily De Stigter (top left, bottom); Catherine Mills (top right).

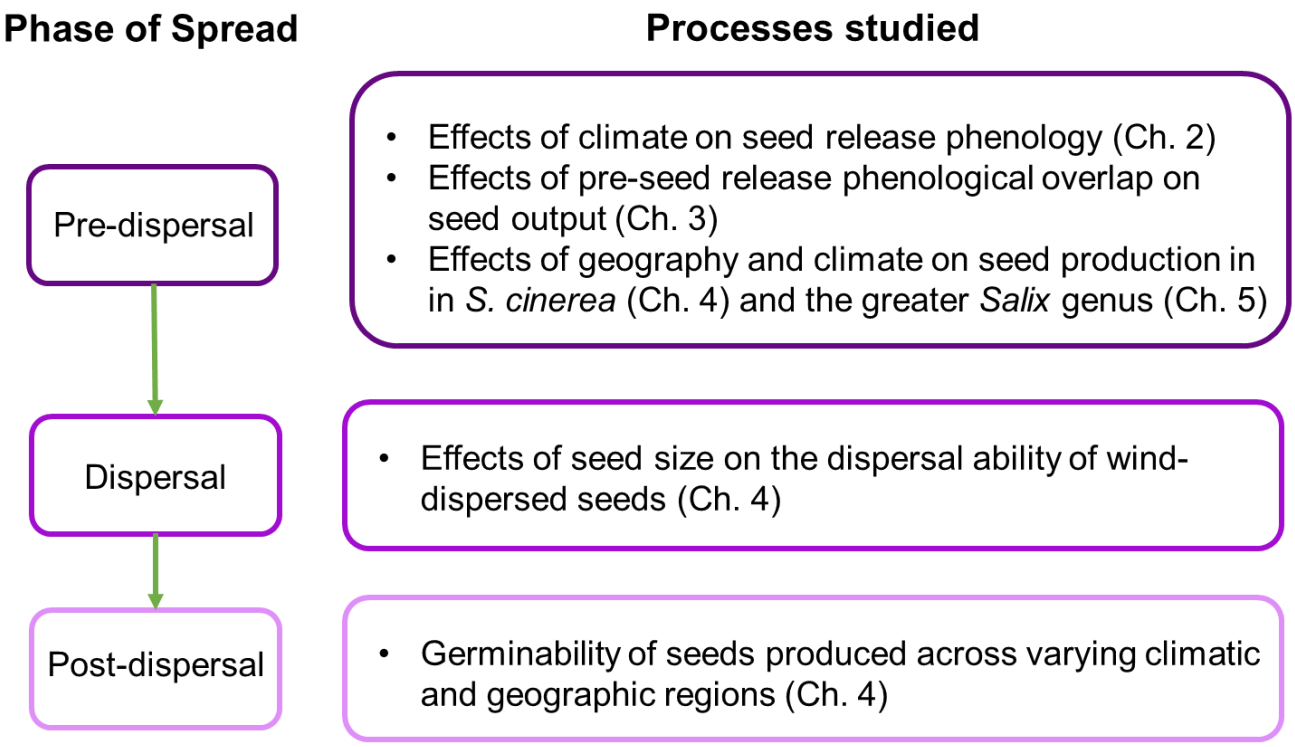

Figure 1.3. Conceptual model outlining the processes studied for each of my four data chapters, as they relate to the phases of spread.

Chapter 2

Duration, but not timing, of flowering phenology changes with elevation in an invasive shrub willow (*S. cinerea*)

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Abstract

Within-species variation in the initiation and duration of phenological events has the potential to influence key reproductive events and the overall spread of the plant. This is particularly pertinent for invasive plants in a warming climate because a shift in phenological timing could result in the invader occupying a different temporal niche, thereby increasing competition with native species for pollinators. Here we studied a widely invasive shrub willow, *Salix cinerea,* in south-eastern Australia. We quantified the initiation and duration of its reproductive and foliar phenological events to examine how the initiation of these events varied across elevation. Phenology was monitored for a two seasons of growth at four low elevation sites (≤410 m) and one high elevation site (1639 m).

We found that the initiation of flower bud break was correlated with heat accumulation, and the duration of flower bud break drove the initiation of all subsequent reproductive phenophases. This implies that, apart from flower bud break, reproductive events in *S. cinerea* are only indirectly affected by cumulative temperature, via its effect on flower bud break, and that subsequent phenological events are relatively insensitive to temperature. We also found that phenological events were initiated under lower cumulative temperatures at high elevation than low elevation, despite the phenophases occurring later in time at high elevation sites. These findings suggest that *S. cinerea* will not be limited by reproductive phenology as the climate changes.

Introduction

It is well established that phenology in plants is heavily influenced by temperature, and a warmer climate causes reproductive and foliation phenology events to happen earlier in time (Spano et al., 1999, Walther et al., 2002, Cleland et al., 2007). Most plant phenology research focusses on the initiation of single phenophase of interest, often across environmental gradients or in warming experiments which aim to replicate climatic conditions under global warming (Post et al., 2008, Hudson and Keatley, 2013). However, not addressing the duration of key events, and the phenophases surrounding those events, may lead to the loss of fundamental information relevant to a species' population demography. For example, the duration and overlap of pollen release and stigma receptivity may affect seed fertilisation rates and fecundity (Dafni and Firmage 2000). Seed maturation conditions and the timing of seed release can affect dispersal ability (Nathan et al., 2002, Heydel et al., 2015) and seed rain (Buechling et al., 2016). Additionally, the duration of growing season length is a strong indicator of growth rate, which is related to overall plant fitness (Myneni et al., 1997). Moreover, the initiation and duration of phenological events may or may not respond similarly to climate warming and thereby affecting species' demographics (Post et al., 2008). What remains unclear is when in a series of phenological events the effects of temperature have the largest influence on the duration and initiation of those events.

Both the initiation and the duration of phenological events are closely linked to surrounding environmental conditions, especially temperature (Cleland et al., 2007, Allen et al., 2014, Zohner and Renner, 2014). The dependence of phenology on temperature can affect the spread of a plant in various ways. The timing of plant phenology, for example, may be tied to plant traits which describe their competitive ability (Rathcke and Lacey, 1985). In male plants the timing of flowering describes when their pollen is available, and thus when competition for pollinators may occur between and within species (Campbell and Motten, 1985). In deciduous plants initial foliation and leaf fall describes the length of the growing season, as well as the period when species' are at risk of herbivory (Mooney and Gulmon, 1982). Growing season length is positively affected by heat accumulation, such that plants in cooler climates have less growth per day, as well as a shorter temporal window when temperatures are acceptable for growth (Chen et al., 2005). Further, plant growth

rate is also directly dependent on the number of days when plants are producing leaves in a season (Myneni et al., 1997). One way to observe temperature effects in-situ is by observing plant phenology across an elevation gradient (Ranjitkar et al., 2013). Plant phenology at high elevation has been shown to lag behind plants at lower elevation due to the cooler temperatures (Schwartz 2013). Similarly, tree growth rates decline as elevation increases, likely in response to shorter growing season length (Coomes & Allen 2007).

Attention to the initiation and duration of phenological events is of particular importance for invasive species under climate change because phenology plays an important role in the success of invasive species (Wolkovich and Cleland, 2011). Invasive species phenology has been shown to influence the timing and abundance of community-level flowering (Wilke and Irwin, 2010). Since different ecosystems can have unique phenological responses to climate change at a community level (Sherry et al., 2007), it is important to focus on understanding invasive taxa to identify whether their ecosystem impact will increase or decrease with warming temperatures (Walther et al., 2002). What remains unclear is the influence of changing temperature on the duration of phenological events, even though this can have substantial impacts on demographics. Here we will focus on an invasive shrub willow species, *Salix cinerea,* which currently occupies a wide climatic range in its invaded distribution. We compare the effects of temperature on phenological initiation and duration across *S. cinerea's* invaded elevation gradient as an analogue for climate change. With this information, researchers will better understand how a warming climate and increasing climate variability may affect the phenology of cool-climate invasive plants. Focussing on variation in the initiation and duration of seed dispersal can have important implications for spatial patterns of spread (Treep et al., 2018) with consequences for spatial targeting of management (Hauser and McCarthy, 2009).

S. cinerea is a dioecious, deciduous, woody shrub which aggressively invades south-eastern Australia, Tasmania, and New Zealand (Ladson, 1997, Cremer, 2003). The species is native to northern Europe, where its phenology has not previously been quantified. In Australia, previous research on *Salix* phenology has been qualitative and disregards effects of temperature (Cremer, 2003). This species is particularly problematic in Australia because it grows along a wide elevation

gradient, invading riparian areas at low elevation and endangered peatlands at high elevation (Moore and Runge, 2012, Hopley and Young, 2015). Unlike most other species of *Salix* introduced to Australia, *S. cinerea* has both male and female individuals established, and can thus reproduce by seed as well as vegetatively (Cremer, 1999). Single populations of *S. cinerea* are capable of releasing millions of light-weight, pappus-bearing, wind-dispersed seeds in a single reproductive season (Hopley, 2011). The demography of this species is likely to be affected by the duration of phenological events, since flowering phenology of males and females must be overlapping, and it is pollinated by insects and wind, both of which are likely to vary seasonally. Since these seeds are thought to often travel long distances (≥50 km) (Hopley, 2011), populations of *S. cinerea* could reasonably disperse their seeds across elevation and into endangered peatlands (Moore and Runge, 2012). Previous research has shown that dispersal patterns are important for the spatial allocation of control effort (Moore and Runge, 2012), with dispersal potential affected by seasonal wind patterns and so likely influenced by phenology.

Here we observe the phenology of *S. cinerea* over time to explore how temperature influences the duration and timing of flowering and leafing events. Specifically, we aim to: 1) quantify differences in the initiation and duration of phenological events across the invaded region, investigating populations at both low and high elevation which experience different temperature conditions and 2) examine whether heat accumulation requirements influence the initiation of primary reproductive events. We expect that date and heat accumulation measures explain variation in the timing or duration of primary reproductive events (bud break, pollen release, and seed release). We expect that heat accumulation will be informative for describing the initiation and duration of phenological events, as well as the timing of preceding phenological events. An improved understanding of how the species phenology might shift with warming temperatures provides useful insight regarding the ability of a species to flourish in a new environments and its likely response to climate change.

Methods

We monitored five mature *S. cinerea* populations across an invaded region in north-eastern Victoria, recording the timing of key reproductive and foliation events.

Study sites

In September 2015 field sites were established in north-eastern Victoria, Australia. At low elevation (292-410m), four sites were established in the Ovens River Catchment along Yarrabula Creek and Buckland River, each containing 21-28 mature individuals (Figure 2.1, Table S2.1). Sites were determined based on researcher accessibility and number of individuals. At high elevation, one site was monitored in Dinner Plain, Victoria (1639m) along a small, unnamed creek and peatland, approximately 50 km southeast of the Ovens Catchment sites. Due to recent major willow removal efforts in accessible regions of south- eastern Australia, suitable study sites were limited and only one site was identified at high elevation containing 10 or more flower-bearing individuals.

Phenological monitoring

Phenological monitoring was conducted over two growing seasons, spanning 20 months from September 2015 to May 2017 with monthly monitoring in season one and weekly monitoring in season two. All statistical analysis on flower phenology includes the second season only (2016-2017), as the first season of data only had monthly measurements taken and therefore would have added unnecessary noise to the analysis. The growing season length analysis included both seasons of data collection, with all observations occurring at the consistent monthly resolution. There were 98 individuals monitored at low elevation and 21 individuals monitored at high elevation sites. To monitor phenophases, the BBCH framework was used which outlines important phenological stages by taxonomic group (Meier, 1997, Koch et al., 2007). Each individual was monitored visually using the BBCH classification scale developed for *Salix* by Saska and Kuzovkina (2010) and outlined in Table 2.2.

Reproduction was measured from August until reproduction had ended (November and January for low and high elevation sites, respectively, defined by 90% of females having dropped 90% of flowers). Flower bud break in males at high elevation (2016-2017) was not monitored from the initiation of the phenophase due to limited access, so the results presented are the minimum duration of the phase. During the 2016-2017 growing season a major flooding event occurred shortly before the initiation of seed release. Additional details describing the flood and phenology data collection can be found in the supplementary materials.

The growing season we quantified was from the start of spring foliation to the end of autumn leaf fall. First foliation was defined by the appearance of visible leaf surfaces protruded from the petiole at 3-4 positions around an individuals' crown (Kehl et al., 2008). Initial foliation was monitored weekly, in tandem with reproductive output monitoring. Autumn leaf drop was also quantified to define the end of the growing season (Post et al., 2008). Leaf fall was monitored fortnightly from March to May in 2016 and 2017. Complete leaf fall (senescence) was defined as ≥90% of leaves lost (Saska and Kuzovkina, 2010). Photographs of the individuals were taken when in full leaf out so that they could be referenced when assigning percent leaf-drop values throughout the season. All phenological events were recorded by the same researcher (E. L. De Stigter) throughout the growing season.

Meteorological measurements

Maximum and minimum temperature data were collected from the Queensland Government's SILO climate data drill function (Table 2.1). SILO provides synthetic, interpolated data based on point observations collected by the Bureau of Meteorology and is accurate to 0.05 degrees of latitude and longitude. The SILO weather data was compared to a small amount of weather data collected from the field sites to ensure that it was a reasonable representation of the site conditions. Data loggers (T-TEC RF version 6; accuracy: ± 0.2°C) were stationed at three of the field sites at Yarrabula Creek, Buckland River, and Dinner Plain (Sites 1, 4, and 5) for between 29 and 112 days in spring, 2016. Linear regression analysis showed the SILO temperature data provided reasonable temperature estimates for the sites (Figure S2.1, Table S2.2-S2.3).

Daily maximum and minimum SILO temperature data was included in models as a potential trigger of reproductive phenological events. To measure heat accumulation, we calculated growing degree days (henceforth referred to as GDD): a cumulative average daily temperature summed from winter solstice during the year of study (June 21, 2016). The GDD calculation includes threshold temperature (THS), minimum daily temperature (MIN), and maximum daily temperature (Pollnac et al.) (Snyder, 1985):

$$GDD = \sum_{i=1} \frac{MAX + MIN}{2} - THS$$

Following previous work on other *Salix* species, the threshold temperature (THS) chosen for the GDD calculations was 0°C (Baskerville and Emin, 1969, Spano et al., 1999, Ruml et al., 2010). If the GDD calculation for any given day was below the threshold temperature, then the value was "reset" to the threshold (THS=0) (McMaster and Wilhelm, 1997). For comparison, we ran all analyses with a threshold of 10°C in the GDD calculation. Based on previous work looking at common threshold GDD values, 0°C and 10°C were both highly correlated with 5°C. As such, we chose 10°C to compare a less similar threshold value with 0°C. There was no substantial change in the results when using THS=10°C (Gordon and Bootsma, 1993) (Table S2.4-S2.5).

Data analysis

A) Initiation, duration, and variation of phenophases across elevation

To determine the probability of a particular reproductive phenophase occurring on a given date, we used ordinal logistic regression analysis. All reproductive phenophases (those in Table 2.2, excluding 11 and 97) were included in the models. We used the dates each individual was monitored in a particular phenophase to predict the probability that each phenophase occurred on a given day (Cornelius et al., 2011). The ordinal logistic regression coefficients estimate the probability that an individual was in a given phenophase, or any preceding phenophase on any given day from winter solstice (June 21) in 2016 to June 20, 2017. To visualise this, we fitted logistic cumulative distribution functions (CDFs) for each phenophase. The logistic CDF of the previous phase then subtracted from the phase of interest to show the probability an individual occurred in each phenophase. We ran separate analyses for male and female individuals at high and low elevation. The four low elevation sites were pooled to increase the power of the models and more clearly show differences between low and high elevation sites. The ordinal logistic regression analysis used the polr function in R's MASS package (version 7.3-47) (Venables and Ripley, 2002). Goodness of fit was determined using McFadden's Pseudo R^2 (Hensher and Stopher, 1979).

We also tested for a difference in the duration of each reproductive phenophase between high and low elevation sites. To do this we ran four one-way

ANOVAs, one for each of the following phenophases at the individual level: male flower bud break (BBm), female flower bud break (BBf), pollen release (PR), and seed release (Myneni et al.). Duration of each phenophase for each individual was taken as the time between the first and last days the individual was observed in that phase. Since phenology was only observed on a weekly basis, it is important to note that these duration values are a minimum calculation, and the phenophases could have started up to 6 days earlier and/or stopped 6 days later. Further, for BBm, since the phase had already initiated before monitoring began, we cannot be certain how much longer the duration really was compared to what is listed here. However, we estimate that less than 10% of male flower buds had burst upon our first visit.

To determine the effect of elevation on the length of the growing season we performed a one-way ANOVA. A Kruskal Wallis test was completed prior to analysis to determine whether plant sex needed to be included as predictor; the results were non-significant, so it was excluded for simplicity (χ=1.5, df=1, *p=0.1*). The total sample size for each sex across elevation, including repeated individuals between years, was as follows: low elevation males: n=67; high elevation males: n=7; low elevation females: n=67; high elevation females: n=14. Low elevation sites were pooled in this analysis to compare low and high elevation sites, rather than between site variability. To account for unequal sample sizes we used the weighted mean method for calculations (Crawley, 2002).

B) Influence of Heat Accumulation on Reproductive Phenology

To determine the relative importance of heat accumulation, measured as GDD, and date in predicting the initiation of phenological events, we fitted generalized linear mixed effects models (GLMMs) to data for the following phenophases: BBf, PR, and SR (R package: lme4 (version 1.1-14)). Bud break was only modelled in females because the four males at high elevation were already in bud break at our first visit in the second (2016-2017) field season. The response variable was the phenophase presence or absence for each individual. Two models were built for each phenophase: one with GDD as a predictor and the other with time (number of days since winter solstice) as a predictor. We compared models of time with GDD to determine which was most effective in predicting the initiation of phenological events based on their goodness of fit (R^2) parameters. All models also

include a categorical elevation predictor variable (low vs. high), and individuals within sites as a nested random effect variable to account for pseudoreplication. Each model was fit with binomial distributions and logit links. Goodness of model fit was measured by calculating the marginal R^2, which describes variance explained by fixed effects, and conditional R^2, which describes the variance explained by both the fixed and random effects (R package: MuMIn (version 1.40.0)) (Nakagawa and Schielzeth, 2013). All statistical analyses and figures were completed using RStudio (version 1.0.143).

Results

A) Timing, duration, and variation of phenophases across elevation

Most reproductive phenology events began and ended later at high elevation than at low elevation. Pollen release began in mid- to late-September at low elevation and began three weeks later at high elevation (Figure 2.2, Table S2.6). Seed release began at all low elevation sites in late-September and began about six weeks later at high elevation. Bud break (Init. BB and Full BB) was the only phase that began at approximately the same time across elevation, in mid-August. However, bud break at high elevation was significantly longer than at low elevation in both males and females. Bud break in males lasted an average of 16 and 58 days at low and high elevation, respectively (F=103.8, df=1, *p*= < *0.001*), and in females lasted an average of 11 and 44 days at low and high elevation (F=63.2, df=1, *p*= < *0.001*) (Figure 2.3, Tables S2.7-S2.8). There was no significant difference between pollen and seed release duration across elevation. Pollen release lasted an average of 21 and 24 days at low and high elevation (F=0.06, df=1, *p=0.80*), and seed release lasted an average of 11 and 15 days at low and high elevation (F=2.02, df=1, *p=0.16*) (Figures 2.2, 2.3, Tables S2.7-S2.8).

In addition to variation between high and low elevation, there was also variation between low elevation populations, including those inhabiting the same river. Across the four low elevation sites, average phenophase initiation dates spanned across 9 days for male bud break, 13 days for female bud break, 11 days for pollen release, 9 days for seed release, and 9 days for foliation (Table S2.7). For all phenophases measured, the Buckland River sites' phenological events occurred before the same events on Yarrabula Creek.

Growing season length differed significantly between high elevation and low elevation sites (F=267.15, df=1, *p*= < *0.001*) (Table S2.9). Low elevation populations had leaves for approximately 50 days longer than high elevation populations (Table S2.7), equating to a 31% longer growing season. Initiation of foliation began an average of 28 days later at high elevation than low elevation. Leaf fall began 17-25 days earlier at high elevation than at low elevation (Table S2.7).

B) Influence of Heat Accumulation on Reproductive Phenology

GDD and Julian date (days since winter solstice) were both significant predictors of the initiation of all phenophases modelled (bud break in females, pollen release, and seed release), but the relationship varied with elevation (Figure 2.4, Table S2.5). Low elevation populations began phenological phases earlier in time than those at high elevation. However, high elevation populations began their phenophases with fewer cumulative GDDs than populations at low elevation (Figures 2.4-2.5). At low elevation we observed pollen release began at 600-750 GDD and seed release was initiated at 1100-1300 GDD. In contrast, high elevation pollen release occurred at approximately 350 GDD and seed release at approximately 600 GDD (Figure 2.5). The initiation of pollen release and seed release were positively correlated with GDD and date (ie: as GDD/date increased, the phenophase was more likely to initiate) (Figure 2.4). The elevation parameter was positively correlated with initiation of all phenophases in the GDD models. Alternatively, elevation was negatively correlated with pollen and seed release initiation in the Julian date models, and had no significant effect on female bud break initiation (z= -0.15, n=878, *p=0.89*) (Figure 2.4). Importantly, the Julian date model for bud break found that there was no difference in the time of initiation across elevation (Table S2.5b). The fixed effects in the GLMMs explained most of the variation in the initiation of phenological events, and there was only slight differences in prediction ability between the GDD and Julian date models (Tables S2.5, S2.10). The Marginal R^2 was slightly higher in the GDD models for bud break (Marginal R^2_{GDD}= 0.90, Marginal R^2_{Date}= 0.88) and pollen release (Marginal R^2_{GDD}= 0.97, Marginal R^2_{Date}= 0.96), but slightly lower for the seed release model (Marginal R^2_{GDD}= 0.96, Marginal R^2_{Date}= 0.98) (Table S2.4).

Discussion

We have quantified the timing and duration of reproductive and foliation phenology at low and high elevation in *S. cinerea's* invaded range of south-eastern Australia. The analysis revealed two novel points of interest. Firstly, the duration of phenological events following flower bud break was consistent across elevation, with only flower bud break duration showing significant differences across elevation. Secondly, we found that while their duration did not differ, pollen release and seed release was initiated at lower levels of heat accumulation at high elevation compared to low elevation. As expected, we also found that foliation began earlier, ended later, and therefore had a longer duration at low elevation, resulting in a 31% longer growing season overall. This change is growing season length across elevation may have a significant impacts on the spread of *S. cinerea* over time and with climate change. Overall, this research suggests that as cooler areas warm due to climate change, they may become more vulnerable to the accelerating spread of fast-growing invasive species like *S. cinerea*.

Flower bud break was the only phenological event monitored which showed no significant difference in initiation date across elevation. Furthermore, flower bud break was unexpectedly the only phenophase that was significantly different in duration at high elevation compared to low elevation. Alternatively, there was little variation in the duration of the phenophases following bud break across elevation. This suggests that the duration of the bud break phenophase is most likely the main influence on initiation of subsequent phenophases, such as pollen and seed release, in these populations, while temperature is contributing primarily via its effect on the speed of bud maturation earlier in the season. These results together suggest that the initiation of flower bud break was not related to temperature, but the duration of bud break is. Previous research has found pronounced variation in the initiation of bud burst between individuals of *Salix* species, largely determined by differences in temperature requirements (Ghelardini et al. 2014; Lennartsson and Ogren 2004; Weih 2009). The lack of variation we found across elevation could be due to the heritability of the initiation of bud break, which has been reported in *Salix viminalis*, a closely related species to *S. cinerea* (Ronnberg-Wastljung and Gullberg 1999). Alternatively, initiation to bud break may have been in response to the consistent photoperiod between low and high elevation sites.

High elevation populations began all of their reproductive phenophases later in time than low elevation populations (except flower bud break), which is consistent with previous research suggesting that cool, high elevation temperatures can significantly delay the onset of reproductive events (Crimmins et al., 2010). In particular, we believe that the delay in phenological events was the result of differences in bud maturation times at low and high elevation, which was driven by temperature differences. This insight is possible because we measured all the relevant phenophases throughout the reproductive season and found the same delay in all of them. Further, all the phenophases were the same length except bud maturation, which suggests that the other phases following bud maturation had a consistent development time. Though we are confident in our insights, there are also other external factors which can affect the onset of phenological events. Another cause for the delay in the onset of these events could be related to the observed difference in size across elevation. Individuals at high elevation were physically smaller than at low elevation (see Chapter 4), and smaller individuals have previously been recorded as flowering later than larger individuals in both herbaceous perennials (*Lotus corniculatus*) (Ollerton and Lack, 1998) and dioecious shrubs (*Ilex leucoclada*) (Torimaru and Tomaru, 2006). Additionally, a spring flooding event at low elevation may have disrupted typical phenological timing and delayed reproductive events and seed set in the year of study. As such, the effect size between low and high elevation may be even larger during an average year of weather than was observed in 2016.

We found that GDD had a significant effect on the initiation of bud break in females, pollen release, and seed release at both low and high elevation. This is consistent with previous research showing heat accumulation is a major driver of the initiation of phenological events in other plant species (Gordienko and Sokolov, 2009, Fernandez-Martinez et al., 2012), as well as seven North American *Salix spp.* (Mosseler and Papadopol, 1989). However, this does not necessarily imply that surrounding temperatures at the time of initiation are the primary cause of initiation, since phenological events are responsive to those which occur beforehand. The initiation pattern observed here appears to be due to the extended duration of bud break. There may also have been an effect of photoperiod on high elevation sites, triggering their initiation before the season was too advanced for the success of

subsequent phenophases (Korner and Basler, 2010). Additionally, there is likely an interaction between temperature and other environmental conditions thought to affect the onset of phenological events that were not measured, including precipitation (Kudo and Ida, 2013) and shading (Cipollini, 2005). There are also potentially intrinsic, genetic factors which may alter the timing of initiation (Ronnberg-Wastljung and Gullberg, 1999), as well as chilling requirements (Korner and Basler, 2010) and/or responses to growing season length in the prior seasons. Future studies could quantify the effects of these factors as well.

In this and several other studies (Chapman et al., 1999, Munguia-Rosas et al., 2013), variation was found in the timing of phenological events among individuals within and among populations (Figure 2.3). Since there was some intra-population variation among sites that were geographically close to each other (Figure 2.3), we can be sure that variation in the initiation and duration of reproductive phenology events was not entirely determined by surrounding regional weather conditions and might be better explained microclimatic effects (Reyer et al., 2013), or the many factors highlighted in the previous paragraph. The variation found between low elevation sites, and between individuals within sites, could also be related to increased shading at certain sites. Personal observation, which is consistent with previous woody plant research, suggests that heavily shaded individuals began and ended their phenophases later than those in full sun (Munguia-Rosas et al., 2013). Within this study, Yarrabula Creek sites (sites 1 and 2) generally had phenological events which occurred slightly later than those at the Buckland River (sites 3 and 4), despite being 80-110 meters lower in elevation. However, the lowest elevation sites (Yarrabula Creek) were more shaded than the Buckland River sites, which may have caused a lag in the populations' phenology. Future studies could expand upon the methods described here by further investigating the effects of shading.

At high elevation we found that pollen release and seed release occurred later in time and at unexpectedly lower cumulative GDD since winter solstice than experienced at low elevation. The reasons for this are unclear, but we propose three possible explanations. Firstly, the discrepancy could be due to local adaptation to elevation differences, echoing previous research on *Salix lapponum* which found that female catkins at low elevation (988 m) had a higher thermal budget compared with those at high elevation (1222 m) (Hill and Hodkinson, 1995). A second explanation

might be that physiological thermal requirements for phenological events at low elevation are met and surpassed before individuals release reproductive material. Alternatively, at high elevation, reproductive events may be forced into initiation once a basal temperature threshold is just met, despite the potential for poor seed development conditions. Early fruit production is adaptive in populations with shorter growing season, but comes at a severe fitness cost (Colautti and Barrett, 2013). Thus, the GDD experienced by *S. cinerea* at high elevation may be representative of necessary basal temperature requirements, rather than ideal conditions for phenophase initiation that are more similar to low elevation temperature conditions. Thirdly, the warmth experienced at low elevation may have forced flowering, while the high elevation population's flowering may have been triggered by photoperiod or chilling effects, since the high elevation individuals never quite experienced the temperatures experienced by low elevation populations at the time of flowering. Overall, this result suggests that as the south-eastern Australian climate warms in upcoming decades the increasing temperatures are unlikely to inhibit phenological processes in *S. cinerea.* However, because we have not measured the non-linear effects of chilling and frosts on phenology at high elevation, these implications require further future investigation.

We determined that the growing season at high elevation was approximately 24% shorter (50 days) than at low elevation (Table S2.7). This was expected, since warmer temperatures result in longer growing seasons and low elevation sites are significantly warmer than high (McMahon et al., 2010). However, this result is important due to its probable impact on the demographics of *S. cinerea* in its invaded range. High elevation populations are likely to experience slower growth rate than low elevation populations as a result of decreased photosynthetic intake (Myneni et al., 1997). This suggests that vegetative spread will be slowed, as well as seed spread, as plants will likely take longer to reach reproductive maturity (Oleksyn et al., 1998). Therefore, in warming climates, growing season lengths and thus growth rates may increase, especially at high elevation, resulting in populations that mature and produce seed more quickly, accelerating spread.

The information presented in this study is applicable to ecological, phenological, and horticultural literature. We have highlighted that the flower bud break phenophase duration appears to be responding to changing temperature.

However, the GDD vs. date reversal in pollen release and seed release suggests that populations are responding to something more than temperature: perhaps shorter growing seasons, day length, or chilling factors. As the phenology of *S. cinerea* and native species shift temporally, it is unlikely that all the members of the community will respond to climate warming uniformly (Sherry et al., 2007). This variation in response may change the competitive dynamic between native and invasive species in a given community. While we do not directly address this issue here, in providing detailed phenological records of *S. cinerea* in its invaded range we have collected the first piece of required information to understand the ecological impact of the species under changing climates.

This study has also quantified the timing of seed release. Understanding the initiation of *S. cinerea* seed dispersal events, and how the timing varies across the elevation gradient, allows managers to anticipate seed dispersal patterns and tailor the spatial allocation of control efforts accordingly (Moore and Runge, 2012). Recently, managers have focussed their control efforts on threatened peatlands, which have high ecological value (Moore et al., 2017). However, the objectives of most previous research focusses on protecting the high elevation peatlands and not slowing spread (Giljohann et al., 2011, Moore and Runge, 2012). As such, for upcoming landscape-scale control efforts, we recommend they be distributed according to the risk of further spread posed by each population. Since population spread potential will vary across a landscape, control efforts should be distributed accordingly.

Conclusion

Most phenology studies that investigate plant responses to temperature focus on the initiation of phenological events in isolation, without also quantifying the duration of these events. Focus on initiation and duration of phenological events allows us to better understand the potential consequences of climate change to invasive plant demographics more completely than initiation information could alone. Here we have developed novel analytical methods to quantify the initiation and duration of phenological events, which we believe will be useful to future researchers interested in intra-specific phenology. The consequences of warming climate on the invasive willow, *S. cinerea,* were clear: the phenological events shifted in time, but only the flower bud break phase had a longer duration at the warmer, low elevation

sites. Based on this information, we believe that *S. cinerea* will be resilient to the warming temperatures caused by climate change, though it is still unclear what the impact on biotic interactions will be, particularly with respect to competition with other *Salix* species. Speculatively, this study may also help to describe the species in its native European range under climate change. We recommend future researchers focussing on invasive plant phenology consider quantifying the duration of phenological events in addition to their initiation.

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Tables

Table 2.1 A summary of climate statistics for the Yarrabula Creek (sites 1 and 2), Buckland River (sites 3 and 4), and Dinner Plain (site 5). Temperature data from interpolated SILO data, as described in methods section. Annual temperatures are from winter solstice in 2016 (June 21) until June 21, 2017. Spring temperatures use only data from September to December, to focus on the primary period when flowering and leaf out are occurring.

Timeframe	Site	Mean (°C)	Maximum (°C)	Minimum (°C)
Annual	Yarrabula Creek	13.3	19.1	7.6
	Buckland River	14.1	20.2	8.1
	Dinner Plain	7.5	11.4	3.7
Spring only	Yarrabula Creek	13.8	19.6	7.9
	Buckland River	14.7	20.8	8.6
	Dinner Plain	7.7	11.7	3.6

Table 2.2 Phenological stages applied during field monitoring. Stages were adapted from the BBCH scale first developed for *Salix spp.* by Saska (2010) and are listed in observed order of occurrence for *S. cinerea*. Sex-specific stages are labelled or otherwise left blank.

Stage	Sex	Abbreviation	Stage title	Description
53		W. Sen	Winter senescence	Senescent. No broken leaf or generative buds.
54		Init. BB	Initial catkin bud break	Buds have broken and the white hairs of young catkins are visible.
55		Full BB	Full catkin bud break	≥50% of the crown has broken catkin buds.
56	M	Init. PR	Initial pollen release	Pollen is visible on at least one developing catkin.
61	F	Init. StRe	Initial stigma availability	Stigmas are distinguishable and separated on 10% or more of catkins.
11			Leaf expansion	Leaf lamina is fully expanded at ≥5 points around the crown.
65		PR / StRe Anth.	Anthesis	Full flowering. Anthesis was reached on ≥50% of catkins. Pollen is visible or stigmas are separated.
69	M	S. Sen	Summer senescence	≥50% of male inflorescences have dropped.
70	F	Init. SR	Initial seed release	Seed release has begun in at least one catkin.
71	F	Full SR	Full seed release	≥50% of catkins are releasing seed.
75	F	S. Sen	Summer senescence	≥50% of female inflorescences have dropped.
97		Dorm.	Dormancy	≥90% of leaves dropped.

Figures

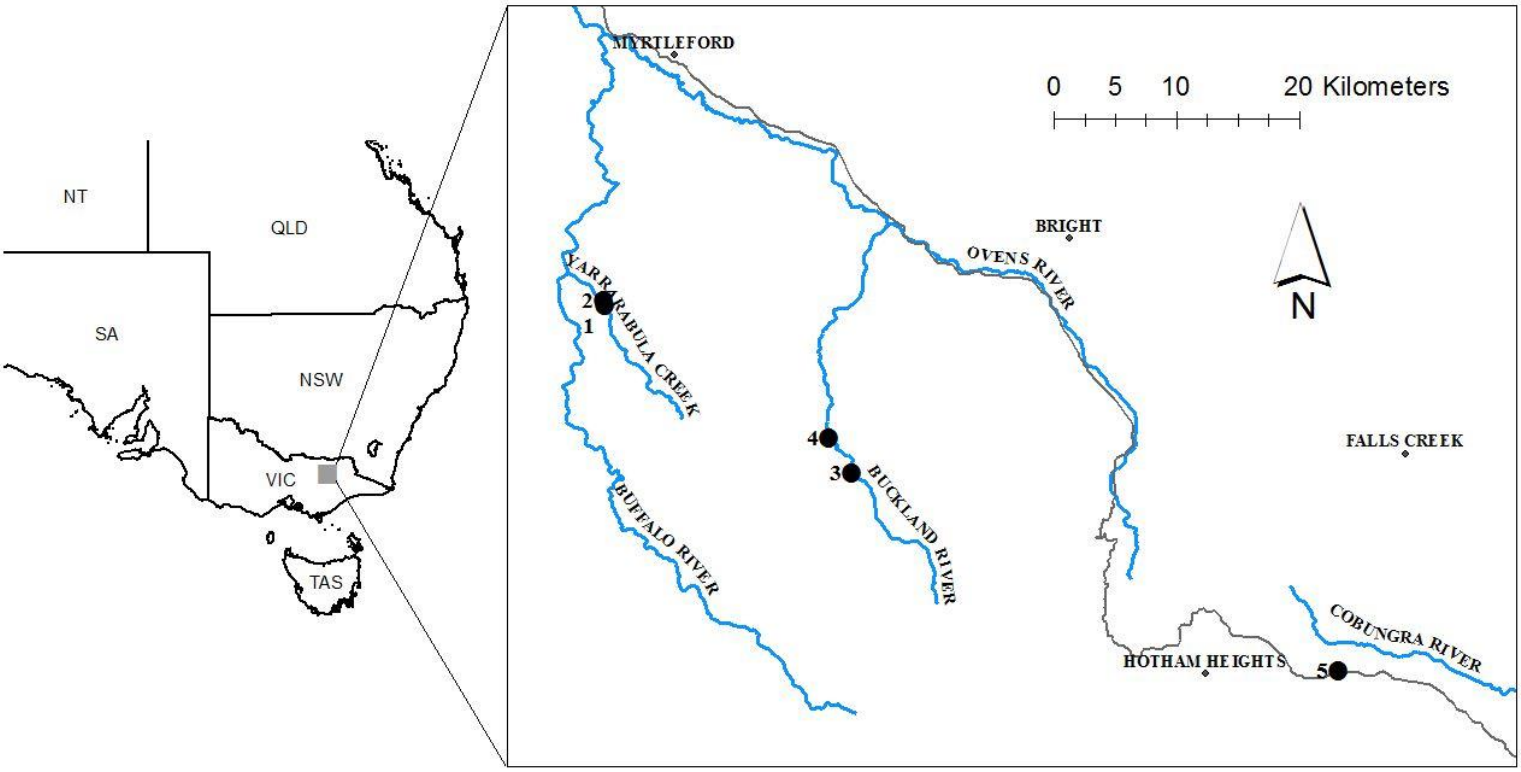

Figure 2.1. Map of field sites in north-eastern Victoria, Australia.

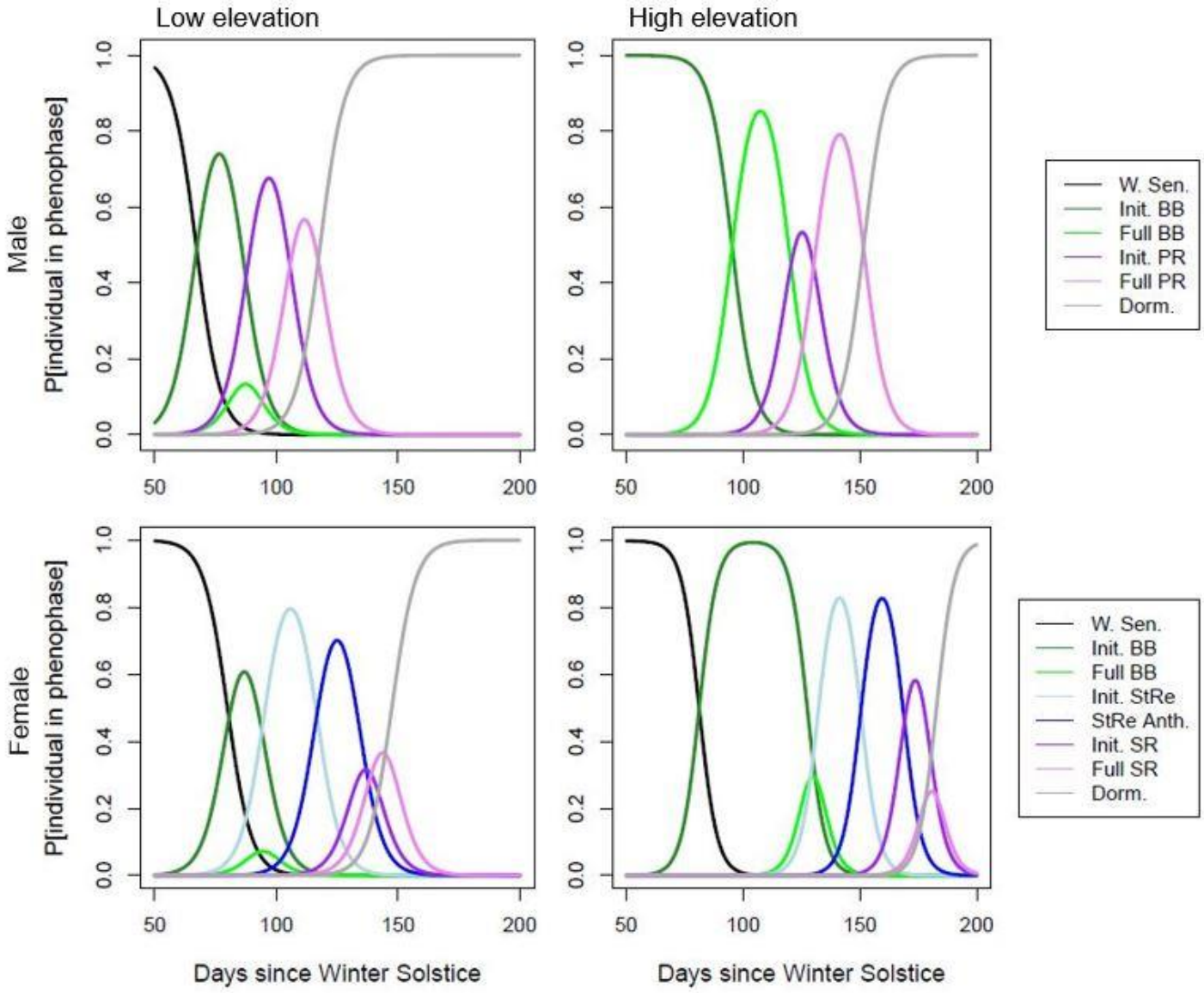

Figure 2.2. The probability of each phenophase occurring over time based elevation and sex. Variation in height of curves depends on the frequency of individuals which were observed in each phenophase. Some individuals were not observed in particular phenophases, which causes curve heights to be shorter. Phenophases correspond with the BBCH codes listed in Table 2.2: W. Sen= winter senescence (53), Init. BB= initial bud break (54); Full BB=full bud break (55); Init. PR=initial pollen release (56); PR Anth.=Anthesis (males) (65); Init. StRe=initial stigma receptivity (61); StRe Anth.= Anthesis (females) (65); Init. SR=Initial seed release (70); Full SR=Full seed release (71); Dorm.=Dormancy (69 in males, 75 in females). Ordinal logistic regression output used to calculate these curves can be found in Table S2.11. Data included from 2016 only.

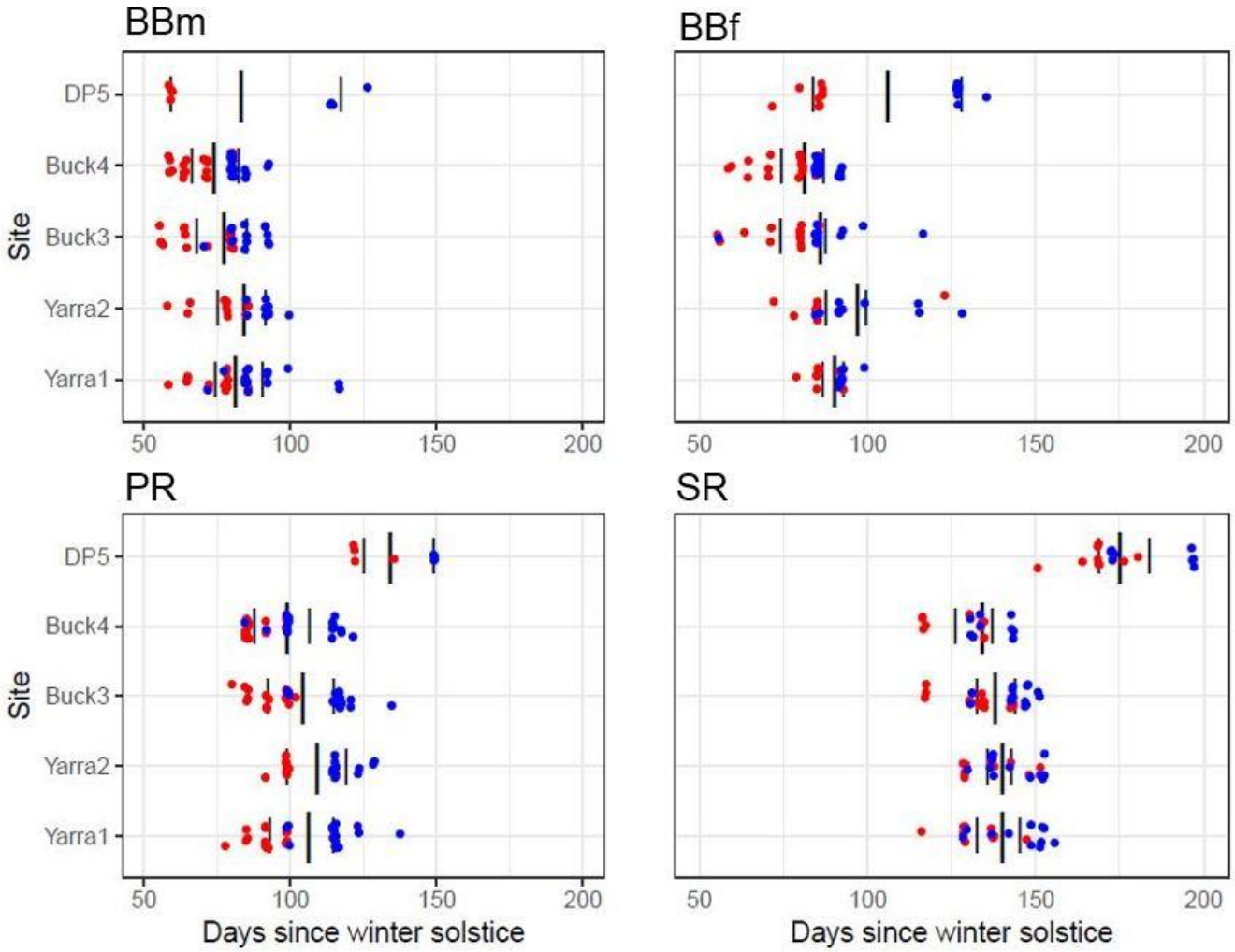

Figure 2.3. Duration of each phenophase at each site. Each panel refers to a particular phenophase: BBm=bud break in males; BBf=bud break in females; PR=pollen release; SR=seed release. Peak dates, as extracted from the ordinal logistic regression analyses, are indicated by the center vertical lines. Left and right vertical lines indicate the average initiation and average end dates at each site. Blue points represent the final date a phenological event was observed in each individual, while red points represent the initial date a phenological event was observed for each individual. The x-axes extend from August 9, 2016 to January 6, 2017, as winter solstice during the year of study in the southern hemisphere was June 21, 2016.

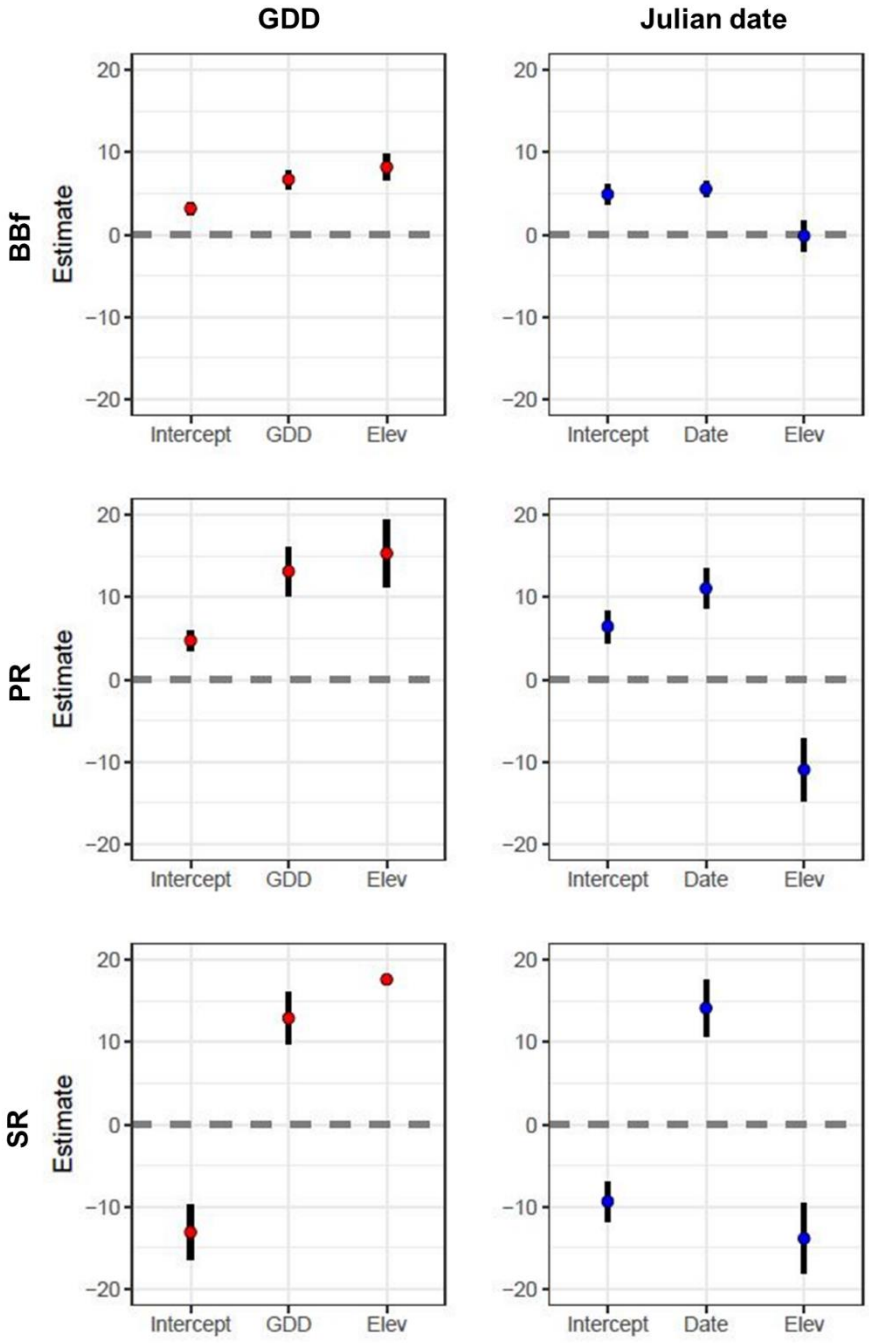

Figure 2.4. Coefficient plots from GLMM analysis comparing influence of GDD or date and elevation on the initiation of bud break in females (BBf), pollen release (PR), and seed release (SR). Red dots indicate models created with GDD as a predictor and blue dots with Julian date as a predictor. Confidence intervals indicate two standard errors above and below the parameter estimate.

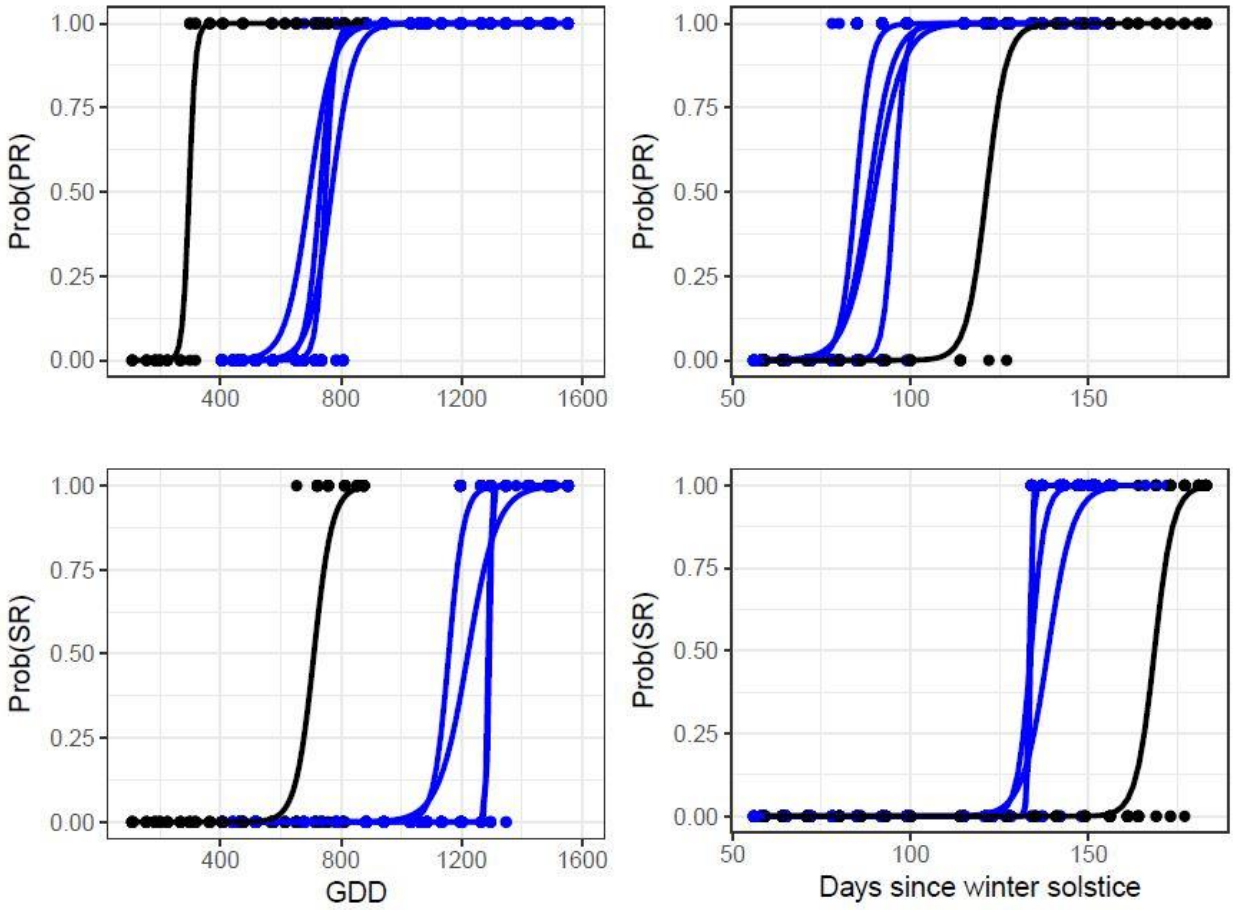

Figure 2.5. The probability of the initiation of each phenophase occurring with respect to heat accumulation, measured in growing degree days (GDD) (left) and time (right). Each blue line represents one of the four low elevation sites, while the black line represents the high elevation site. The curves represent the probability of each phenophase occurring over time or with increasing GDD. Points represent a particular date of monitoring for each individual. The high elevation individuals occur later in time, and when fewer GDDs have accumulated.

Chapter 3

New methods reveal incompatibility of flowering phenology across elevation causes lowered seed output in an invasive, dioecious shrub

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Abstract

The magnitude of a plant's seed output may depend on the degree of phenological overlap between male pollen release and female stigma receptivity. Because phenological events are strongly influenced by climatic conditions, this overlap may vary across elevation gradients and affect the opportunity for seed fertilisation and subsequent seed output. Here we observe an invasive willow (*Salix cinerea*) in its introduced range under climatic conditions largely warmer than its native range. We quantified the flowering phenology and seed output of populations of *S. cinerea* at low (≤410 m) and high (1640 m) elevation in Victoria, Australia. New applied ordinal logistic regression modelling methods reveal that warm, low elevation populations had an 18% longer period of overlap between pollen release and stigma receptivity than those at cool, high elevation. This increase in overlap at low elevation indicates an increased opportunity for seed fertilization. Low elevation populations also showed approximately six times the amount of seed set as the high elevation population. This suggests that the cool, high elevation temperature conditions influenced pollen availability at the time of stigma receptivity, leading to lowered pollen acquisition and seed output overall.

Introduction

Phenology is an increasingly important focus of research as ecologists attempt to better understand how it is influenced by anthropogenic climate change. The majority of phenology research focusses on interspecific relationships (e.g.: pollinator mismatch (Hegland et al., 2009, Solga et al., 2014) and species' interactions with their food (Visser and Both, 2005)) or on particular key developmental events in single species (e.g.: leaf-out or seed release (Inouye, 2008)). Very little attention has been given to quantifying intraspecific variation in phenology across a range, or how that variation may affect demography and range expansion over time (Miller-Rushing et al., 2010).

In particular, intraspecific phenological variation may be important to the fecundity of a plant species (Dafni and Firmage, 2000). The period of phenological overlap when male pollen is released and when female stigmas are receptive to that pollen has the potential to affect overall seed output and subsequent population dynamics (Espirito-Santo et al., 2003, Kudo and Ida, 2013). This is particularly important in dioecious species because male-female synchrony is required for successful seed fertilization (Campbell and Reece, 2002, Herrero, 2003). Additionally, *Salix* pollen is generally short-lived, with one species (*S. lapponum*) having a maximum of 15% pollen germination in the first 72 hours post-collection (Pogorzelec et al., 2015). As such, it is important that there is tight phenological synchrony between males and females to optimize the opportunity for seed fertilization and subsequent reproductive success (Dafni and Firmage, 2000). Despite its potential to significantly affect plant fecundity, the phenology of pollen release and stigma receptivity is often not mentioned in reviews about the effects of climate change on phenology (Cleland et al., 2007) or plant reproduction (Hedhly et al., 2009).

The timing of phenological events is closely tied to surrounding climatic conditions, which can alter the initiation and duration of pollen release and stigma receptivity (Chapter 2)(Schwartz, 2013). Warm temperatures tend to accelerate, while cool temperatures often slow pollen tube growth and pistil degeneration (Herrero, 2003). Similarly, precipitation has been shown to have an effect on flowering times (Inouye et al., 2003). A similar response of male and female stages likely gives plants plasticity to withstand changing environmental conditions, ensuring the arrival of pollen to the ovary (Hedhly et al., 2005). However, in some cases

pollen germination responds positively to increasing temperatures, while stigma receptivity responds negatively (Hedhly et al., 2005). Temperature clearly affects the duration of stigmatic receptivity in woody plants, such that the stigma loses its capacity for pollen adhesion, penetration, and germination. This effect is apparent as early as the first day after anthesis, even when there are no visible signs of stigma degeneration (Hedhly et al., 2003). This suggests that the synchrony of pollen release and stigma receptivity has the potential to break down outside of typical ranges encountered by each plant species. If environmental conditions are different enough across a landscape to alter the synchrony of pollen release and stigma receptivity, fecundity may vary as well.

This potential for phenological disruption could be especially important for plants that have been introduced outside of their native range, and are therefore growing under novel climatic conditions. As such, non-native species' phenology may be more vulnerable to intraspecific asynchrony and experience lowered fecundity as a result. However, this has not been tested empirically. To address these concepts, we focus on a dioecious willow species, *Salix cinerea,* native to northern Europe and widely invasive in south-eastern Australia, Tasmania, and New Zealand. In its invaded range, *S. cinerea* causes severe erosion to riparian and endangered peatland ecosystems (Ladson, 1997). More than 80 *Salix* species have been introduced into Australia, most of which only have one sex present and reproduce vegetatively or by hybridisation (Holland-Clift, 2004). However, *S. cinerea* has both sexes present and can reproduce by seed as well (Cremer, 1999). *Salix* species are pollinated largely by insects, and occasionally by wind (Argus, 1986, Hopley et al., 2015). Genetic paternity assignment testing of *S. cinerea* found that more than 75% of female individuals are fertilised by pollen that has dispersed from populations outside of their own (Hopley et al., 2015). Single populations of *S. cinerea* have been estimated to release more than a million tiny, wind-dispersed seeds in a given year (Hopley, 2011). Previous research has found that the initiation of *S. cinerea's* reproductive phenological events occur later in time at high elevation (Chapter 1), suggesting that there may be other unidentified phenological differences across elevation.

In this study we aimed to examine whether varying climatic conditions affected the synchrony between male and female flowering and if this had consequences for fecundity. Specifically, we aimed to answer the following questions:

1. Is there variation in the overlap of pollen release and stigma receptivity in *S. cinerea* between low and high elevation?
2. Does phenological overlap correlate with seed production at low and high elevation?

To address these questions we observed populations of *S. cinerea* in Australia at low and high elevation to quantify the phenological synchrony of the species in response to varying bioclimatic conditions. This research provides novel insights to the importance of intraspecific phenology and its influence on species demography at a landscape scale.

Methods

Study sites

Study sites were established in north-eastern Victoria (350 km northeast of Melbourne, Australia) where the *S. cinerea* invasion is arguably the worst in continental Australia (Cremer, 2003). At low elevation (292-410 m), four sites were established in the Ovens River Catchment along Yarrabula Creek and Buckland River (Chapter 1 Figure 2.1), each containing 21-28 mature (seed or pollen producing) individuals. Individuals were only measured if the main stem was located ≥ 1 m from the neighbouring individual. Site size and number of individuals within each site were determined based on safe researcher accessibility to the individuals (river depth and speed). At high elevation, one site was monitored in Dinner Plain, Victoria (1639 m) along a small, unnamed creek approximately 50 km southeast of the Ovens Catchment sites. The number of sites monitored was constrained by the inaccessibility of upland *S. cinerea* sites.

Phenological monitoring

Phenological monitoring occurred throughout the growing season from mid-August, 2016 to early January, 2017 (mid-winter until mid-summer) on a weekly basis. Monitoring included visual assessment of each individual. Low elevation sites were visited from mid-August until late November when >90% of flowers had dropped off of >90% of individuals. The high elevation site was monitored from the time it was accessible in mid-September until January, when >90% of their flowers had dropped. In total, 98 individuals at low elevation and 21 individuals at high elevation were monitored.

When monitoring reproduction, each individual was classified into stages adapted from the BBCH monitoring scheme developed by Saska and Kuzovkina (2010): pre-bud break (no BB), initial bud break (init. BB), late bud break (>50% BB), initial pollen release (init. PR), full pollen release (>50% PR), initial visibility of receptive stigmas (init. StRe), initial seed release (init. SR), full seed release (>50% SR) and senescence (>90% drop) (Chapter 2: Table 2.2).

Reproductive output and size

Reproductive output was measured for each individual by visual assessment of two researchers. The crown was visually divided into three equal bands agreed on by the two researchers, then catkins were counted in each third of the crown independently. After data collection, the researchers' measurements were averaged together by crown level and then the three crown levels were summed together for a total catkin per crown measurement. To estimate the number of seeds per tree, mature catkins were collected from each individual and kept in paper bags in the sun to prompt their seed release. Between two and five catkins per individual were selected which were in the optimal phenological stage for analysis, with all capsules still present and not yet dropped from the fruit, and at least two capsules which had burst open but not shed their seed. The number of capsules per catkin were counted, and two burst capsules were selected for dissection and seed counting. The number of seeds per capsule were averaged between the two capsule counts, then multiplied by the number of capsules per catkin to get the number of seeds per catkin. This value was multiplied by the number of catkins per individual. Low elevation seed set was likely affected by a flood which occurred shortly before seed release at low elevation (detailed description in supplementary materials). Dozens of catkins were ripped from the study individuals and were therefore not measured. As such, the seed set discrepancy between low and high elevation may be larger than is described here.

Crown volume of each individual was calculated by measuring the height, length, and width, of each individual in November, 2016. Height (h) was measured by photographing each individual with a three meter PVC pole in frame for scale. ImageJ software was used to calculate height from the known PVC pole size. Length (w_1) and width (w_2) values were collected by measuring the longest extension of the

crown in the field, then the width of the crown perpendicular to the length measurement. To calculate the volume we used the ellipsoid volume equation: $Volume = \frac{4}{3}\pi(h \times w_1 \times w_2)$.

Data analysis

A) Synchrony between phenophases

To estimate the proportion of individuals that were overlapping at low and high elevation between pollen release and stigma receptivity phenophases over time, we fit ordinal logistic regression (OLR) models using the phenological observation field data. Within each model, *S. cinerea's* major phenological events were encoded into an ordered response variable, including bud break, pollen release, stigma receptivity, seed release, and winter senescence (more details in Table S3.1). Julian date since winter solstice (June 21, 2016) is the single independent variable. We calculate the proportion of individuals (*f*) that were in the pollen release or stigma receptivity phases on a given Julian date (*t*) as:

$$f(t) = \frac{1}{1 + e^{Dt-b}} - \frac{1}{1 + e^{Dt-a}}$$

where *t* is the number of Julian days since winter solstice, *D* is the OLR model coefficient value for Julian date, *a* is the OLR intercept for a given phenophase state change, and *b* is the OLR intercept for the phenophase state change following *a*.

We developed a new method to calculate overlap between phenophases which accounts for both the number of days overlapping and the number of individuals in the phenophases of interest on a given date. This method calculates the proportion of overlapping area between the models describing pollen release and stigma receptivity at a given elevation. First, we calculated the intersection point of the two curves, i.e. the point at which $f_P(t) = f_S(t)$ (P=pollen release, S=stigma receptivity). Then, we used the integral of $f_P(t)$, $F_P(t) = \frac{1}{D}[-b - a - \ln(1 + e^{Dt-b}) + \ln(1 + e^{Dt-a})]$, to calculate the area under the right tail of the pollen release curve from the intersection point. We used the corresponding procedure to calculate the left tail of the stigma receptivity curve from the intersection point. Adding these two areas together gave us the total overlapping area which describes the number of individuals that are releasing pollen when stigmas are receptive. Lastly, we

integrated the stigma receptivity curve from 0 (winter solstice: June 21, 2016) to 365 (June 21, 2017) to determine the total area under the curve. To determine the proportion of stigma receptivity that is overlapped by pollen release, we divided the area of pollen release-stigma receptivity overlap by the total stigma receptivity area. This method was repeated for both low and high elevation, with the four low elevation sites grouped into one analysis.

B) Reproductive output variation

We used generalised linear models to look for associations between the overlap of pollen release and stigma receptivity and an individual's reproductive output. The response variable was seeds per individual. Since 43% of individuals from high elevation had no seed set in the capsules sampled, we averaged the number of seeds per capsules across each site before calculating the number of seeds per individual. This method assumes that the lack of set seeds in a catkin is not representative of the entire shrub and there is some low number of seeds per capsule, on average, per individual. Explanatory variables were the phenological overlap period, represented by the proportion of days that stigmas were receptive to pollen and pollen was available out of the total number of days stigmas were receptive (as calculated in the OLR models). Other explanatory variables included a categorical elevation variable (low and high) and the volume of each individual. The model was fit using a negative binomial family to minimize overdispersion and had a sample size of 57 catkins total, including 43 from low elevation and 14 from high elevation. Both the OLR and generalised linear models were fitted using the MASS package (version 7.3-49) in R (Team, 2015).

Results

A) Synchrony between phenophases

On average, low elevation pollen release and stigma receptivity overlapped by 36.25 days (SE=3.40 days). The four low elevation sites had between 29 and 42 days of overlap between pollen release and stigma receptivity. At high elevation there were only 14 days of overlap. Based on the absolute number of days overlapping between sites, the proportion of the total stigma receptivity phenophase that was overlapped by pollen release was 88.4% at site 1, 64.3% at site 2, 100% at site 3, 69.0% at site 4, and 33.3% at the high elevation site 5. Using the OLR models

and integrating the area under the low elevation and high elevation curves we found slightly different levels of overlap since the OLR models account for the number of individuals in each phase on a given date. The OLR models show that 42% of stigma receptivity overlapped with pollen release at low elevation (Figure 3.1, Table S3.2). By contrast, at high elevation only 24% of stigma receptivity overlapped with pollen release when accounting for the number of individuals in each phenophase at each site.

B) Reproductive output variation

The high elevation site produced significantly fewer seeds relative to the four low elevation sites. At low elevation we found an average of 4.03 seeds set per capsule across all four sites, while at high elevation only 0.68 seeds set per capsule. At high elevation we estimated that each individual produced approximately 13,000 seeds on average (SE=275, calculated at the site level), while low elevation sites produced an estimated 140,000 at site 1 (SE= 53,000), 62,000 at site 2 (SE= 23,000), 268,000 at site 3 (SE= 11,000), and 29,000 at site 4 (SE= 10,000) (Figure 3.2). On average, low elevation sites produced approximately 13.5 times as many seeds as the high elevation site did.

Our negative binomial GLM which assessed seed output as a function of phenological overlap, shrub volume, and elevation, found that all three model parameters were significant (Table 3.1). The overall model fit was good (Pseudo R^2 =0.47). Phenological overlap and volume were positive relative to seed output (overlap coefficient= 0.32 (SE=0.08), *p=0.003*; volume coefficient = 0.001 (SE=0.0002), *p=0.003*), while elevation had a strongly negative relationship with seed output (coefficient= -6.46 (SE=2.41), *p=0.01*).

Discussion

In this study we have shown that low elevation populations of *S. cinerea* have approximately double the overlap between pollen release and stigma receptivity than at high elevation. This degree of overlap is also correlated with seed production: low elevation populations produce more seeds per individual. Because phenology is highly responsive to temperature (Walther et al., 2002, Cleland et al., 2007), we

expected that *S. cinerea* would have a longer period of overlap between its pollen release and stigma receptivity phenophases in the high elevation Australian range, since those are more similar to the conditions in its home range (Supplementary Materials). However, we found the opposite: in conditions the high elevation conditions similar to the average European climate there was less overlap and less seed production than at low elevation. This research suggests that *S. cinerea* is actually more fecund in regions that are on average warmer than in the current native range of the species, and hence those low elevation populations have greater potential to spread by seed than the populations at high elevation.

Phenological overlap and reproductive output

On average, the high elevation site had 22 days fewer overlap between pollen release and stigma receptivity than low elevation sites and an 18% lower degree of overlap. This discrepancy is somewhat surprising since the phenological overlap between pollen release and stigma receptivity in seven other *Salix* species was nearly 100% in their native North American range (Mosseler, 1989). This suggests that the phenological timing of pollen release and stigma receptivity vary in response to their new surrounding environmental conditions. Pollen development and seed fertilization are thought to be the most sensitive reproductive stages to temperature stress, which can cause asynchrony between male and female reproductive development (Zinn et al., 2010). However, Chapter 2 suggests that bud maturation rate it the most sensitive to temperature. Another explanation for increased phenological overlap and seed output at low elevation could be a result of genetic drift (Maron et al., 2004), or perhaps rapid adaptation (Prentis et al., 2008) to the broad-scale climatic variation. Other research has suggested that elongated pollen release and stigma receptivity phenophases appear to shift in accordance with insect pollinator availability (Beardsell et al., 1993). It is common for non-native plants to lack effective pollinators compared to native plants (Parker, 1997), however since no research has been completed on pollinator limitation in the alpine study region we cannot make conclusions about its effect on *S. cinerea*. In the future, the phenological data collection could be improved by lengthening the duration of the study and observing consistency between years. Previous research has found that at least six years of data are required to accurately detect phenological cycles, and even longer for species with large amounts of variation in their phenological patterns

(Bush et al., 2017). However, despite imperfect resource availability, this study still collected novel data which was sufficiently robust to describe the phenological overlap of an ecologically significant invasive species during the year of study.

The low elevation populations had approximately six times as many seeds set per capsule than at high elevation, with an average of 4.03 seeds per capsule at low elevation and only 0.68 seeds at high elevation. Interestingly, other research on *S. cinerea* completed in the same low elevation region of Australia, including one of the same river catchments, found an average of 5.46 seeds set per capsule (Hopley, 2011). This suggests a non-negligible amount of between-year variability in seed production, and that our year of study was perhaps below average for seed production overall. Given that there was a flood during our year of study (Supplementary Materials), this is likely true. To our knowledge, there have not been any studies which quantify the amount of seed produced by *S. cinerea* in its native range, however, studies on other *Salix* species in their native range found higher seed set rates than were found here. Sacchi and Price (1992) found 5.84 seeds per capsule in *S. lasiolepis*, while *S. alaxensis* had 8 seeds per capsule (Fox, 1992). Alternatively, Shafroth et al. (1994) observed non-native populations of *S. rubens* and found only 3.47 seeds set per capsule, which was attributed to a low male to female ratio in the study region. This may also be the case for *S. cinerea,* as there are at least twice as many males as females in both the native (Alliende and Harper, 1989) and invasive (Hopley et al., 2015) ranges, and a female dominated sex ratio can affect pollen limitation and severely reduce seed set (Wilson and Harder, 2003, Davis et al., 2004). However, pollen immigration is quite extensive for *S. cinerea* in south-eastern Australia, with between 27-48% of pollen emigrating from its site of maturation (Hopley et al., 2015). As such, pollen limitation is unlikely to be the result of low pollen dispersal ability.

Although our results suggest that phenological overlap is likely causing the variation in reproductive output between high and low elevation, there are a number of other reasons which could explain the variation. For example, higher seed set found at low elevation may have been facilitated by warmer conditions (Merow et al., 2017). Alternatively, when accounting for volume, the high elevation site produced an approximately average number of catkins per shrub compared to the low elevation sites. This lack of variation across sites may be due to a “bet-hedging”

strategy of high elevation individuals to over-produce catkins in order to increase the plant's probability of being visited by pollinators (Ashman et al., 2004). Previous research has found that high elevation herbaceous individuals prioritised their reproductive output over growth in size by reserving three times as much of their above ground biomass for flowers compared with low elevation individuals (Fabbro and Korner, 2004). Prioritising reproductive output at high elevation is sensible for *S. cinerea* since the surrounding canopy is less dense, making light accumulation less of a focus for growth, and leaving resources available for catkin production. Moreover, the low elevation sites may also be more conducive to seed production due to higher rates of disturbance from farming on riverbanks where they grow, allowing for lowered native species competition and increased light availability. Alternatively, there is certainly lower intra-genus competition at high elevation, since *S. cinerea* is the only *Salix* species which has colonised in alpine Australia (McDougall et al., 2005).

One limitation of this study is that pollen viability and stigma receptivity were not physiologically measured. Instead, it was assumed that pollen was viable when it was released and continued to be viable throughout the period of stigma receptivity (Saska and Kuzovkina, 2010). This assumption could cause an overestimate in the amount of overlap if the end of the pollen release phenophase actually had shorter periods of viability than our coarse visual estimates could reflect. Similarly, stigma receptivity was identified based on fruit capsule size, and may not have perfectly described the timing of when stigmas were truly receptive to pollen. Previous research on pear trees (*Pyrus communis*) found that stigmatic receptivity by each carpel develops sequentially in flowers, rather than all at once, giving the flower an extended period of stigma receptivity (Sanzol et al., 2003). If this were the case for *S. cinerea,* female individuals may have had only a small proportion of early-developing receptive stigmas when it was classified by our categorical scale to the stigma receptive phenophase. Fortunately, the phenological estimates were recorded by the same researcher consistently throughout the study, so although the absolute values have some uncertainty, the relative values are more reliable.

The relatively high asynchrony between pollen release and stigma receptivity at high elevation indicates that *S. cinerea* populations at high elevation may be spreading more slowly than at low elevation due to lowered seed output.

Furthermore, *S. cinerea* populations at high elevation have a shorter growing season, causing slower growth rates (Chapter 2) (Myneni et al., 1997), which may further exacerbate the low seed output discovered here. This is positive for invasion control, since the current control of *S. cinerea* is focussed on protecting threatened high elevation peatlands (McDougall et al., 2005, Moore and Runge, 2012). These results suggest that managers may consider expending more of their resources to remove low elevation populations to avoid a high annual influx of seedlings.

Although high elevation sites may only be producing a fraction of the amount of seed as is produced at low elevation, it is likely that a non-negligible amount of seed is being transported between high and low elevation. About 10% of *S. cinerea* seeds are thought to disperse more than 50 kilometres before establishment (Hopley, 2011) and, consistently, seed dispersal potential is considered the most important factor for managerial decision-making in *S. cinerea* invasion into alpine peatlands (Moore and Runge, 2012). Previous research on *S. cinerea* in Victoria, Australia found that removing the top 20% of seed producing sites could drop overall seed production by approximately 50% (Hopley, 2011). Since a smaller quantity of seeds are being produced at high elevation sites, the top 20% of seed-producing sites most likely exist at low elevation. Future research might develop models to assist managers in identifying priority control sites which account for the lowered seed output at high elevation. However, since *S. cinerea* can reproduce both by seed and vegetatively (Cremer, 2003), it is possible that the lowered seed output at high elevation will have only a small effect on the amount of overall willow biomass that occurs at high elevation.

Conclusion

We have used an invasive willow, *S. cinerea*, to determine how within-species phenological incompatibility can occur in response to temperature conditions that are dissimilar from their native range. Furthermore, we developed a new method to quantify overlap between phenophases which accounts for both the number of days overlapping and the number of individuals in the phenophases of interest on a given date. To our knowledge, this is the first study of its kind which incorporates the number of individuals into phenophase overlap measurements. This method is useful

to others wishing to quantify variation in phenological overlap for sites differing in population sizes.

In conclusion, this research provides support that low fecundity in non-native plants may be a result of sub-optimal phenological overlap. We also show that the degree of phenological overlap has significant effects on intraspecific fecundity, and therefore overall demography. Previously, there have been few studies which empirically identify the relationship between phenology and demography, particularly within a species (Miller-Rushing et al., 2010), and this research highlights its importance.

Acknowledgements

We thank our funders: the Australian Research Council Linkage Program and the Holsworth Wildlife Research Endowment. The research was permitted by DELWP to Dr. Joslin Moore (permit number 10007867). We wish to thank the environmental managers from Parks Victoria, DELWP, and several Victorian Catchment Management Authorities for their logistical support and assistance in identifying willow populations to monitor. We thank Melodie McGeoch for helpful comments in the early stages of the manuscript.

Tables

Table 3.1. Parameter estimates of generalized linear models for number of seeds per individual predicted by the proportion of stigma receptivity overlapped by pollen release, shrub volume (m^3), and elevation (low, high). Seed per capsule counts were averaged across site to account for a high number of zeros in the high elevation individuals, but individual variation was still accounted for through variation in catkin and capsule numbers. The stigma receptivity-pollen release overlap values were extracted from the ordinal logistic regression curves, with low elevation having 42% overlap and high elevation 24% overlap. The elevation predictor was categorical, including just low and high levels. The model was fit using a negative binomial family to account for overdispersion and had a sample size of 57 individuals. Psuedo R^2 was calculated as 1-(residual deviance/null deviance).

Model	GLM coefficient [CI lower, CI upper]	t value	*p-value*	Psuedo R^2
Intercept	6.95 [5.76, 8.27]	9.55	<0.001	0.47
Overlap	0.17 [0.09, 0.24]	3.59	<0.001	
Volume	0.001 [0.0003, 0.002]	3.22	0.001	
Elevation	-2.60 [-4.49, -0.33]	-2.13	0.03	

Figures

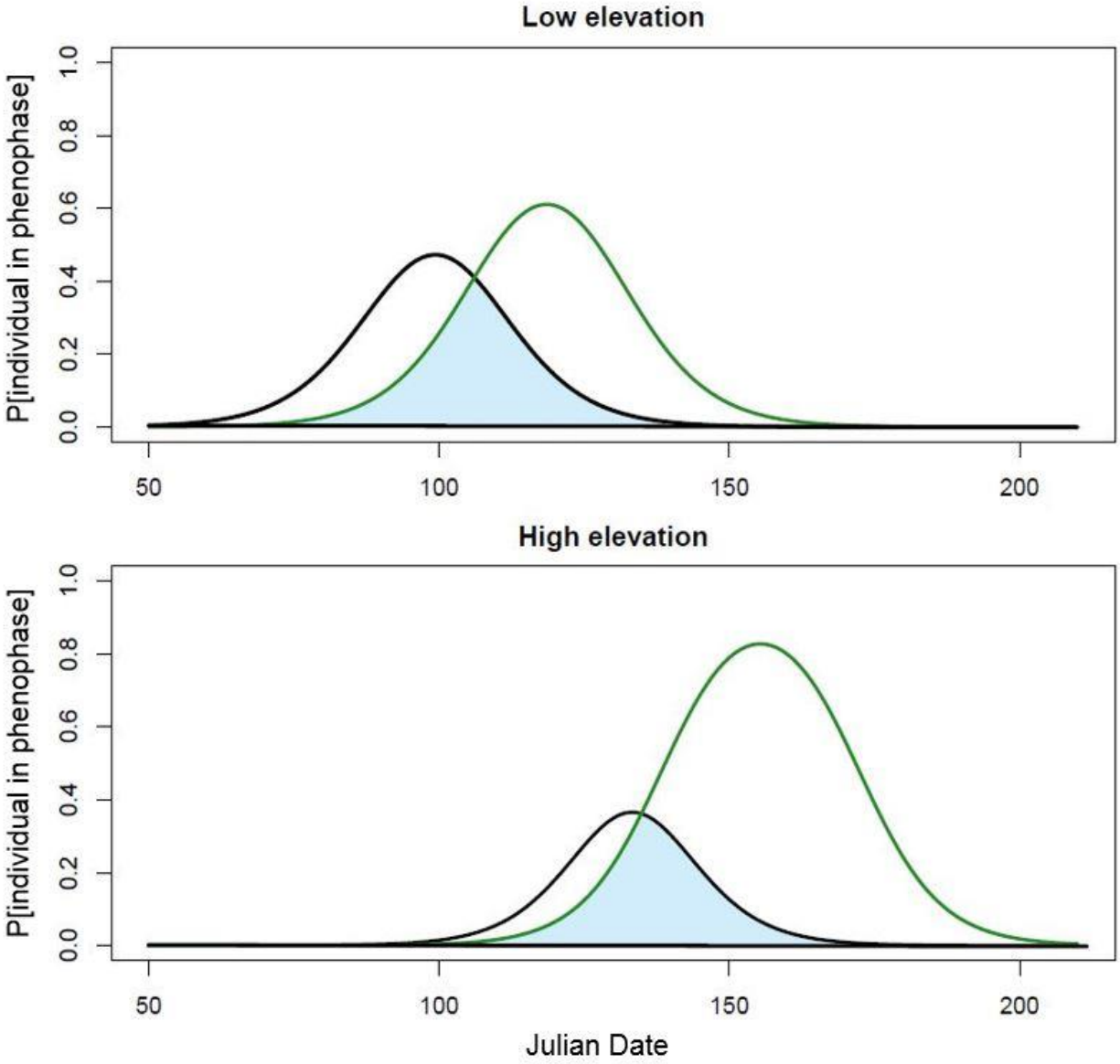

Figure 3.1. Ordinal logistic regression curves showing the probability through time of pollen release (black line) and stigma receptivity (green line). The x axis describes Julian date since winter solstice (June 21, 2016), and the y axis describes the proportion of individuals in a given phenophase at low (top) or high (bottom) elevation. The proportion of stigma receptivity overlapped by pollen releasing individuals was derived from these models and is shaded in light blue.

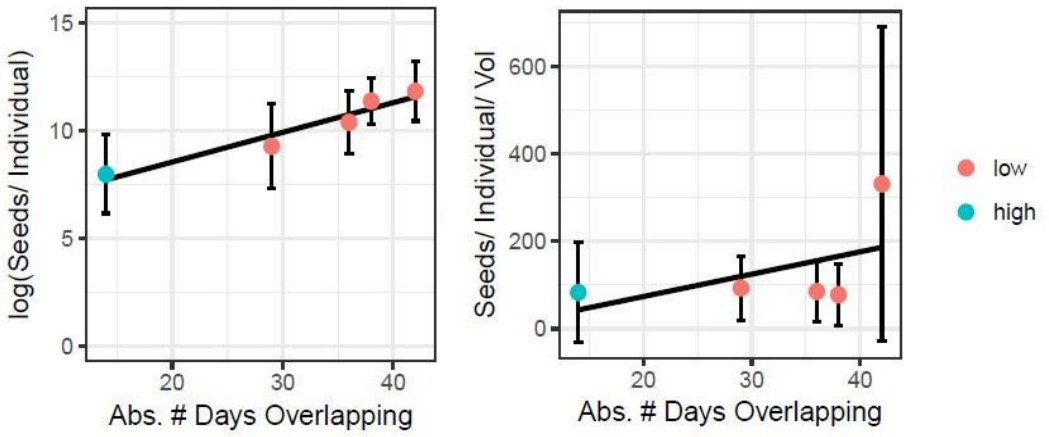

Figure 3.2. Depiction of relative trends across all sites with respect to phenological overlap and seed output. Phenological overlap was quantified by site by the absolute number of days pollen release and stigma receptivity were overlapping. Seed output was calculated per female individual. Best fit regression lines are included to show overall trend. The right panel reports seeds produced per individual per unit volume (m^3). The left panel has excluded one outlier from 302 m and one from 402 m in the left panel and the right panel has exclued one outlier from elevation 302 m and two at 410 m.

Chapter 4

Effects of elevation and seed size on the competition-colonization trade off in a small-seeded invasive species (*S. cinerea*)

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Abstract

According to the well-studied competition-colonization hypothesis, there is a trade-off between the ability to establish and dispersal distance in seeds of different sizes. Per this hypothesis, larger seeds generally have higher competitive ability. Smaller seeds, however, are generally capable of dispersing farther than large seeds, and thus have higher colonization ability. However, many wind-dispersed seeds have dispersal-mediating appendages, like pappi, which help them to travel long distances. In these cases, it is unclear whether the seed competition-colonization hypothesis will hold since dispersal distance may be more influenced by the shape and size of the pappus than the size of the seed. If the dispersal ability of pappus-bearing seeds are negatively correlated with their mass, then we might assume the hypothesis holds true. Similarly, it is unclear whether there is improved competitive ability in the larger seeds of small-seeded species.

We examined evidence for this size/dispersal trade-off in an invasive willow, *Salix cinerea*, assessing the correlations between seed size, competitive ability (measured as proportion of seeds germinated) and the potential for dispersal (measured as terminal velocity of the seeds and their pappi) within its invaded south-eastern Australian range. We found that terminal velocity was not correlated with seed mass, indicating that pappus presence confounds the relationship between seed size and dispersal distance. Furthermore, there was no observed difference in germinability across the size classes. These findings suggest that the competition-colonization hypothesis does not necessarily hold for plant species with seed attachments that facilitate dispersal or that have very small seeds. Future researchers should consider the effects of pappi on dispersal ability when considering the competition-colonization trade-off hypothesis for wind-dispersed species.

Introduction

Plants produce seeds of varying sizes, with seed size affecting long-term fitness, including a plant's size and probability of flowering (Michaels et al., 1988, Halpern, 2005). It has been observed that there is a seed production trade-off, where larger seeds tend to produce larger seedlings with a higher probability of establishment success (Westoby et al., 1996). Alternatively, smaller seeds dispersed by wind are generally able to disperse farther than larger, heavier seeds, increasing their competitive ability (Primack, 1987, Eriksson, 1999, Turnbull et al., 2004, Lehtila and Ehrlen, 2005, Lonnberg and Eriksson, 2013). This trade-off between the lower competitive ability of smaller seeds and their longer dispersal ability is described by the competition-colonisation trade-off. The competition-colonization hypothesis was originally intended to be utilised in plant community research comparing competitive ability between species (Levins and Culver, 1971). However, we argue the importance of comparing intraspecific trade-offs as well, particularly in early-successional species. Early-successional species often have little competition from other plant species upon arrival at a site for establishment, meaning much of their primary competition may be from members of the same species (Cain et al., 2008). As such, the within-species variation in seed size vs. germinability may significantly alter the subset of individuals in that species which establish in a new habitat. Hence an intraspecific trade-off might have important implications for the spread of plant populations.

Seed germination rates often vary with environmental conditions to which the mother plant is exposed (Wulff, 1986), and according to the conditions at the post-dispersal site of germination. Previous research has found that maternal origin may modify seed mass effects as well (Gonzalez-Rodriguez et al., 2011). Mother plants subjected to sub-optimal temperatures throughout a growing season have less stored energy available to allocate towards producing large seeds with higher probability of germination (Wulff, 1986, Biere, 1991). This may result in fewer seeds overall, or seeds produced with less available endosperm, resulting in a shorter period of time to find a habitable germination site before perishing from lack of nutrients (Mauseth, 2008)**.** Germination rates can also be affected by the region seeds are deposited post-dispersal. Extreme temperatures in post-dispersal sites can decrease germination rates, with risk of drought, extreme heat (Hou et al., 2014), and frost affecting seed germination and seedling lifespan (Leiblein-Wild et al., 2014). Since temperature has a strong effect on seed germinability (Stevens et al., 2014), we expect temperature changes across elevation will also influence the rate of germination.

The location of mother plants can have a significant effect on seed size, especially with respect to temperature and precipitation during the season of and prior to seed

maturation (Valencia-Diaz and Montana, 2005). Similarly, nutrient levels available to mother plants can affect seed sizes (Wulff, 1986, Vaughton and Ramsey, 1998), along with shading (Michaels et al., 1988). These factors can vary across elevation, and, expectedly, it has been previously shown that seed size can correlate negatively with elevation (Lord, 1994). What remains unclear is whether these different temperature regimes, specifically during the spring reproductive season, affect overall seed size and/or germinability of seeds produced in the low or high elevation populations. This information is particularly important if there is variation in seed sizes across the distribution of a species.

Many wind-dispersed seeds also have pappus, or other dispersal-mediating appendages, which assists their dispersal (Cain et al., 2008). Particularly in species with little variation in the size of their seeds, seed dispersal is heavily influenced by the shape of the pappus (Chrtek et al., 2018). Despite variation in seed size, we might expect wind-dispersed seeds have approximately equivalent dispersal abilities due to their attached pappi. In order to provide counter-evidence for the competition-colonisation trade-off, the correlation between seed mass and dispersal ability would need to be anything other than negative; a lack of correlation or a positive correlation would both suggest that smaller seed mass does not increase dispersal ability. Thus, there would be no trade-off between seed size and dispersal distance. Similarly, if larger seeds do not germinate at a higher rate than small seeds, there would be no clear trade-off in the competitive ability of larger seeds. We expect that dispersal ability is dependent on pappus attachment, though it is unclear whether germination rates are dependent on seed size for particularly small-seeded species.

In this study our focal species, *Salix cinerea,* is a highly invasive plant found in riparian zones of south-eastern Australia and New Zealand (Cremer, 1999). The species has been under management for over two decades, costing land managers millions of dollars for its control (Cremer, 1999). Previous research on *S. cinerea* has recognized dispersal ability as the most important factor for identifying priority control sites for management (Moore and Runge, 2012). The lightweight, anemochorous seeds of *S. cinerea* are pappus-bearing and have been recorded traveling more than 50 km by wind and between catchment systems (Hopley, 2011). Upon observation, *S. cinerea* seeds are most commonly released from their capsules as single seeds with attached pappi, or in clusters of seeds loosely entwined within their pappi. What remains unclear is whether there is variation in the mass of *S. cinerea* seeds, and whether that variation outweighs the effects of the pappus on the seed's dispersal ability. Additionally, it is unknown where across the invaded landscape there are relatively productive seed sources based on their levels of seed germinability and/or dispersal ability. This information would prove useful to managers when attempting to optimise their resources, and builds on the research of Moore and Runge (2012), by

identifying variation in dispersal ability of *S. cinerea* seeds produced across their invaded distributions.

This study examines the competition-colonization trade-off in a small-seeded species across *S. cinerea's* invaded distribution at low and high elevation. Additionally, we aim to better understand how the germinability of the species varies across elevation in its invaded range, based on its high dispersal potential at a landscape scale. To assess dispersal potential, we measure terminal velocity because it is strongly correlated with spread rate and is thought to well-describe dispersal ability (Caplat et al., 2012). Since terminal velocity is negatively correlated with dispersal distance, we would expect a positive correlation between terminal velocity and seed mass if the competition-colonisation trade-off were true. To better understand the competition-colonisation trade-off in *S. cinerea*, we asked:

1. Is there variation in *S. cinerea* seed size at low vs. high elevation?
2. Is there variation in the size and proportion of seeds that germinate across elevation, and depending the conditions of their germination site?
3. Is terminal velocity consistently correlated with seed size?

The competition-colonisation trade-off has never, to our knowledge, been studied intra-specifically in a small-seeded species across its invaded range. Furthermore, this study provides crucial information for optimising control efforts of *S. cinerea* in its invaded range based on the germinability of seeds across a landscape.

Methods

Study sites, seed collection, and sorting

Seeds were collected from four sites at low elevation (280-410 m) in the Ovens River Catchment of north-eastern Victoria, Australia. High elevation seeds were collected from one population growing along a small unnamed creek in Dinner Plain, Victoria (1640 m). Between two and five female individuals were randomly selected for measurement and catkin collection from each site, based on mature catkin availability at the time of collection. Length and width of each female individual was measured. Height was measured by photographing individuals from trunk base to apex of the crown with a meter stick for scale. ImageJ software was used to measure the heights of each individual, based on its length relative to the meter stick. We recorded the number of male individuals at each site within 10 meters of the first and last female individual at each site. Each site contained at least 10 mature male individuals.

15 to 20 catkins were haphazardly collected from the lower-hanging branches of each individual. One catkin was randomly selected per individual to measure five capsule lengths and widths across the widest point, the number of capsules per catkin, and catkin length and width. Catkins were stored in direct sunlight in an enclosed space until seed abscission occurred (Hopley and Young, 2015). Catkin collection occurred at low elevation on November 7, 2017, and on December 5, 2017 at high elevation to account for phenological differences in seed release times (Chapter 2). Once seeds abscised, they were kept in an ice-filled cooler, then transferred to a 4°C cold room for storage for up to 30 hours prior to the germination experiment.

On the day following catkin collection, seeds with pappi removed were sorted by mass into three size classes (small, medium, and large) using a Zig Zag seed aspirator. Aspirators sort seeds according to their mass by blowing wind at a user-defined speed upwards as seeds fall from above. We used consistent settings (220, 280 volts) to determine the proportion of seeds within each site that fell into each of the size categories. Low and high elevation seeds were sorted separately.

Seeds from each size class were divided in half to be germinated in each of the 16°C or 22°C cabinets. The cabinet temperatures were chosen to reflect the average daily spring temperatures during the time of seed release in December at high elevation (Dinner Plain, Victoria) and in November at low elevation (Myrtleford, Victoria) (Chapter 2)(Meteorology, 2018). The cabinets were set to have 14 hours of light, as is consistent during the months of November and December in Victoria. Each quantity of seed was weighed then plated in Petri dishes lined with moistened filter paper and wrapped in Parafilm (Hopley and Young, 2015). Each Petri dish was photographed so that the number of seeds could be counted digitally using ImageJ software (Schneider et al., 2012) (Figure S4.1). The ImageJ snipping tool was used to create a cropped images that contained only seeds. Colour saturation of the image was then adjusted so that the outline of each seed was distinguishable from its neighbours. Then particle analysis (seed counting) was completed with the size set from 25 pixels^2 to infinity, so that dust and debris particles were not counted as seeds.

Previous research has found no germination one week after sowing *S. cinerea* seeds (Hopley and Young, 2015), so petri dishes were kept in the 16°C and 22°C cabinets for 11 days and monitored every second day. Seeds were considered germinated when their cotyledons had opened or there was radicle elongation and visible rootlets (Mortlock, 1999). Cabinet temperature and light consistency were measured before the start of the study. Cabinet temperature was measured using T-TEC RF version 6 data loggers (accuracy: ± 0.2 C° and ± 3% relative humidity) to ensure the temperature was correct and consistent. Light

consistency between cabinets was measured using a light meter, and there were no notable differences between each of the four corners or any of the shelves. However, to mediate any small variations, we rotated the trays of Petri dishes after every monitoring.

To determine the mass of *S. cinerea* seeds, an additional 1,884 seeds were collected from low elevation sites and sorted by size using the same voltage settings on the Zig Zag aspirator. Only low elevation seeds were measured because the seed aspirator sorted seeds by pre-determined small, medium, and large settings. As such, the small, medium, and large high elevation seeds would certainly have the same average masses as at low elevation. Different seeds were used than in the germination study so that the germination study could commence more quickly and avoid seed death prior to the initiation of the experiment. Each size class was split into five groups of seed, which were then individually counted and weighed to obtain an average mass for each size class. The sample included 725 small seeds, 580 medium seeds, and 579 large seeds.

Terminal velocity

Terminal velocity was calculated as a proxy for dispersal potential by gently separating a single abscised seed from a catkin, careful to retain any pappus connected to or encapsulating the seed. The pappus-bearing seed was then dropped into a clear 2L graduated cylinder. The falling seed was recorded on an iPhone 7 using a timestamp app (Timestamp Camera), then viewed frame-by-frame in a slow-motion app (Slowmo) to determine the length of time required for the seed to fall 25 cm. The top 25 cm of the graduated cylinder were not measured so that the seed to could attain its terminal velocity before measurement. We found <10% variation in seed fall times when five seeds were each replicated five times to assess precision of the methods. Using the known distance the seed fell and time required to fall we calculate terminal velocity (V_t = Distance/Time). After seed terminal velocity was measured, pappi were removed and individual seeds were weighed using a high-accuracy Quantum Scientific Shimadzu scale. To ensure accurate measurements, we reset the scale to tare between each seed. If the scale could not settle at 0.00000 g, then the tare mass was recorded and subtracted from the measured seed mass. Viability of seeds was tested after mass measurements were taken using a cut test, which determines that the coloration of seed embryos are consistent with healthy, germinable seeds. The terminal velocity experiment was completed on 30 seeds collected from low elevation including: 18 large, 7 medium, and 5 small seeds.

Data analysis

To determine whether there was variation in seed size we completed a one-way ANOVA with seed size class as the categorical predictor and seed mass as the response.

Following the ANOVA we completed a Tukey test to compare the differences between the mean size classes. Next, to better understand the effects of seed size, maturation location, and cabinet temperature on the proportion of seed germination, we developed a binomial generalised linear mixed effects model (GLMM) with a logit link. The model was fit using the Laplace Approximation by maximum likelihood. A Petri dish (observation-level) random effect was included to account for the overdispersion (Harrison, 2014). Interaction variables were included between each of the three predictors. Additionally, confidence intervals were calculated around the proportion of seeds germinated between seed size, maturation location, and cabinet temperature groups for comparison. Finally, to determine the effect of terminal velocity on seed size, we completed a linear regression analysis between terminal velocity and seed mass. The independent terminal velocity variable was centred and scaled, then assumptions for the model were checked; the data was normally distributed, independent, and had no issues of non-homogeneity of variance.

Results

Confirming the efficiency of the seed sorting technique, seed mass differed significantly among the small, medium, and large size categories ($F=482$, df=2, 1,881, $p<0.001$) (Table 4.1a). Each seed size class was significantly different the others (Tukey test, $p<0.05$). Medium seeds were, on average, 15% heavier than small seeds (difference=$1.4x10^{-5}$, CI=$1.1\ x10^{-5}$-$1.6\ x10^{-5}$) and large seeds were 18% heavier than medium seeds (difference=$1.8x10^{-5}$, CI=$1.6\ x10^{-5}$-$2.1\ x10^{-5}$). Plants at low elevation produced a smaller proportion of large seeds than those at high elevation. There were 26% more large seeds produced at high elevation compared with low elevation. Similarly, there were 7% more small and 13% more medium sized seeds produced at low elevation than at high elevation (Table 4.1b).

Germinability was highly variable among plates within treatments and among treatment means, ranging from a mean of only 17% germination for small seeds from the high elevation site at 16°C to 72% for large seeds from high elevation at 22°C (Table 4.2). Pooled across elevation and temperature treatments, germinability was (mean $\pm$ SE): 0.20 ± 0.09 for small seeds (n = 415), 0.30 ± 0.06 for medium seeds (n = 1,672), and 0.38 ± 0.06 for large seeds (n = 3,515). Pooled across elevation and size classes, 32.1% of seeds germinated at 16°C and 35.4% at 22°C. Overall, 32.3% of seeds collected at low elevation germinated, while 47.8% of seeds from the high elevation site germinated (Figure 4.1-4.2).

There was no significant effect of seed size, elevation, or temperature factors on the proportion of seed germination, and the model had relatively low predictive ability (Marginal R^2=0.07). The largest effect size in the GLMM was for the effect of small seeds produced at

high elevation (est.=-2.17, z-value=-1.7, *p=0.09*) on the proportion of seeds germinating (Table S4.1) (Figure 4.3). The next largest effect size was of the interaction between small seeds produced at high elevation in the 22°C cabinet (est.=1.15, z-value=0.7, *p=0.49*). Seeds produced at high elevation germinated 26% more frequently in the 22°C cabinet (mean=0.62, var=0.05, n=446) than in the 16°C cabinet (mean=0.36, var=0.09, n=538), while there was only a 1.4% difference between 16°C and 22°C for low elevation seeds (16°C: mean=0.32, var=0.05, n=2,302; 22°C: mean=0.33, var=0.05, n=2,316) (Figure 4.1).

Seed mass was not a significant predictor of terminal velocity in the linear regression model (coefficient estimate= 0.25, t=1.3, df=28, *p=0.19*) (Figure 4.4). The model explained a very small amount of the variation in the data (Multiple R^2=0.06).

Discussion

Competition-Colonisation Trade-off

We found little evidence to support the competition-colonisation hypothesis in the pappus-bearing, small-seeded species *S. cinerea*. Under the competition-colonization hypothesis, we would expect to see larger seeds with consistently higher germination rates than medium and small seeds. Instead we found no detectable variation in germinability among the small, medium, and large size classes, suggesting that increased seed size does not confer a clear advantage with respect to germination rates. Similarly, our results show that terminal velocity, a proxy for dispersal distance, was not correlated with seed mass, which suggests that there is not a trade-off between seed size and dispersal distance, as would be expected under the competition-colonisation trade-off (Jakobsson and Eriksson, 2003).

Typically there is a positive correlation between seed mass and terminal velocity (Greene and Johnson, 1993, Greene and Quesada, 2005, Hahn et al., 2013), but our results were not consistent with this finding. Our results are consistent with recent research suggesting that variation in terminal velocity can be largely attributed to variation in seed mass, pappus length, and their interaction (Chrtek et al., 2018). If both germinability and dispersal are unrelated to seed mass, we might expect to find nearly uniform size among the seeds of *S. cinerea*, the predicted optimum in basic models of offspring size-number trade-offs (Smith and Fretwell, 1974, Haig and Westoby, 1988). Indeed, previous research tends to suggest that there is a strong stabilising selection for the seed size so that seeds are largely invariable (Turnbull et al., 1999). What we found instead was relatively large variation in seed size. However, seed size is likely to have fitness consequences other than germinability and dispersal; it can influence seed longevity and seedling establishment rates and longevity (Silvertown, 1981). The ability of a seed to survive following dispersal but

before germination and establishment, including the size of the nutrient subsidy available to a seedling, would affect its competitive ability. Colonisation rates would also be affected by seed longevity depending on the distance of dispersal and how long a seed is transported prior to arrival at its establishment site. Thus, longevity may be a third factor to take into account when considering an intraspecific competition-colonisation trade-off.

To our knowledge this is the first study of its kind to consider the competition-colonisation hypothesis for an individual species, rather than an assemblage of species. From the information gathered in this study, we believe it is important not to consider a competition-colonisation trade-off as a categorical, binary alternative. Rather, species may exist on a continuum of this trade-off, and there may be multiple variables included in that trade-off. For some species this trade-off may be very important, such as late-successional colonisers. Alternatively, for species such as *S. cinerea* which are early-successional with wind-dispersed, pappus-bearing seeds, compromises between competition and colonisation may not be significant to their population growth or persistence. Instead, a third variable, longevity, may be included in this trade-off for species which do not have a clear competition-colonisation trade-off related to seed size. For wind-dispersed species, rather than larger seeds having an increased likelihood of germination immediately post-seed release, the larger seeds may benefit in an ability to survive longer before germination in harsh conditions.

The lack of relationship between dispersal ability and seed size may be found in other wind-dispersed species which bear a pappus, and also likely in other species with any wind-dispersal-appendage. A large proportion of the angiosperm world includes anemochorous species with dispersal attachments that come in many forms (Howe and Smallwood, 1982) and may also imperfectly reflect the competition-colonisation trade-off. Seeds from taxonomically large plant families including Asteraceae, Salicaceae, and Asclepiadaceae frequently bear pappi to assist in dispersal, with Asteraceae alone making up approximately 9% of flowering plant species world-wide (The Plant List, 2013). Additionally, other wind-dispersed plants, such as Acer (Sapindaceae), Dipterocarpaceae and Brassicaceae, bear wings on their fruits (1993), and variation in seed size within or among species in these groups may have little affect on dispersal. Based on the results of this study we know one wind-dispersed plant does not conform to the competition-colonisation hypothesis, and future studies might aim to consider the relative prevalence of this trade-off intraspecifically for wind-dispersed species, and in particular for those with small seeds, or dispersal-mediating appendages.

Similarly, we speculate the competition-colonization trade-off is likely to affect other dispersal syndromes to varying degrees due to their significant variation in seed mass, size, and shape (Liu, 2014). Dispersal in hydrochorous plants, for example, is unlikely to be affected by seed size, assuming seeds are being dispersed in a moderately-sized water body. In fleshy-fruited species dispersed by animals, the influence of the competition-colonization trade-off may depend both on the method and vector of dispersal. Seed size for endozoochorous plants, dispersed by seed ingestion and defecation, may be irrelevant, since fruit size is selected for by animals, and larger fruits do not necessarily produce larger seeds (Michaels et al., 1988). This likely is only true to an extent though; myrmechorous (ant-dispersed) seeds can only disperse as far as an ant can carry them, which limits the size they can grow and effectively disperse. Alternatively, the seed size of epizoochorous species (accidental seed dispersal by animals via attachment to fur or otherwise) may have no effect on seed dispersal, depending on the size of the animal vector. Large mammals are unlikely to notice within-species variation of medium-sized seeds stuck to their fur and attempt to remove them. Alternatively, a song-bird may be quicker to notice and attempt to detach a seed stuck to its minute body. Spininess of the seed, as well as the protectiveness of the animals' coat would also likely affect seed dispersal distance by an animal. Epizoochorous species are also those which may be influenced significantly by seed longevity, rather than classic establishment or dispersal abilities. There are a number of factors related to morphological seed features and seed dispersal vectors which would have an effect on the applicability of the intraspecific competition-colonization trade-off in a large proportion of, and perhaps even a majority, of flowering plant species (Figure 4.5).

Elevation effects

We expected to find smaller seeds at high elevation due to the colder environment, but actually found that a higher proportion of large seeds produced at high elevation compared with low. Among nine species of herbaceous *Pedicularis,* high elevation populations did not have smaller seed mass than those at low elevation, relative to their variation in biomass (Guo et al., 2010). Similarly, variability in seed mass of the shrubs *Cytisus scoparius* and *Ulex europaeus* could not be attributed to broad scale differences in elevation (Buckley et al., 2003). However, there was significant variation in germinability between seeds produced at high and low elevation, despite the lack of variation in seed size. High elevation seeds had higher germinability overall for all seed size classes and in both maturation temperature cabinets. Additionally, seeds matured at high elevation had a higher proportion of large and medium sized seeds than produced at low elevation. Higher germination in seeds produced at high elevation has been identified before in two heathland species (Vera, 1997). This may be because high elevation seeds produce more endosperm

overall to increase their longevity in the response to increased environmental stress (Arshad et al., 2017). Seeds produced at low elevation may not require as much stored nutrition since conditions are likely to be favourable for germination shortly after their release from fruit. This may affect the spread of *S. cinerea* across elevation, with high elevation seeds having larger seeds with higher germination rates compared to those produced at low elevation.

Unexpectedly, we found that seeds produced at high elevation had significantly higher germinability in the warmer, 22°C cabinet, emulating low elevation spring conditions compared with the 16°C cabinet emulating high elevation conditions (Figure 4.1), as well as a higher proportion of large seeds compared to low. In its native northern European range, the climate *S. cinerea* experiences is more similar to that of high elevation than low elevation sites in this study (Chapter 3). As such, this result is unexpected since *S. cinerea* is adapted to conditions similar to those at high elevation. One explanation could be that high elevation individuals are producing seeds that are more resilient towards harsh environmental conditions and profiting from the warmer, low elevation conditions. Alternatively, the low elevation populations may have rapidly evolved to their warmer climate, requiring only a minimalistic seed coat and less endosperm due to optimal conditions in spring for rapid germination. Consistently, there is not a consensus that populations in invaded ranges are more successful than in their native range (Bossdorf et al., 2005). Previous research on two invasive shrubs found that one of the two species had larger seeds in its invaded range, and the other had no consistent differences in seed size between the native and invaded ranges (Buckley et al., 2003). These results suggest that seed size may not have adapted rapidly to the invaded range conditions. Fortunately for managers, long-distance seed dispersal by wind is relatively uncommon (Nathan et al., 2002), so the vast majority of high elevation seeds are unlikely to disperse down to low elevation and experience higher germination rates. However, the species is also capable of hydrochory, which may present more of an issue in cases of high elevation seeds being transported downstream to low elevation.

There was substantial observed variation in germinability of small seeds produced at high elevation, with seeds in the warm, 22°C cabinet having 27% higher germinability compared with the 16°C cabinet. The small seeds are likely not cold-resistant, possibly due to thinner exocarps providing less cold protection, and resulting in lowered germination rates. This may further explain why high elevation sites produced far fewer seeds than did low elevation sites, as was found in Chapter 2. High elevation sites, due to worse conditions for germination, must produce larger seeds overall. This phenomenon is commonly described as a growth-survival trade-off, where seed energy investment varies according to surrounding conditions, with high elevation populations producing fewer, but larger, seeds.

Low elevation seeds instead produce a higher quantity of smaller seeds. The growth-survival trade-off has been seen previously in the height growth performance of two *Pinus spp.* (Chuine et al., 2006), as well as the tree-ring growth of *Pinus contora, Abies lasiocarpa,* and *Picea glauca* (Miyamoto et al., 2010), in response to varying temperature gradients, where slower growth was observed in trees growing in stressful environments. The same trade-off has also been observed in 103 tree species in Panama in response to light availability, rather than temperature stress (Wright et al., 2010). Light availability may be an important and interesting factor to look into for future research, since light levels could not be measured in this study, but likely vary considerably between populations. Further, *Salix* species are highly responsive to surrounding light conditions, which might make the trade-off effects more pronounced it there is consistent variation in light availability between high and low elevation populations (Cremer, 2003).

Combining the overall proportion germination across elevation, with the average amount of seed produced by site at low vs high elevation (as estimated in Chapter 3), we see that low elevation sites are still producing more seeds that are likely to germinate (13,160 seeds*0.323 prop. germination= 4250 seeds likely to germinate per individual) compared with the high elevation site (8,020 seeds*0.478 prop. germination= 3830 seeds likely to germinate per individual). Although the difference in seeds likely to germinate between high and low elevation is not large, there are significantly more populations of *S. cinerea* at low elevation compared to high. Currently, managers are focussing their control efforts on high elevation regions in south-eastern Australia. However, it is likely that the distribution expansion of *S. cinerea* is predominately spreading from low elevation seeds based on their high level of production, and since the populations are much more plentiful and expansive. As such, we recommend managers consider shifting some of their control efforts to large populations at low elevation that are producing enormous quantities of seed.

Conclusion

Our study indicates that greater research attention should be given to phenotypic trade-offs in woody plants and, in particular, those with small seeds and dispersal-mediating appendages. We have shown that the competition-colonization trade-off does not hold true for one wind-dispersed species, which suggests that other plants, perhaps with other dispersal syndromes, may also not abide by the trade-off within species as they may be expected to. Future researchers could look at variation in applicability of the trade-off in species with other dispersal syndromes, or could focus on early- vs. late-successional species, as this may also affect the applicability of the trade-off.

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Tables

Table 4.1. The average mass of each size class (a) was determined by weighing, individually counting, then dividing the number of individual seeds by the total group mass to obtain an average mass for each size class. Average mass was calculated from seeds collected at low elevation only, and separate from the seeds used in the germination study, but sorted using the same, fixed aspirator settings. For the germination study, the proportion of small, medium, and large seeds (P(size)) for low vs. high elevation varies as a result of the sorting methods completed by the aspirator (b). The fixed setting that the seeds were sorted at was consistent between high and low elevation, and therefore shows differences in the proportion of small, medium, and large seeds across elevation.

a)

Size class	Sample size	Mass (grams)
Small	725	9.9×10^{-5}
Medium	580	1.1×10^{-4}
Large	579	1.4×10^{-4}

b)

	Low	High
Sample size	4618	984
P(Small)	0.10	0.03
P(Medium)	0.32	0.13
P(Large)	0.58	0.84

Table 4.2. Effects of temperature conditions, seed size, and maturation location on germinability of *Salix cinerea*. Standard deviation of the average maximum proportion of seed germination refers to the variation between plates.

Maturation location	Cabinet temp.	Size class	Prop. germinated Mean	SD	# of Petri plates	# of seeds
Low elevation	16°C	Small	0.48	0.24	9	177
		Medium	0.41	0.26	10	807
		Large	0.38	0.20	13	1353
	22°C	Small	0.51	0.28	8	210
		Medium	0.37	0.17	10	735
		Large	0.43	0.24	13	1336
High elevation	16°C	Small	0.17	0.28	3	12
		Medium	0.64	0.14	4	87
		Large	0.52	0.30	6	439
	22°C	Small	0.68	0.37	4	16
		Medium	0.69	0.14	4	43
		Large	0.72	0.20	6	387

Figures

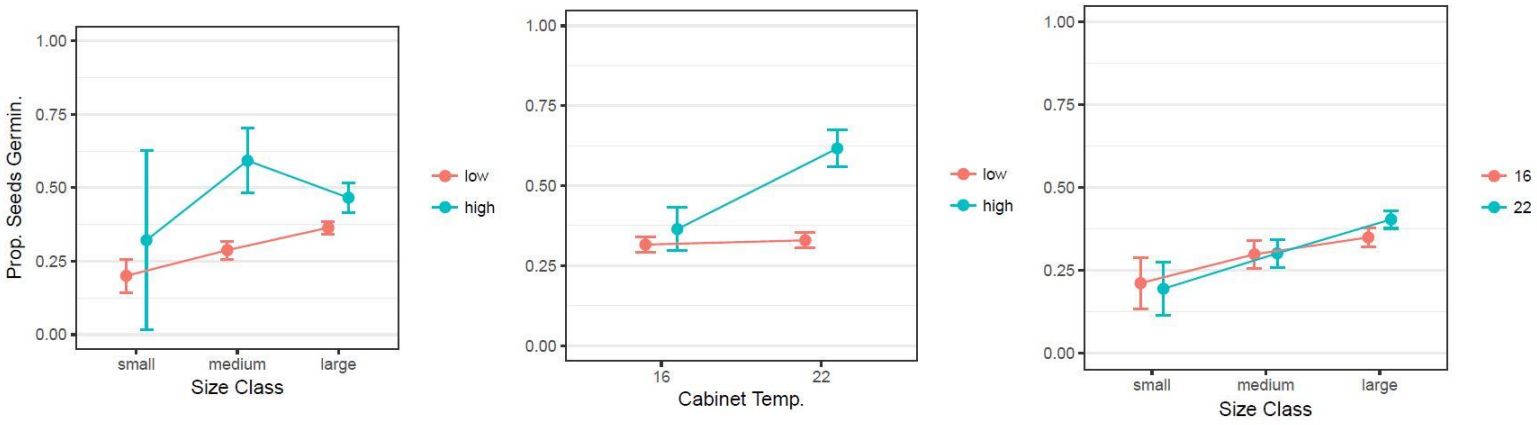

Figure 4.1. Interaction plots describing the proportion of the total number of seeds which germinated for each group. Standard errors were calculated for each group as such: $SE = \frac{(proportion\ germinated * (1 - prop.germ.))}{total\ number\ of\ seeds\ in\ group}$ (Crawley, 2002). 95% confidence intervals were then calculated around the total proportion of seeds germinated per group and presented here.

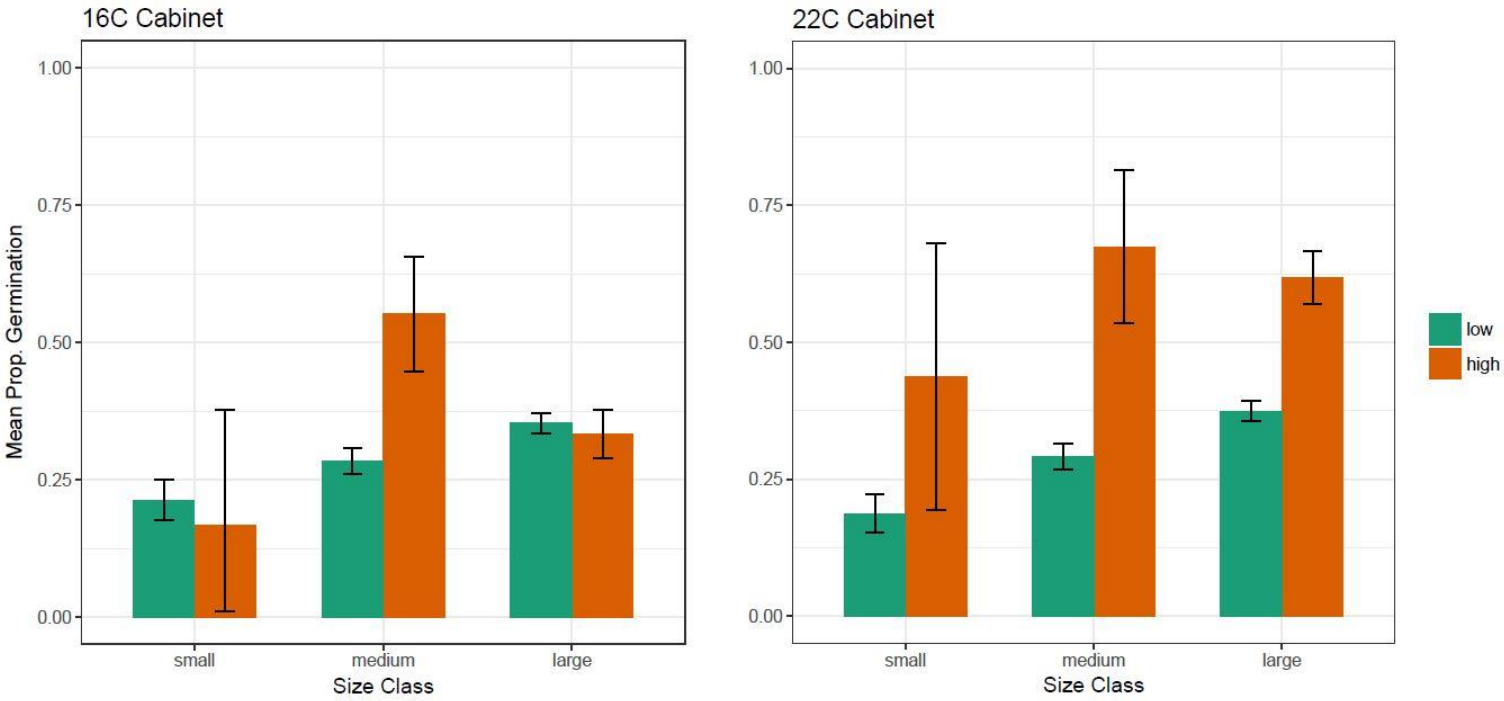

Figure 4.2. Variation in mean proportion germination for each seed size class, separated by those matured at low or high elevation, and germinated in either warm or cool conditions. 16°C cabinet emulates average daily conditions at high elevation Victoria, Australia, while the 22°C cabinet emulates average daily low elevation conditions. Confidence bands are two standard errors above and below the mean proportion germination.

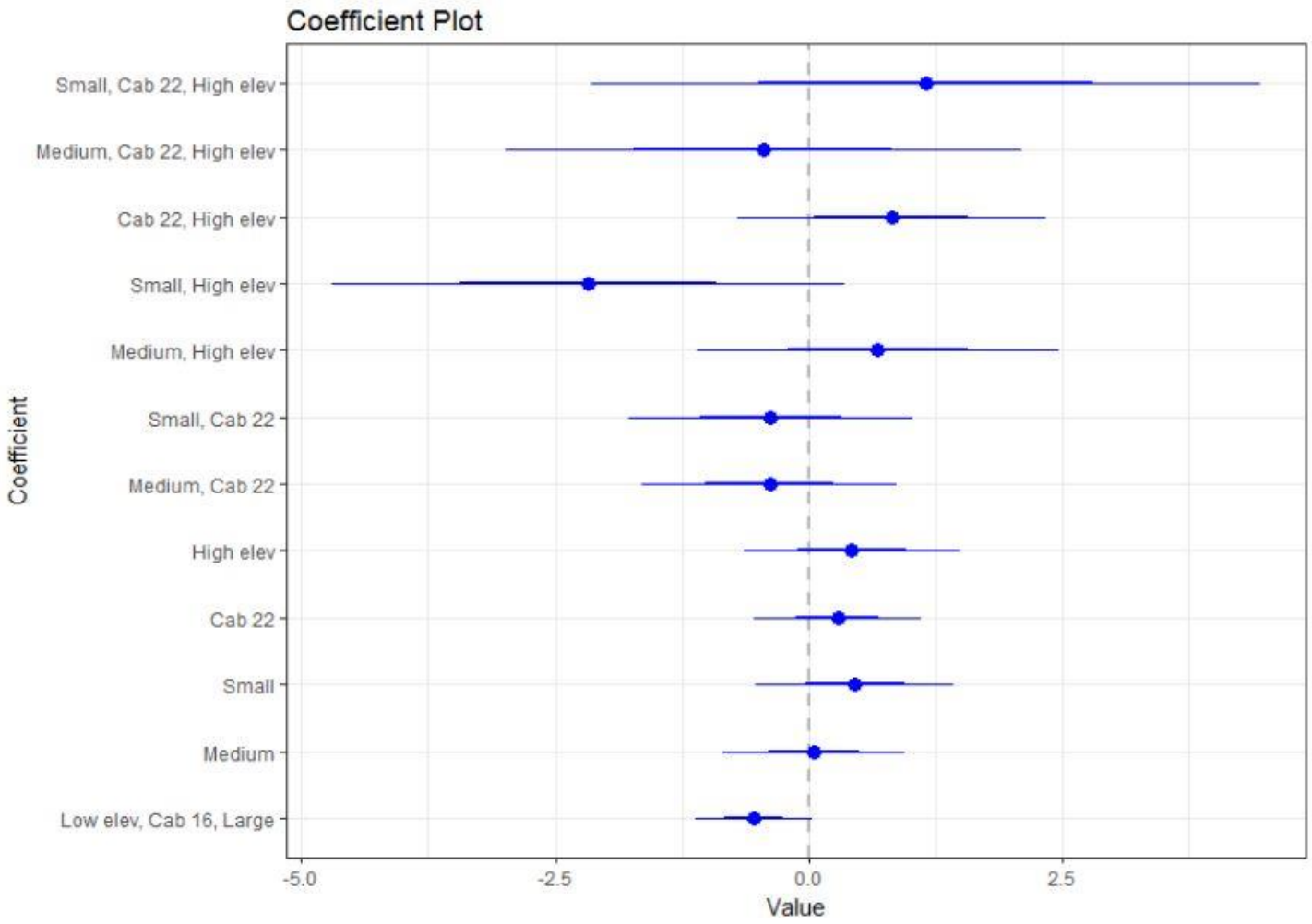

Figure 4.3. Coefficient plot for the binomial generalised linear mixed effects model, including the interactions between each variable. The response variable was germination success per Petri plate (n=90), predicted by maturation location (low vs high elevation), seed size (small, medium, large), and germination cabinet temperature (16°C or 22°C). The cool cabinet temperature is representative of average high elevation spring conditions, while the 22°C cabinet is representative of average low elevation conditions. The intercept is the variable the other coefficients are compared to: large seeds matured at low elevation in the 16°C cabinet. Coefficients whose error bars cross the vertical x=0 line are not significantly different from the intercept variable (α=0.05).

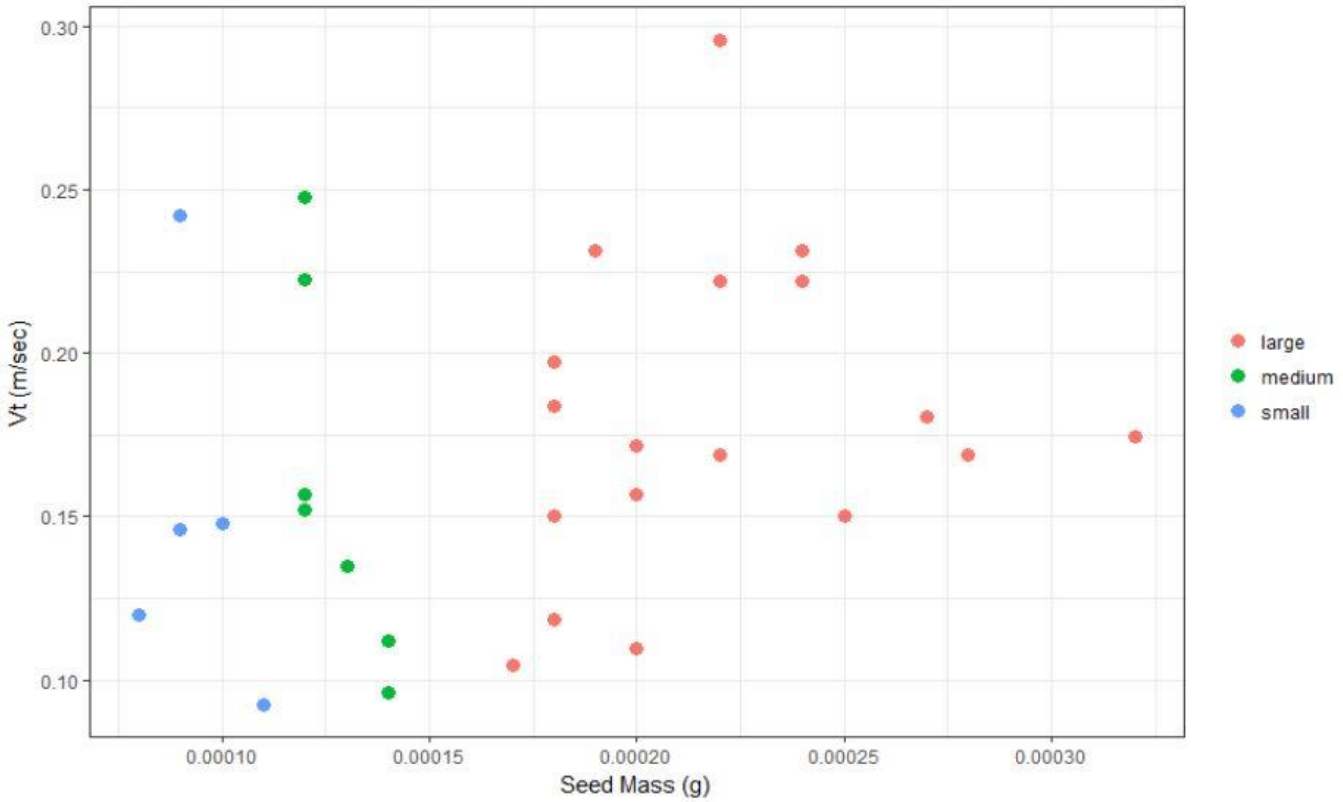

Figure 4.4. *S. cinerea* seed mass for 30 seeds collected at low elevation with pappus removed. Terminal velocity (Vt) was calculated from measurements on seeds still bearing their dispersal-mediating pappus. Seed size classes are colour according to the ranges identified for the germination study.

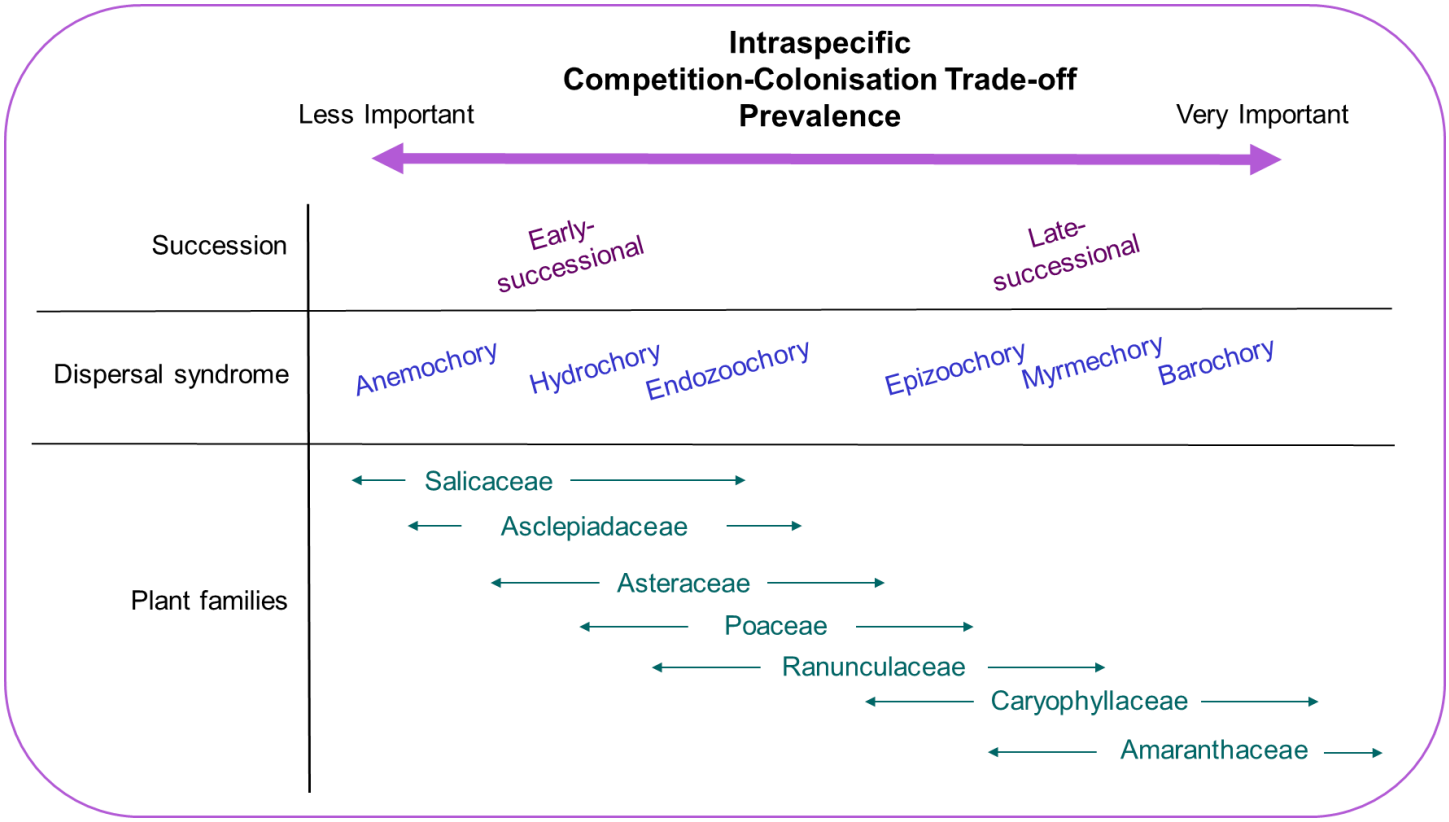

Figure 4.5. Intraspecific variation in the competition-colonisation hypothesis may have varying effects on plants depending on their colonisation strategy and their dispersal syndrome. This schematic diagram aims to function as a starting point for considering how various colonisation strategies may vary according to how applicable the competition-colonisation trade-off may be. Plant families have breadths listed to suggest that competition-colonisation trade-off may apply in varying amounts to different taxa within a family. Plant family dispersal syndromes are as follows: Amaranthaceae: anemochory, barochory, epizoochory; Ascelpiadaceae: anemochory; Asteraceae: anemochory, epizoochory; Caryophyllaceae: barochory; Ranunculaceae: epizoochory; Salicaceae: anemochory; Poaceae: anemochory, epizoochory (Liu, 2014). Definitions of seed dispersal syndromes in supplemental materials (Table S4.2).

Chapter 5

Variation in fecundity across geographic and climatic gradients in the willow (*Salix*) genus

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Abstract

Since fecundity is vital to the spread of a plant species, it is important to determine whether there is variability in how fecund a species may be across its distribution. Furthermore, depending on climatic conditions, an individual is likely to produce variable amounts and quality of seed. To better understand the effect of climate and geography on plant fecundity we observed trends in catkin length variation (as a proxy for fecundity) across 17 willow (*Salix*) species ranges using data from over 800 herbarium specimens collected from North America, Europe, and Australia.

We found that the climatic and geographic models explained an approximately equal amount of variation in fecundity. Elevation and diurnality, a measure of monthly temperature variability, both had a significant negative correlation with catkin length at the genus-level; however the effect sizes were quite small. Diurnality was also the most common climatic variable at the species-level, while latitude was more important than elevation at the species-level. Additionally, we found that the invasive vs. native range variable was significant to catkin length in three of five species studied when geographic and climatic variability were mathematically controlled for. These results suggest that there was little evidence of consistent large-scale environmental effects at the genus- or species-levels which affected fecundity, regardless of the range (native or invasive) of the specimens. Species fecundity is likely constrained by factors other than physical location or climate.

Introduction

Fecundity is a key demographic trait which affects a plant's spread potential (Burns et al. 2013). The spread potential of a plant species is, in part, related to the intensity of propagule pressure (Simberloff 2009). As such, the variability in fecundity may be influential to a species' invasion potential. Furthermore, information about within-species variation in fecundity across its distribution may be used to describe the environmental or geographic factors which affect fecundity. It is already known that fecundity is likely to vary across a plant's distribution in response to varying climatic conditions, including temperature and precipitation (Angert 2009, Buechling et al. 2016). Similarly, geographic location can be used to describe fecundity: some species fecundity varies with relative position within their distributions (Brussard 1984, Angert 2009) while fecundity is unaffected by range position in other species (Kluth and Bruelheide 2005). Geography may also be informative as a summary measure of climatic variation not captured by individual temperature or humidity variables. In order to better understand within-species variation in fecundity, we might focus on the variation in fecundity as it relates to surrounding geographic position and climatic conditions.

The spreading of species' distributions is particularly important for invasive plant species. Invasive plants are often more fecund in their introduced range compared with the native range, which is true for a number of reasons. The evolution of increased competitive ability (EICA) hypothesis states that the introduced range is not home to natural enemies, freeing up the plant's resources to be funneled into biomass production (Blossey and Notzold 1995). Additionally, female output is often favored in invasive plants to increase their early population growth and probability of colonization to newly occupied sites (Pannell 1997). Lastly, and most relevant to our study, there is evidence that environmental gradients across a landscape promote genetic differentiation in non-native plants, and therefore rapid evolution, to encourage their fecundity (Maron et al. 2004). Previous research observing invasive populations across latitude found that most variation in traits was explained by differences in the environmental region, suggesting that invasive populations are responsive to the conditions they inhabit (Kollmann and Banuelos 2004). However, despite the likelihood that non-native plants will have varying fecundity across their distribution compared to their native range, there have been few observation of the effects of environmental gradients on non-native plant fecundity and even fewer

comparing species in their native and invaded ranges. This is likely because of the logistical difficulty of completing field reproductive output assessments on a species in both its native and invaded ranges, and across its distribution.

Invasion biologists have long assumed that invasive species have enhanced performance in their introduced range relative to home range (Blossey and Notzold 1995), and there is a growing knowledge-base which supports that assumption. A meta-analysis comparing invasive plants with non-invasive plants found that invasiveness was associated with an increase in performance-related traits (van Kleunen et al. 2010b). However, it is unclear whether this trend is consistent between members of the same species in their native and introduced range. Some studies have found invasive plants are on average larger and more fecund than native plants (Parker et al. 2013, Jelbert et al. 2015). Another study examining grassland ecosystems found that grasses showed no difference between native and introduced range abundance, while forb abundance tended to be lower in the away ranges (Firn et al. 2011). What remains unclear is whether fecundity varies according to the environmental conditions experienced in the native and invaded ranges. By comparing the traits of species in their native and introduced ranges, we can better understand how likely a species is to be successful in its invaded range based on the surrounding conditions and potentially whether invasive species have undergone evolutionary change after their introduction (van Kleunen et al. 2010a).

Salix is a dioecious, woody plant genus with female trees which produce long, tubular capsule-bearing flowers in pendant inflorescences called catkins. Out of more than 330+ *Salix* species worldwide, dozens are considered invasive in Australia and New Zealand, and several in North America and Europe (Isebrands and Richardson 2014). The genus produces tiny seeds that are primarily dispersed by wind and have been estimated to travel farther in a larger proportion of seeds than has been estimated in any other plant genus (Hopley 2011). Understanding fecundity in *Salix* is particularly important because dispersal has been identified as the most important factor in determining optimal control efforts in an invasive member of the genus (*S. cinerea*)(Moore and Runge 2012).

Here we aim to better understand how fecundity might vary with geography, temperature, and moisture, and whether this variation is consistent between the genus' native and invaded ranges. We compare catkin lengths as a measure of fecundity using herbarium records collected in North America, Europe, Australia, and

New Zealand. Catkin length is used to measure seed output because longer catkins have been shown to produce more seeds in one invasive willow species (*S. cinerea*) (Figure S5.1), and information regarding catkin length is easily accessible from historical records. Specifically, for 17 species of *Salix,* we aim to answer the following questions:

1. Is there an overarching species- or genus-level response of fecundity to geographic and climatic conditions?
2. Do invasive species have higher fecundity in their native or invaded range?

The first aim examines both inter- and intraspecific variation. The second aim, comparing native and invasive ranges, is inherently intraspecific. We focus on geographic variables to account for all environmental changes together described by an individual's physical location. Climate variables more specifically describe temperature and moisture only. We expect that fecundity will vary across geographic and climatic conditions at the species-level, rather than a genus-level response, in response to the varying optimal conditions for each of the 17 species. Additionally, we predict that invasive species will have higher fecundity in their invaded range due to increased environmental pressure driving the genetic pool.

This study uses herbarium records from three continents in a novel approach to determine whether invasive plant populations are more fecund across environmental gradients compared with their native range. Herbarium records are the ideal method of data collection for a study of this magnitude due to their global spatial coverage, extensive temporal coverage, and information regarding species presence and traits (Vellend et al. 2013). By incorporating phylogeny and comparing 17 members of a specific genus we can understand how much variation there is in the fecundity of closely related taxa, and how that might vary across their geographic distributions.

Methods

Species of interest

Seventeen *Salix* species were included in this study based on their global distribution and the quantity of available specimens (Table S5.1). To compare between home and away ranges, species were selected that were naturalised outside of their native continent. Selected species are native to either Europe or

North America and naturalised in Australia, North America, and/or Europe (Table S5.1). Four additional North American *Salix spp.* were selected that do not have established populations outside of their native range for the purpose of elongating the latitudinal gradient of study. The nine species that are naturalised in Australia were only selected if both male and female individuals exist in the country. Other taxa were excluded from the study if they did not add length to the latitudinal gradient, had a small number of specimens available in the herbaria visited, and/or if they were not commonly naturalised outside of their native range. All varieties and subspecies were included in data collection for each species, however hybrids were excluded.

Phylogeny was also considered when selecting species for study; only members of the two most common *Salix* sub-genera, *Salix* and *Vetrix,* were selected. The sub-genus *Salix* has been recently split into three sections: *Salix, Protitea*, and *Longifoliae*, all of which were included in this study. *Salix* and *Protitea* are phylogenetically highly similar, but separated biogeographically: *Salix* members are native in Europe while *Protitea* members are native to the Americas (Lauron-Moreau et al. 2015). *Longifoliae* is the more distantly related section of the *Salix* subgenus (Lauron-Moreau et al. 2015) (Figure S5.1). Three members of each sub-genus were selected that were native to Europe (six total: three subgenus *Salix* (section *Salix*) and three subgenus *Vetrix*). Native North American species include 11 species total: six in subgenus *Salix* (four in section *Protitea* and two in *Longifoliae*), and five in subgenus *Vetrix*. North American species distributions spanned the United States and either spread north through Canada or south through Central America, but *S. humboldiana* is distributed through South America as well.

Herbarium data collection

Herbaria to visit were chosen based on the number of specimens available for each of the species of interest. The following 12 herbaria were visited for data collection in Australia, Canada, France, New Zealand, Sweden, the United Kingdom, and the United States: National Herbarium of Victoria, Australian National Herbarium, the Herbarium of NSW, the Canadian Museum of Natural History in Ottawa, Muséum National d'Histoire Naturelle in Paris, the Allan Herbarium, Auckland War Memorial, Uppsala University Herbarium, Kew Botanic Gardens,

California Academy of Sciences Herbarium, University of California Jepson Herbarium, and the New York Botanic Gardens Herbarium.

Specimens were excluded from the study if they did not include the following information: a month and year of collection, a specific location of collection, at least one catkin in the seed release phase, if the written labels were illegible, or if the specimen was explicitly listed as cultivated to avoid the confounding influence of assisted growing conditions. If the day of the month was not listed, then the 15th day of the recorded month was used for analysis. Specimens were collected at approximately the same stage of catkin development to ensure comparability. Phenological stages were established according to *Salix* BBCH (Biologishe Bundesanstalt, Bundessortenamt and Chemical Industry) codes (Saska and Kuzovkina 2010). Catkin lengths were only measured on mature catkins that had begun releasing seed, and thus in specimens classified according to the BBCH system as indicating initial (BBCH code 70) or full seed release (BBCH code 71).

Longer catkins produce more capsules (r=0.85) and therefore more seeds overall (Figure S5.2). Thus, catkin length can be used as a proxy measure of fecundity. To estimate catkin length on each specimen, the five longest catkins were measured on each specimen. If there were multiple branches on the specimen, at least one catkin was measured per branch until five catkins had been measured. In order to measure the reproductive portion of the inflorescence only, catkins were measured from the point where the petiole meets the start of the seed capsules to the tip of the inflorescence. The following information was also recorded for each specimen: number of catkins present on the specimen, number of catkins releasing seed, BBCH flowering codes, and elevation of the collection, if listed. Catkins were only counted and measured if they were greater than 1cm in length, to avoid inclusion of aborted inflorescences. Occasionally catkins were pressed on top of each other and indistinguishable for measurement without causing damage to the specimen, so they were excluded from measurement. All data collection was completed by the same researcher for consistency of catkin length measurements and phenophase coding. In the home ranges we collected between 8 and 83 specimens per species and between 15 and 71 specimens in the away ranges. In total, we use data from 819 specimens, out of approximately 23,000 handled across all herbaria.

Geographic location matching

Locations of collection which did not already contain latitude were georeferenced using the location description listed, identified in Google maps, and recorded to the minute (latitudinal resolution of 1.85-1.87 kilometers, longitudinal resolution of 0.32-1.85 kilometers) (Figure 5.1). Once all the specimen records had an associated latitude, a digital elevation model was used to best estimate the elevation at the specimen's location. 337 herbarium records had elevation already listed, but for those that did not, elevation values were identified from a digital elevation model. We used the geo-tiff model from ETOPO in WGS 84 (https://www.ngdc.noaa.gov/mgg/global/global.html). Climatic variables at the location of each specimen were extracted from the interpolated WorldClim database at a resolution of 0.033° (about 4.8 kilometers) (Fick and Hijmans 2017).

Data analysis

We took account of phylogenetic relationships in interspecific analyses by initially assessing the degree of phylogenetic signal for female catkin length among the 17 species in our sample. We used a *Salix* phylogenetic tree based on *matK* and *rbcL* sequences (Figure S5.1) (Lauron-Moreau et al. 2015) and assessed phylogenetic signal by Pagel's λ parameter for tree transformation (Pagel 1999). Under the assumption of Brownian evolution, the expected covariance among trait values will be proportional to the branch lengths shared between species in the tree structure (Symonds and Blomberg 2014). Pagel's λ scales branch lengths in the tree to best account for the actual interspecific distribution of measured trait values. At λ = 1, species are as similar as expected under the Brownian evolution assumption. At λ < 1, there is less similarity than expected, and at λ = 0 the phylogeny contributes no information on the pattern of trait correlation among species.

We used the pgls (phylogenetic generalized least-squares) routine in the R package *caper* (Orme et al. 2013) to find the maximum likelihood estimate of λ for mean catkin length among the species in our sample. Phylogenetic statistical methods like pgls resolve to ordinary least-squares methods when using a phylogeny rescaled to λ = 0 (Symonds and Blomberg 2014), so if λ = 0 phylogeny can be disregarded in models.

Effect of geographic and climatic parameters

Broad effects of geographic and climatic variables on average catkin length at the genus-level were assessed using mixed effects models. Two models were developed, one containing geographic variables (latitude and elevation) and another containing climatic variables. We chose four of the 19 available WorldClim variables based on their correlation matrices, incorporating a measure of temperature (mean annual temperature), precipitation (annual precipitation), temperature variability (diurnality), and precipitation variability (precipitation seasonality), since climatic variability has been shown to affect plant fecundity, as well as mean climate parameters (Scheepens et al. 2018). The diurnality variable is the mean diurnal range: sum(monthly maximum temperature-monthly minimum temperature)/12, where larger values indicate wider differences between the mean maximum and minimum temperatures. Precipitation seasonality is the coefficient of variation of mean month precipitation, calculated from the ratio of the standard deviation of the monthly total precipitation and the mean monthly total precipitation (O'Donnell and Ignizio 2012). Both models also included a random intercept variable for species. Normality and homogeneity of variance was assessed and no data transformations were required. Parameters were centered and scaled for ease of interpretation. The absolute value of latitude was included in the model to account for hemisphere differentiation. Marginal and conditional R^2 values were calculated for each mixed effects model to describe the variance explained by the fixed effects only and the fixed and random effects together. All analyses were completed in R version 3.1.2 (Team 2015) and used the following packages for data synthesis (tidyverse, ggplot2) and analysis (nlme, MuMIn, raster, maps).

Next, we identified whether catkin length was affected at the species-level by geographic and climatic parameters. The principal challenge in our analysis is not the usual one of detecting statistical significance in a hypothesis-testing framework but rather avoiding overspecification of a multiple regression model (Burnham and Anderson 2002). This is particularly so because the WorldClim and geographic explanatory variables under consideration are correlated among themselves; indeed, it would be surprising if they were not. For example, mean annual temperature and elevation are negatively correlated, where temperature decreases with increasing

latitude (r= -0.82). As such, we completed separate model selection for the climatic and geographic variables, to determine their effects independently.

Our first step in model selection was therefore to define a limited, but still large, set of biologically reasonable candidate models. We calculated a null model fitting only an intercept and all possible univariate regression models for catkin length as a function of the geographic (latitude and elevation) and climatic (mean annual temperature, annual precipitation, diurnality, and precipitation seasonality) variables for each species. We also calculated all possible bivariate models for each species, involving all pair-wise combinations for each of the four climatic and the two geographic explanatory variables. Interaction effects between climatic or geographic variables were excluded from these models as the biological importance was tenuous. More complex models (trivariate and quadvariate) were not included, due to low sample sizes. For each model we determined the value of its Akaike Information Criterion corrected for small sample size (AICc). The lowest values indicate the models with the best empirical support, and we used the convention that models differing in AICc by less than 2 units have a similar degree of support (Kass and Raftery 1995, Burnham and Anderson 2002). Once the models with the lowest AICc scores were identified, we ran linear or multiple linear regression models to identify the the effect size of each of the environmental variables.

Home/Away range effects

We repeated the AICc model selection techniques including a home vs. away range variable. Five out of 17 species had adequate sample sizes for inclusion in the home vs. away range analysis: *S. alba* (n_{home}= 30, n_{away}= 50)*, S. cinerea* (n_{home}= 46, n_{away}= 71)*, S. fragilis* (n_{home}= 8, n_{away}= 27)*, S. purpurea* (n_{home}= 33, n_{away}= 15)*,* and *S. viminalis* (n_{home}= 35, n_{away}= 20)*.* Similar to the analysis excluding the home/away range variable, we calculated all possible univariate and bivariate models for each species, involving all pair-wise combinations for each of the four climatic and two geographic explanatory variables plus the home/away range variable. Additionally, for each species we calculated models with all combinations of one or two interaction variables between each climatic or geographic variable and the home/away range parameter, resulting in 60 candidate models per species, including 12 geographic models and 48 climatic models. The geographic and climatic models with the lowest

AICc scores were identified and summarised to identify the strength of the home/away range variable. Lastly, a simple Welch's t-test was completed for each species to compare mean catkin lengths between the home and away ranges.

Results

Phylogeny

There was no phylogenetic signal for catkin length. Maximum likelihood estimates of Pagel's λ were λ = 0.0000 using the phylogeny of Lauron-Moreau et al. (2015), indicating no phylogenetic signal. The likelihoods for this value did not differ from those for λ = 0, and likelihood ratio tests did not reject the hypothesis of λ = 0 ($P \approx 1$) for the phylogeny. We therefore ignore phylogeny and report ordinary least squares regression or linear mixed model results for inter- and intraspecific relationships.

Effect of geographic and climatic parameters

In our geographic genus-level mixed effects model, we found elevation had a significant negative correlation (t= -3.87, df= 564, *p<0.001*) with catkin length, while latitude and the latitude and elevation interaction variables had no significant relationship (latitude: t= -0.21, df= 564, *p=0.86*; latitude-elevation interaction: t= -0.10, df= 564, *p=0.92*) (Figure 5.2, Table S5.2). The fixed effects of the geographic genus-level model had low predictive ability for catkin length ($R^2_{marginal}$=0.03), but the random effect of species added considerable predictive power ($R^2_{conditional}$=0.34). In our climatic genus-level mixed effects model, we found that diurnality (t= -2.42, df= 563, *p=0.02*) had a significant effect on catkin length, while mean annual temperature (t= 0.65, df= 563, *p=0.53*), annual precipitation (t= -0.34, df= 563, *p=0.74*), and precipitation seasonality (t= -0.10, df= 563, *p=0.93*) did not. Similar to the geographic model, the fixed effects of the genus-level climatic model also had low predictive ability for catkin length ($R^2_{marginal}$=0.02), and the species random effect increased the predictive power ($R^2_{conditional}$=0.33). The variance and standard deviation of the species random effect variables for both the climatic and geographic models were larger than any of the fixed effect size variables (geographic model species RE variance= 0.71 (std. dev= 0.84), climatic model species RE variance=

0.72 (std. dev= 0.85)). The strength of the random effect variable suggests there are considerable differences among species in mean catkin length values, and that species-specific analyses are required.

Following the genus-level analysis, we identified the top models with greatest support for each of the species. Of the candidate models considered, 15 of the geographic models were univariate and 13 out of 17 of the climatic models were univariate (Table 5.1). 16 out of 17 species had at least one other candidate model that was within 2 AIC_c units of the best supported model (Table S5.3). As such, these AIC_c differences place many of the remaining models within the range of empirical support substantially equivalent to that of the best models (Burnham and Anderson 2002) (Table S5.3). Seven out of the species top (not null) climatic models included diurnality, five of the top models included annual precipitation, five included precipitation seasonality, and four included mean annual temperature. Of the geographic models, 12 species included latitude in their top models and seven included elevation. Of the 17 species, five of their top climatic models were better (more than 2 AIC_c units difference) than the null model in predicting catkin length. Five of the top geographic models were more informative than the null model, though not necessarily for the same species where the climatic model was significant (Table 5.2).

Home/Away range effects

Two of the five top climatic models were better than the null model, and one of the five top geographic models was better than the null (Table 5.2-5.3). Of the five species, two of the top climatic models were better than the null model in predicting catkin length, and the range parameter was significant included in a top model for three species (Table 5.3). Only one of the top five geographic models were significant, and none had a significant range predictor. None of the top geographic models contained an interaction between range and either geographic variable (latitude or elevation). Of the best (not null) geographic models, none were bivariate, with four top models containing elevation and only one containing latitude. Of the univariate, bivariate, and one- or two-interaction candidate models, three of the five top climatic models (excluding the null) included one interaction with the range variable, and only one species had a bivariate top model. None of the top climatic

models (excluding the null) contained both temperature and precipitation variables. Four out of five species had at least one other candidate model that was within 2 AIC_c units of the top home/away range model, including one of the species' climatic models and one of the geographic models (Tables 5.3, S5.4). Three of five top climatic models contained mean annual temperature. Two top climatic models contained precipitation seasonality and the interaction between precipitation seasonality and range (Table 5.2).

Four of the five species had no significant difference between their native and invaded range mean catkin lengths (*S. cinerea*: t=0.98, df=95, *p=0.35, S. fragilis*: t= -.05, df=12, *p=0.96*, *S. purpurea*: t=2.04, df=25, *p=0.05*, *S. viminalis*: t=0.78, df=38, *p=0.44*). *S. alba* had significantly longer catkins in the invaded range than in the native range (t=2.78, df=75, *p=0.006*) (Figure 5.3).

Discussion

Our study suggests that ecologically and phylogenetically similar species that inhabit similar ecosystems do not necessarily have consistent responses to their large-scale environment that are reflected by fecundity levels. When all species are pooled together, elevation (geographic) and diurnality (climatic) were significantly correlated with catkin length. However, their effect sizes were small and the models explain only a small proportion of the variability in the data (Marginal $R^2_{climatic}$= 0.02, Marginal $R^2_{geographic}$= 0.03). When including species differences, the top climatic and geographic models explained much more of the variation in catkin length (Conditional $R^2_{climatic}$= 0.33, Conditional $R^2_{geographic}$= 0.34). Seven of 17 *Salix* species we studied had climatic and/or geographic variables that predict catkin length, our proxy measure of fecundity, better than a null model. Diurnality was the most common climatic variable in top species-level models, followed by annual precipitation, precipitation seasonality, and mean annual temperature. In the geographic top models, 12 species models included latitude, while only seven included elevation. Based on larger R^2 values in 15 out of 17 species' models when comparing between top climatic and geographic models, climatic variables may be more efficient parameters to describe broad-scale patterns of fecundity variation in the *Salix* genus. When comparing catkin length between the home and away range, only one out of five species had significantly longer mean catkin lengths in the

invaded range, while all other species showed no difference. Additionally, when accounting for geographic and climatic variables, for four out of five species the null models were equivalent or better predictors of catkin length than those including geographic and climatic variables. This suggests that, overall, there are few differences in fecundity between native and invaded ranges in the five *Salix* species studied.

Climatic and geographic variation

When pooling all *Salix* species together, as expected, there was a significant negative relationship between catkin length and elevation: in warmer, low elevation populations, catkins were longer. Mean annual temperature, however, had no significant correlation with catkin length. Extensive research suggests that warmer conditions during the period of reproductive bud maturation leads to increased plant fecundity (Woodward et al. 1994, Houle 1999, Piovesan and Adams 2001, Roland et al. 2014). The climatic data used here may have been too coarse to capture micro-climatic differences, possibly obscuring the positive correlation we expected. Small samples of some species may also have resulted in non-detectable signals. Unexpectedly, there was also not a significant correlation between latitude and catkin length. We would have expected higher latitudes to have smaller catkins overall. The *Salix* genus is largely temperate (Isebrands and Richardson 2014). Consequently, temperate species in this study were highly oversampled, making large-scale latitudinal trends difficult to detect. More interestingly, the most important climatic predictor at both the genus- and species-level was diurnality, the mean difference between maximum and minimum monthly temperatures (O'Donnell and Ignizio 2012). The correlation between catkin length and diurnality was negative, suggesting that increasing amplitude of monthly temperature variation yields shorter catkins, producing less seed. Previous research on wheat (Semenov and Porter 1995), soybean, and maize (Riha et al. 1996) yields for agricultural applications found the same effect in experimental studies: an increase in daily temperature fluctuations reduces grain yields (Wheeler et al. 2000). However, the fixed effects had very low predictive power (marginal $R^2_{geographic}$=0.03, marginal $R^2_{climatic}$=0.02) and extremely small effect sizes (elevation coefficient= -0.25, diurnality coefficient= -0.07), which was to be expected based on the resolution of both the geographic and climatic variables. Similarly, our measure of fecundity, catkin length, is likely influenced by a variety of other genetic, biotic, and abiotic factors.

Among the 17 *Salix* species studied here, there was a considerable lack of similarity in the amount of variation in catkin length explained by either the geographic or climatic models ($0.02 \leq$ Multiple $R^2 \leq 0.32$). Since we found no phylogenetic signal related to catkin length, we expect that environmental conditions must account for much of the variation in catkin length between species. Certainly, each *Salix* species' catkin lengths respond differently to surrounding biotic and abiotic conditions, and also have unique levels of phenotypic plasticity. Previous research on 11 *Arabidopsis thaliana* genotypes found that the developmental timing of temperature stress affects plant fecundity, and that genotypes differed substantially in their response, which is consistent with our results on closely related taxa (Scheepens et al. 2018). Scheepens et al. (2018) also found that precipitation variability in the climate of origin was positively related to trait plasticity, which was in the top (not null) model describing catkin length for five of the species in this study. Since climate warming is forcing rapid adaptation of plant populations, and *Salix* species are all responding differently to climate, it is difficult to determine how *Salix* species will adapt to the changing climate. It is possible that some species will be overwhelmed by the magnitude of climate change occurring to adapt quickly enough for persistence under new environmental conditions (Jump and Penuelas 2005). This could have drastic consequences for the geographic distribution of more slowly adapting species in the upcoming warming centuries. However, since the many members of the genus are capable of and frequently do produce hybrids, the genus may produce more hybrid crosses with higher fitness than their parents under climate warming. This is consistent with previous findings which suggest that hybridization stimulates plant invasion success (Prentis et al. 2008).

In addition to variation in the amount of catkin length variability explained by the geographic and climatic variables, there was also considerable variation among species in which variables were the most important to catkin length. Diurnality was in the top (not null) models for the highest number of species, where increasing variation in temperature lowers catkin length. Previously, research has shown that increasing repetitions of extreme temperatures can cause seed output to be lowered (Moot et al. 1996). One reason this may be is because plants respond to temperature anomalies as if they are independent of one another (Porter and Semenov 2005), which, when occurring in close succession, can affect meiosis and pollen grain development (Wallwork et al. 1998). Since extreme weather events are

increasing with climate change (Coumou and Rahmstorf 2012), the frequency of temperature anomalies will increase and could potentially affect seed output. However, 16 out of 17 species had more than one statistically equivalent top model (AIC_c difference < 2), including 15 of the climatic species models and 13 of the geographic species models. Therefore, there is little indication that increasing temperature anomalies will have any effect on seed output at all. We recommend that future research aim to collect more precise temperature and precipitation data to more accurately describe the effects of temperature anomalies on fecundity. Our results may have primarily been dictated by the level of precision that the data was collected at, and the scale at which the study took place. These results also suggest that there is not consistency within the *Salix* genus in the fecundity responses of individual species to temperature, precipitation, or their variability. Researchers sometimes focus their studies on either mean values or their variability in climate, but not both. We recommend researchers consider using both average measures and measures of variability when completing correlational analyses between plant demographic traits and climatic variables.

Home-Away range differences

There was no significant difference in catkin length for four of the five species between ranges, though the invaded range catkins of *S. alba* were significantly longer than those in the native range. Previous research suggests that invasive plant populations generally have increased sexual reproduction when their life history strategy is reliant on fecundity, rather than survival, which increases propagule pressure thereby supporting invasion (Burns et al. 2013). Since the *Salix* genus is early-successional in its home range (Isebrands and Richardson 2014), we would expect that species on margins of their distribution might produce more seed than those in the established native range, in order to optimize the transport of seed to open, disturbed habitat (Angert 2009).

In four out of five species the range variable is an equivalently useful predictor of catkin length as the null model, which entirely excludes the geographic, climatic, and range variables. In the fifth species, the null model is a better predictor of catkin length. Three of the species had significant interactions between the top climatic variables and the home-away range. Overall, there is little evidence which supports consistent differences between *Salix* fecundity in their native vs. invaded ranges as they relate to climatic or geographic variables. Our results suggest that we might

expect invasive and native *Salix* population fecundity to respond approximately equally under climate warming. This is inconsistent with previous research which suggests that small introduced populations of invasive species may be in a worse position to respond to climate change because the populations have fragmented habitats, which leads to decreased gene flow and a slower rate of adaption that could lag behind the rate of climate change (Jump and Penuelas 2005).

Caveats

The data utilized in this research is quite coarse, and the climatic and geographic variables could not be descriptive of microsite variation between herbarium records. Temperature and precipitation variability, as derived from daily mean values, may not capture the temperature fluctuations relevant for responses of fecundity (Scheepens et al. 2018). Similarly, catkin length as a proxy for fecundity is less precise than other measures of fecundity because there is not a perfect positive correlation between catkin length and seed output. Ideally, field measurements of seed output would be more accurate, however given the scope of the study this would be impossible. Previous field measurements on *S. cinerea* in its invaded range found that seed set per capsule at high elevation Australia is actually about 1/4 the amount as at low elevation Australia (chapter 3), in addition to catkins being shorter in length and fewer catkins produced per crown (Figure S5.3). Furthermore, catkin length is likely affected by a number of variables other than its surrounding climate, such as biotic conditions, nutrient or light availability, and genetic limitations. However, since there were significant limitations to precision, the ability of our models to explain >10% of the variability in the catkin lengths of multiple species is striking and certainly ecologically significant. Furthermore, since this research was completed at a global scale across a large number of species, it would not be feasible to conduct in a more precise, field-based study without employing researchers from multiple countries and institutions. As such, this study is an example of the trade-off between data precision and collection opportunity to acquire broad-scale data and answer important ecological questions.

Conclusions

Our results suggest that there were no consistent large-scale environmental processes at the genus- or species-levels which affected fecundity, regardless of the range of the specimens. As such, the fecundity of the *Salix* species in this study must be constrained by factors other than physical location or surrounding climatic

conditions. However, our research also suggests that if researchers were able to collect more precise data we might see that surrounding environment would explain more of the variability in catkin length than was exhibited here. Though these results are rough approximations of what precise field data might tell us, it was an accomplishable first step in understanding the influence of climate, geography, and home vs. away range on fecundity in an ecologically and culturally important plant genus.

Acknowledgements

We thank the curators of the herbaria visited around the globe for their knowledge and hospitality of resources and space. We thank the Holsworth Wildlife Research Endowment and the Denis and Maisie Carr award for funding the travel to the herbaria visited. Thanks also to Ros Gleadow, Marie Keatley, and Kay Hodgins for elegantly enforcing this study to reach its potential.

Tables

Table 5.1. Top models for each species without the home/away variable included, and excluding the null model. Species name is bolded if top model is at least 2 AIC_c units less than the null model for either the climatic or geographic models. * indicates significance of the variable when α=0.05. Adjusted R^2 values are listed for each model to account for the difference in number of variables between the two models. The greater R^2 between the geographic and climatic models are written in bolded text. Species in purple text are those included in the home/away analysis as well. The following species had null models as their top geographic model: *S. alaxensis, S. alba*, *S. cinerea, S. eriocephala, S. exigua, S. fragilis, S. lasiolepis, S. purpurea.* The following species had null models for their top climatic model: *S. alaxensis, S. alba, S. cinerea, S. fragilis, S. lasiolepis, S.purpurea*.

	Climate models						Geographic models			
Species	AIC_c 2 units less than null	Adj. R^2	Mean Annual Temp.	Diurn.	Annual Prec.	Prec. Seas.	AIC_c 2 units less than null	Adj. R^2	Lat.	Elev.
S. alaxensis		**0.06**		●				0.02	●	
S. alba		**0.06**			●			0.0007	●	
S. bebbiana	●	**0.09**			●*			0.005	●	
S. bonplandiana		0.07		●	●		●	**0.08**	●	
S. caroliniana	●	**0.26**	●*		●		●	0.03		●
S. cinerea		**0.01**		●				0.009		●
S. eriocephala		**0.11**			●	●*		0.06		●
S. exigua	●	**0.21**	●*	●*				0.01	●	
S. fragilis		**0.36**		●				0.02		●
S. humboldtiana		0.06	●				●	**0.07**	●	●
S. interior		**0.11**		●*			●	0.10	●	●*
S. lasiolepis		**0.02**				●		0.003	●	
S. nigra	●	**0.11**				●*		0.009	●	
S. pentandra		**0.03**	●*					0.008	●	
S. purpurea		**0.04**				●		0.003	●	
S. scouleriana	●	**0.13**				●*		0.003	●	
S. viminalis		**0.01**		●*				0.004		●

Table 5.2. Top models generated from AICc model selection techniques on all univariate and bivariate predictors for each climatic and geographic variable sets. Interactions between each variable were also included in the candidate models. All models included the home/away range variable. Top models were selected and their results are presented here. Model significance is noted if top model is at least 2 AIC_c units smaller than the null model (in Model column). * indicates significance of the variable or model when alpha=0.05. Adjusted R^2 values are listed for each model to account for the difference in number of variables between the two models. The greater R^2 between the geographic and climatic models are listed in bolded text. If an interaction variable was included in the top model it is indicated by an "I" in the column of the variable that it was interacting with. The following species had null models as their top geographic model: *S. alba*, *S. cinerea, S. viminalis.* The following species had null models for their top climatic model: *S. alba, S. viminalis.*

	Climate models							Geographic models				
Species	Model	Range	R^2	Mean Ann Temp	Diurn	Ann Prec	Prec Seas	Model	Range	R^2	Lat	Elev
S. alba		*	0.09	●						**0.10**	●	
S. cinerea		*	**0.04**				●*+I*			0.02		●
S. fragilis	●		**0.32**	●*+I*	●*					0.12		●*
S. purpurea	●	*	**0.12**				● +I*	●		0.11		●
S. viminalis			0.04	●						**0.05**		●

Table 5.3. Top linear regression models predicting catkin length including geographic variables (elevation and latitude) or climatic variables (mean annual temperature, diurnality, annual precipitation, and precipitation seasonality). Tops models include those within 2 AICc units of the best model with the lowest AICc score and that known not to be significantly better than the best model. Models on the left include the climatic or geographic variables and the data from the home range only. Models on the right include both the home and away data, as well as a categorical variable for home/away range. For the home-only models, 10 climatic candidate models and 3 geographic models were considered. For the home/away models, there were 32 climatic candidate models and 8 geographic models. Geographic models are colored in blue for ease of reading. Abbreviations are as follows: MAT=mean annual temperature, AP=annual precipitation, Diurn=diurnality, PS=precipitation seasonality, Lat=latitude, Elev=elevation.

	Home Only			Home/Away		
Species	Top Clim	Top Geo	Other top models	Top Clim	Top Geo	Other top models
S. alba	**Null**	**Null**	Lat	**Null**	**Null**	N/A
S. cinerea	**Null**	**Null**	Diurn, AP, PS, Elev	PS+range+PS:range	**Null**	PS+Diurn+range +PS:range **Null**
S. fragilis	**Null**	**Null**	N/A	MAT+Diurn+range+MAT:range	Elev+ range	MAT+Diurn+range +Diurn:range Lat+Elev+range **Null**

S. purpurea	**Null**	**Null**	PS	PS+range+PS:range	Elev+ range	AP+range, PS+AP+PS:range, PS+MAT+PS:range, Diurn+range+Diurn:range, MAT+range, PS+range
S. viminalis	**Null**	**Null**	Diurn, PS, Elev Lat Elev+Lat	**Null**	**Null**	Elev+ range

Figures

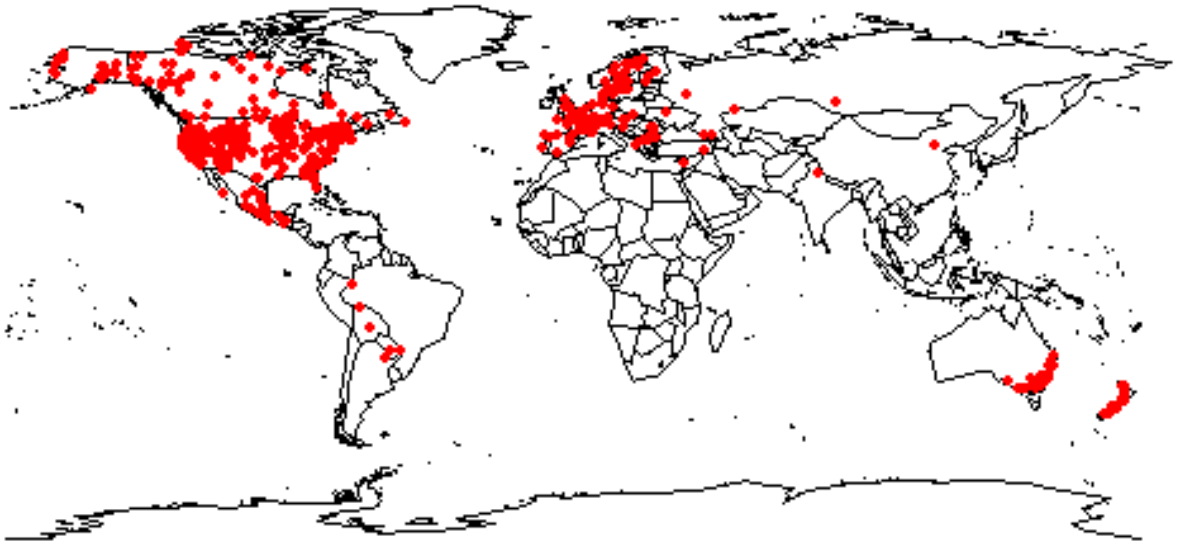

Figure 5.1. Map of herbarium specimen locations, including species' records in their home and away ranges. Each dot represents one herbarium specimen.

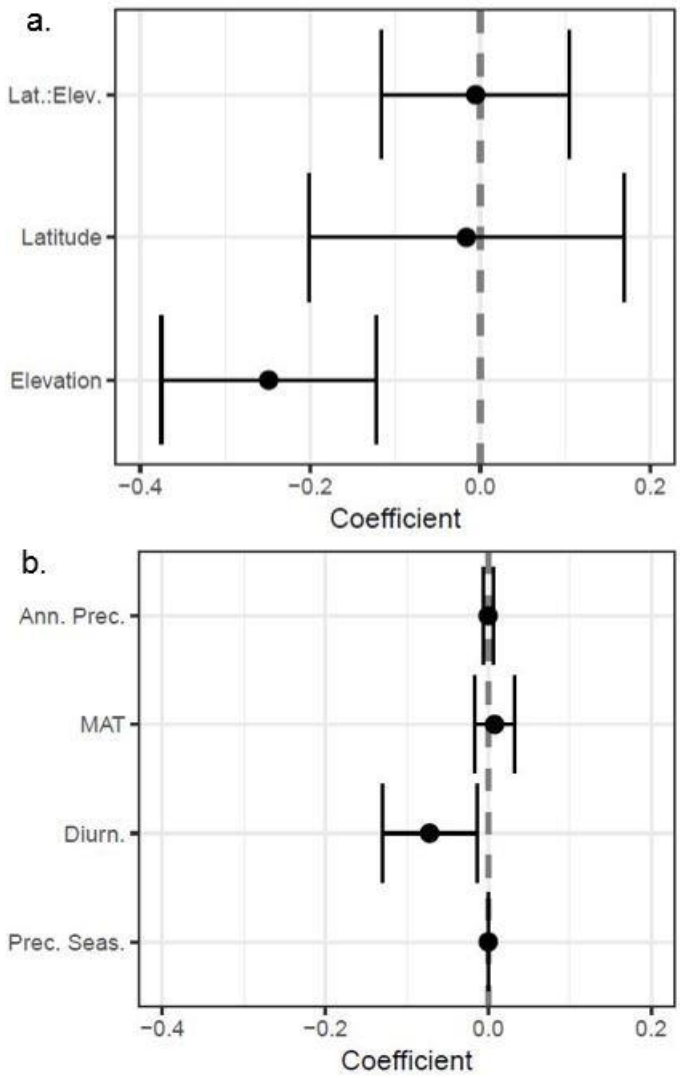

Figure 5.2. Mixed effects model coefficient plots of catkin length as a function of a) geographic variables and b) climatic variables. Geographic variables include elevation, latitude, and their interaction. Climatic variables, derived from WorldClim (Fick and Hijmans 2017), include mean annual temperature, diurnality, annual precipitation, and precipitation seasonality.

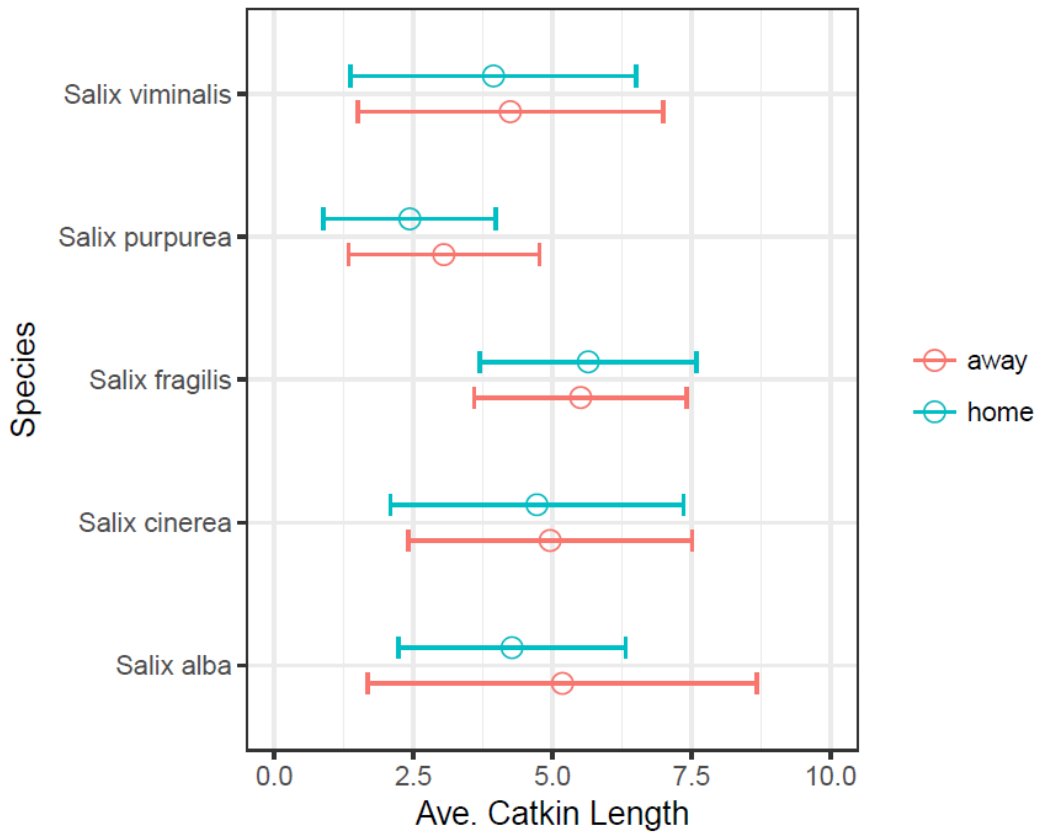

Figure 5.3. Average catkin length for five *Salix* species, between their home and away ranges. *S. alba* was the only species with significantly longer catkins in the invaded range (t=2.78, df=75, *p=0.006*). Species sample sizes are as follows: *S. alba*: n_{home}= 30, n_{away}= 50, *S. cinerea*: n_{home}= 46, n_{away}= 71, *S. fragilis*: n_{home}= 8, n_{away}= 27, *S. purpurea*: n_{home}= 33, n_{away}= 15, and *S. viminalis*: n_{home}= 35, n_{away}= 20).

Chapter 6

General Discussion

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The overall aim of this thesis was to better understand the phenology and reproductive ecology of *Salix cinerea* in its invaded range, and especially in the range of study: Victoria, Australia. I intended to better understand how surrounding abiotic conditions, including climate and geographic location, might influence the fecundity and spread rate of the species across its Victorian distribution. In Chapter 2 I quantified the phenology of *S. cinerea,* a task which had not previously been empirically recorded in the literature. Chapter 2 also identified differences in heat accumulation requirements of the species between low and high elevation, which may help describe the species phenology under global warming. The third chapter considered specific *S. cinerea* phenophases more closely, identifying that pollen release and stigma receptivity overlap was indicative of seed fertilization rates, and was strongly correlated with seed output between low and high elevation. Chapter 4 compared the quality of the seed output of *S. cinerea* between low and high elevation, comparing seed sizes and germination rates. I found that high elevation individuals produced seeds with about 15% higher overall germination rates than those at low elevation. However, high elevation individuals still accounted for fewer seeds germinating than those at low elevation due to the vast difference in seed output between high and low elevation individuals, as well as the higher number of individuals at low elevation overall. Finally, Chapter 5 considered 17 species in the *Salix* genus more broadly to identify variation in fecundity across geography and climate, and in between native and invasive populations of five species. I found that variation in fecundity was more significant between species than across the genus. This suggests that there were no consistent large-scale environmental effects at the genus- or species-levels which affected fecundity, regardless of the range of the specimens. Species' fecundity must be constrained by factors other than physical location or climate.

I have also provided unique insights to the broader fields of invasion biology, plant ecology, and climate change ecology. With respect to climate change, these chapters suggest that willows appear to adapt sufficiently well to warmer climates. This is shown from the successful shifting of reproductive events in response to warmer conditions (Chapter 2), as well as the longer period of phenological overlap at warm, low elevation (Chapter 3). Additionally, *S. cinerea* has a longer growing season (Chapter 2), indicating faster growth rates (Myneni et al., 1997) and

produces considerably more seed overall (Chapter 4) in the warmer, low elevation climate. Overall, these results suggest that the spread potential of *S. cinerea* is likely to be higher at low elevation, under warmer conditions. However, the higher germination rates of high elevation individuals (Chapter 4) suggests that there are complexities in the system that cannot only be attributed to abiotic conditions. This thesis provides further evidence to the growing body of literature which suggests that invasive plants may be more resilient to climate change than species without qualities which allow them to be successful invaders, including rapid growth rate and production of large quantities of seed (Dukes and Mooney, 1999, Theoharides and Dukes, 2007, Blackburn et al., 2011).

Phases of Spread

In the introduction of this thesis (Chapter 1), I outlined the three phases of spread (pre-dispersal, dispersal, and post-dispersal) which were to be addressed in the upcoming chapters (Figure 1.3). In my four subsequent chapters, I provided novel insights into the effects of climate and geographic location on the pre-dispersal phase of spread. In particular, my thesis focussed on the pollen and seed release phenology and seed output aspects of pre-dispersal. Broadly, we found that both climate and geographic location, notably elevation, and likely their interaction, have a significant effect on both phenology and seed output. Interestingly, we found that seed output is affected by climate and geographic location significantly in *S. cinerea* (Chapter 4), but climate and geography were not consistently significant predictors of seed output among other members of the genus (Chapter 5). This may be related to *S. cinerea's* relatively wide geographic and habitat range. Since *S. cinerea* is the only known species of the genus to naturalize outside of riparian zones (Cremer, 2003), it may be more sensitive to climate in non-riverine habitats.

Additionally, in Chapter 4 I identified new insights into the dispersal and post-dispersal phases of spread for *S. cinerea.* I found that the size of *S. cinerea's* anemochorous seeds did not appear to have a direct effect on their dispersal capacity, suggesting that the competition-colonisation trade-off may not be especially relevant in discussion of wind-dispersed seeds. The post-dispersal phase of spread was addressed by quantifying germinability of *S. cinerea* seeds produced at low and high elevation, and grown under conditions mimicking those found in spring at low

and high elevation. We found that germinability was affected both by the location the seeds were produced and the conditions under which they are sown. Generally, this research suggests that all three phases of spread are linked to surrounding climatic conditions, indicating that future research focussed on *Salix* spread should incorporate at least basic metrics of climate into its analysis.

Management Implications

Of the many important findings in this thesis, the most readily applicable are those related to management practices of *S. cinerea* in Victoria, Australia. Catchment managers are given limited financial resources, and therefore require ample planning and information about the species of interest in order to optimise their control efforts, minimizing the spread and impact of the species of interest as effectively as possible (Giljohann et al., 2011). I found several important pieces of information throughout this thesis which could help inform Victorian catchment managers where to allocate their resources in future control seasons.

In Chapter 2, I identified that high elevation populations have 20% shorter growing seasons than at low elevation, and thus have slower growth rates (Myneni et al., 1997), indicating that they will spread vegetatively more slowly. Furthermore, I found in Chapter 4 that the spring conditions at low elevation are more conducive to seed germination than at high elevation. This suggests that more low elevation seeds will experience optimal germination conditions than seeds matured at high elevation. Hopely (2011) found that only approximately 10% of *S. cinerea* seeds disperse further than 50 km, indicating that most of the seeds produced at high elevation will stay in approximately high elevation conditions.

In Chapter 3 I found that there is significantly more pollen available at low elevation compared with high elevation, likely as a result of increased overlap between pollen release and stigma receptivity. The increased level of phenological overlap resulted in an increased level of seed fertilization, which was highly correlated with seed output. Although I found in Chapter 4 that there are slightly higher germination rates overall at high elevation compared to low, there are still more seeds likely to germinate per tree at low elevation due to the significantly higher number of seeds produced. This, combined with the lower number of *S. cinerea* populations at high elevation overall (due to less high elevation land mass,

as well as the planting of *S. cinerea* in low elevation farmlands (Cremer, 2003)), suggests that the primary sources of germinable seeds are originating from low elevation, which is also home to the fastest growing shrubs across the landscape.

State-wide management, as has been mentioned previous chapters, is currently focussed primarily on removing *S. cinerea* populations from the vulnerable and threatened peatland ecosystems at high elevation (McDougall et al., 2005, Moore and Runge, 2012). Similarly, previous research has largely focussed on managing threats to alpine peatlands (Moore and Runge, 2012), however managers are also controlling *S. cinerea* throughout eastern Victoria where the species threatens waterways. Individual catchment management authorities have had varying approaches to *S. cinerea* removal, with some just targeting sections of the catchment most important to recreational activities and others aiming to eradicate the species entirely from their rivers of jurisdiction. Unfortunately, because the species' seeds frequently disperse long distances (Hopley, 2011) and broken branches can re-root after traveling downstream (Cremer, 1999), it would be in the best interest of the affected catchment management areas to develop a coordinated *S. cinerea* eradication strategy which accounts for the interconnectedness of their river systems. The entirety of my research might suggest that in order to minimise the spread of *S. cinerea* at a landscape-scale, state-wide managers could direct their research funds and efforts to remove low elevation populations with large, highly fecund female individuals.

Ecologically, it is somewhat surprising that *S. cinerea* appears to be more fecund at low elevation compared to high. As described in Chapter 3, the species' native range in northern Europe is climatically more similar to high elevation than low elevation. This may be a result of competition between the hundreds of *Salix* species in the native range of *S. cinerea*, where dozens of species with similar ecological niches compete for the same habitat (Isebrands and Richardson, 2014). *S. cinerea* may also be less successful at high elevation because it is often growing outside the ecosystem type common for the genus. Exposure to wind and frost during the growing season as a result of their location at high elevation, for example, may affect the success of high elevation populations (Inouye, 2008). Additionally, most high elevation populations of *S. cinerea* are growing in wetlands and bogs, rather than the usual riparian zones where the other 80+ species in Australia are found (McDougall,

2007). Furthermore, at high elevation, the riparian areas are generally less disturbed from logging, fire, and other human-mediated activities than at low elevation, making establishment of the early-successional species more difficult (McDougall et al., 2005).

The discrepancy between success at low and high elevation may also be the result of a growth-survival trade-off: the phenomenon in which plants allocate more energy resources towards survival in harsh conditions and more energy towards growth in benign conditions (Wright et al., 2010). This was precisely what was identified in *S. cinerea* in Chapters 2-4: a higher proportion of large seeds were produced with higher germinability at high elevation in sub-optimal conditions, and low elevation populations in warm conditions produced large individuals with more seed. Previous research on woody trees along temperature gradients also found that height (Chuine et al. 2006) and tree-rings (Miyamoto et al., 2010) both exhibited slower growth under stressful temperature conditions. This observation is consistent with the findings made throughout my thesis, suggesting that growth and survival are both dependent on *S. cinerea's* surrounding climate. With respect to management, my thesis still recommends a focus on surrounding environmental conditions to gauge the likely success of *Salix* populations.

Certainly, there are a multitude of factors which influence the direction of management efforts for *S. cinerea* (Moore and Runge, 2012). I hope that this research will help managers to have a clearer understanding of the issue at hand when deciding how to optimally allocate their resources for the control of *S. cinerea*.

Future research

The research conducted in this thesis answered many important questions regarding the ecology of *S. cinerea,* but also introduced new questions that would be interesting to pursue in the future.

The Haig-Westoby Equilibrium with a non-native twist

One research idea that stemmed from my thesis involved the classic Haig-Westoby equilibrium, which suggests that pollen limitation is the result of a disequilibrium between pollen acquisition and resource acquisition (Haig and Westoby, 1988). When equilibrium is forced out of sync by a change in pollination

system or the resource environment, chronic pollen limitation can occur (Ashman et al., 2004). Resources include a variety of plant requirements acquired from the environment- soil nutrients, light, heat, etc. Alternatively, pollen acquisition requires an adequate number of pollinators available, as well as energy provided by the plant to produce pollinator-attracting nectar, as *Salix* species do (Isebrands and Richardson, 2014). A pollination environment not conducive to retaining the pollen-resource equilibrium could include increased competition for pollinators and/or limited pollinator availability, possibly as a result of stochastic environmental perturbations (Ashman et al., 2004). The resource environment can cause a shift from equilibrium if there is a change that causes poor nutrient availability or unfavourable weather conditions, such as extreme weather disturbances like flooding or fire. Alternatively, both the resource and pollination environment can be altered by introducing a species into an entirely foreign environment, as non-native species are. In Chapter 3, I found that lowered *S. cinerea* seed output was associated with decreased phenological overlap between pollen release and stigma receptivity, suggesting that its phenology has been disrupted in the invaded range. This change in phenology caused less pollen availability during the period of stigma receptivity, resulting in overall pollen limitation which was only indirectly a result of changes in resource acquisition, via their effect on phenology. Based on those results, I propose that there may be a more sophisticated version of the Haig-Westoby equilibrium theory beyond a simple trade-off of pollen and resource acquisition when applied to non-native plant species.

For species introduced into ecosystems in which they have not evolved, I believe that pollen and resource acquisition are out of equilibrium. Additionally, I hypothesize that non-native plants initially obtain fewer resources from their environment, which can result in lowered pollen and resources overall. Specifically, lowered heat accumulation at high elevation may be affecting the timing of flower bud break (Chapter 2). This affects the timing of reproductive events and causes reduced overlap between pollen release and stigma receptivity, thus limiting pollen availability, and lowering pollen acquisition. Therefore, not only is the equilibrium off balance, but there is also a result of fewer pollen and resources available overall in

the non-native range (Figure 6.1). Additionally, if other resources are limited in the non-native populations of *S. cinerea*, such as soil nutrients or non-essential symbiotic relationships, this might explain the sub-optimal (<100%) overlap in pollen release and stigma receptivity at both low (42% overlap) and high elevation (24% overlap). Most commonly, pollen limitation hypotheses are tested using pollen supplementation experiments: the seed output of control plants is compared to those that have been given additional pollen. If plants with added pollen produce more seed than the controls, it is assumed that the plant's reproduction is limited by pollen reception (Ashman et al., 2004, Knight et al., 2005). Unfortunately, because I did not specifically test pollen limitation of *S. cinerea* in Chapter 3, I do not have sufficient evidence that this disequilibrium can be applied accurately to non-native species. This could be tested at a later date by completing pollen supplementation experiments paired with measures of phenological overlap in, ideally, both the home and away ranges. Following that data collection, a climate comparison between the home and away ranges of study could narrow down the effects of resource acquisition related to heat accumulation. In the future the Haig and Westoby (1988) equilibrium theory applied to non-native plants would be a worthwhile focus of study for theoretical and invasion ecologists interested in differences between intraspecific native and invasive plant fecundity.

Phenological overlap in native vs. invaded range

Another interesting research imperative stemmed from the findings of Chapter 3. I found that there was a lower level of pollen release and stigma receptivity overlap at high elevation, where climatic conditions are more similar to the native range of *S. cinerea* than at low elevation. What remains unclear is why the climate conditions more similar to the native range of the species resulted in lower phenological overlap and fecundity. In order to better understand this phenomenon between the native and invaded distributions it would be necessary to identify whether there is variation in phenological overlap of *S. cinerea* across the climatic range of its native populations. When collecting the herbarium data for Chapter 5, I also gathered specimens in the pollen release and stigma receptivity phenophases for two *Salix* species (*S. cinerea* and *S. nigra*). This data will allow me to identify phenological overlap variation across the latitudinal distributions of *S. cinerea* in its native and invaded ranges, and across the native range of *S. nigra*. Furthermore, I

can use the catkin length data of the herbarium specimens, as done in Chapter 5, to replicate the study findings of Chapter 2, comparing phenological overlap levels to relative catkin lengths, as a measure of fecundity. This comparison will provide more information about the prevalence of variation in phenological overlap between native and invaded ranges of a common native species in Europe, and a species of high management importance in Australia and New Zealand. Additionally, this study will provide novel insights to how the species' phenological overlap is likely to shift in response to climate change, based on the wide climatic variation experienced across the native and invaded distributions.

More completely, in addition to the phenological overlap data analysis, completing a population viability analysis (PVA) may resolve the unlikely result of low elevation sites producing higher phenological overlap and fecundity, while high elevation sites had higher seed fitness. Simple calculations in Chapter 3 suggested that there were indeed more viable seeds produced per individual at low elevation, however a PVA would more accurately incorporate aspects of growth rate into the basic statistics calculated here. By combining the large-scale phenological overlap analysis with a PVA future research could disentangle the effects of climate on the fecundity of invasive *S. cinerea*.

Conclusion

In conclusion, my thesis has highlighted the importance of climate and geographic location for the phenology and reproductive ecology of an invasive plant. Given the rate of spread in *S. cinerea,* and its high capacity to influence native ecosystems, it is of urgent importance to alert managers how best to efficiently and effectively remove populations with especially high potential for spread (Cremer, 2003, Moore and Runge, 2012). My research provides novel insights regarding where major seed sources might exist across the landscape of north-eastern Victoria, Australia. Similarly, I have established novel insights to classic ecological themes as they are related to invasive, wind-dispersed plants. In particular, I have provided useful information about the competition-colonization hypothesis, phenological overlap, and fecundity's response to varying environmental conditions. There remain a number of open questions related to these topics, and the

management of *S. cinerea* in its invaded range, however I hope to inspire future researchers to continue aiming to fill these knowledge gaps.

Figures

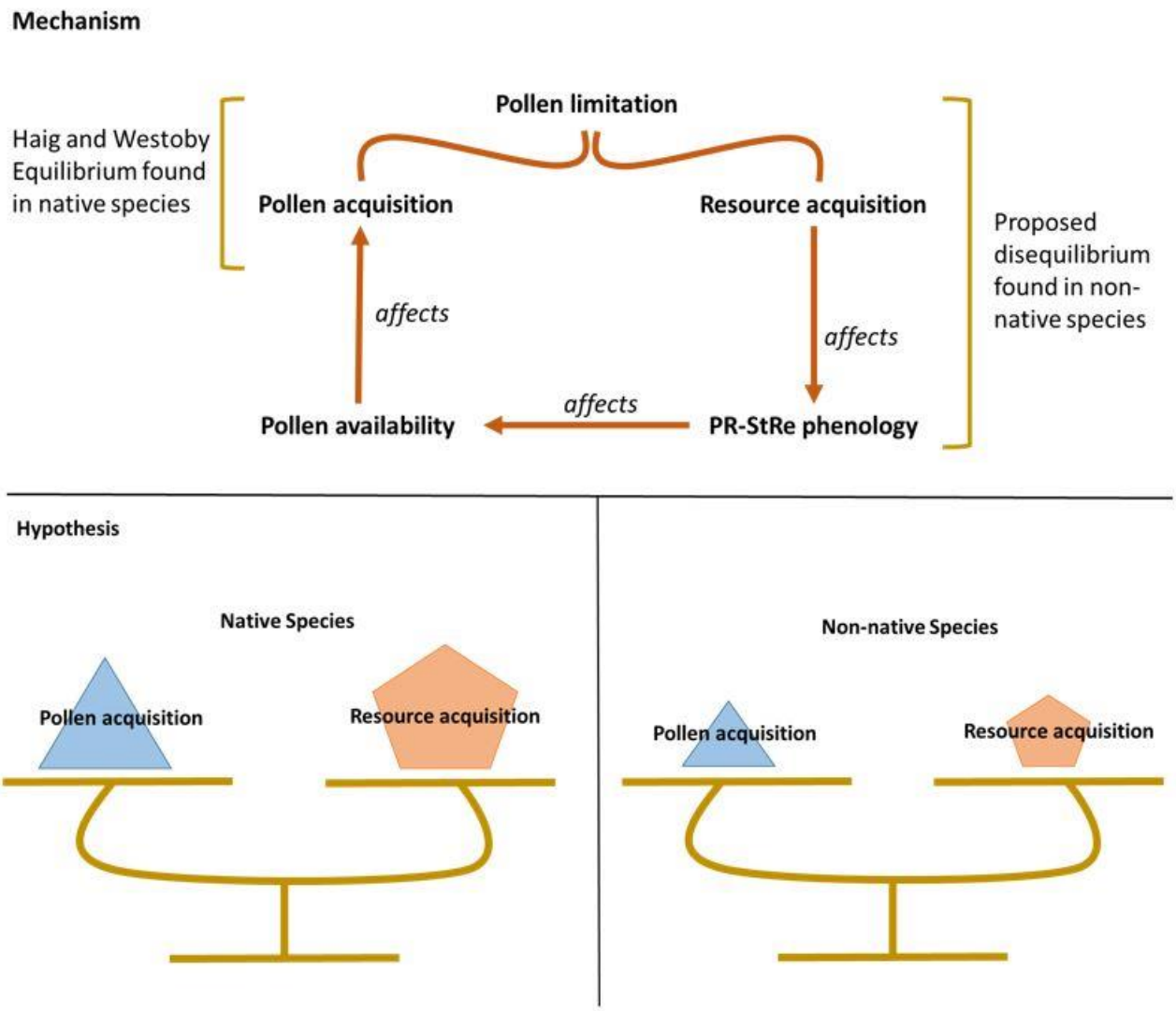


Figure 6.1. Conceptual model of the Haig and Westoby pollen-resource equilibrium for native plants (Haig and Westoby, 1988), compared to my proposed hypothesis of disequilibrium in non-native plants as a result of varying phenological overlap across the non-native distribution (top). I expect (bottom) that native species have a larger amount of pollen and resources as a result of having evolved in the environment they reside, and therefore an ability to utilise resources more effectively than non-native species. Since non-native species have not evolved in response to their surrounding environment, there is potential for resource acquisition to be lower, causing lowered pollen release and stigma receptivity overlap, and therefore decreasing pollen acquisition, regardless of pollinator availability. PR-StRe phenology refers to pollen release and stigma receptivity phenological overlap.

Literature Cited

Allen, J. M., Terres, M. A., Katsuki, T., Iwamoto, K., Kobori, H., Higuchi, H., Primack, R. B., Wilson, A. M., Gelfand, A. & Silander, J. A. (2014) Modeling daily flowering probabilities: expected impact of climate change on Japanese cherry phenology. *Global Change Biology,* **20,** 1251-1263.

Alliende, M. C. & Harper, J. L. (1989) Demographic-studies of a dioecious tree: Colonization, sex and age structure of a population of *Salix cinerea*. *Journal of Ecology,* **77,** 1029-1047.

Angert, A. L. (2009) The niche, limits to species' distributions, and spatiotemporal variation in demography across the elevation ranges of two monkeyflowers. *Proceedings of the National Academy of Sciences of the United States of America,* **106,** 19693-19698.

Argus, G. W. (1986) The Genus *Salix* (Salicaceae) in the Southeastern United States. *Systematic Botany Monographs,* **9,** 1-170.

Arshad, M. S., Farooq, M., Asch, F., Krishna, J. S. V., Prasad, P. V. V. & Siddique, K. H. M. (2017) Thermal stress impacts reproductive development and grain yield in rice. *Plant Physiology and Biochemistry,* **115,** 57-72.

Ashman, T. L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T. & Wilson, W. G. (2004) Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology,* **85,** 2408-2421.

Baskerville, G. L. & Emin, P. (1969) Rapid estimation of heat accumulation from maximum and minimum temperatures. *Ecology,* **50,** 514-&.

Beardsell, D. V., Knox, R. B. & Williams, E. G. (1993) Breeding system and reproductive success of *Thryptomene calycina* (Myrtaceae). *Australian Journal of Botany,* **41,** 333-353.

Becklin, K. M., Pallo, M. L. & Galen, C. (2012) Willows indirectly reduce arbuscular mycorrhizal fungal colonization in understorey communities. *Journal of Ecology,* **100,** 343-351.

Biere, A. (1991) Parental effects in *Lychnis flos cuculi*: Seed size, germination and seedling performance in a controlled environment. *Journal of Evolutionary Biology,* **4,** 447-465.

Blackburn, T. M., Pysek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarosik, V., Wilson, J. R. U. & Richardson, D. M. (2011) A proposed unified framework for biological invasions. *Trends in Ecology & Evolution,* **26,** 333-339.

Blossey, B. & Notzold, R. (1995) Evolution of increased competitive ability in invasive nonindigenous plants - a hypothesis. *Journal of Ecology,* **83,** 887-889.

Borchert, R., Rivera, G. & Hagnauer, W. (2002) Modification of vegetative phenology in a tropical semi-deciduous forest by abnormal drought and rain. *Biotropica,* **34,** 27-39.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E. & Prati, D. (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia,* **144,** 1-11.

Broennimann, O., Fitzpatrick, M. C., Pearman, P. B., Petitpierre, B., Pellissier, L., Yoccoz, N. G., Thuiller, W., Fortin, M. J., Randin, C., Zimmermann, N. E., Graham, C. H. & Guisan, A. (2012) Measuring ecological niche overlap from occurrence and spatial environmental data. *Global Ecology and Biogeography,* **21,** 481-497.

Brussard, P. F. (1984) Geographic patterns and environmental gradients - the central-marginal model in *Drosophila* revisited. *Annual Review of Ecology and Systematics,* **15,** 25-64.

Buckley, Y. M., Bolker, B. M. & Rees, M. (2007) Disturbance, invasion and re-invasion: Managing the weed-shaped hole in disturbed ecosystems. *Ecology Letters,* **10,** 809-817.

Buckley, Y. M., Downey, P., Fowler, S. V., Hill, R., Memmot, J., Norambuena, H., Pitcairn, M., Shaw, R., Sheppard, A. W., Winks, C., Wittenberg, R. & Rees, M. (2003) Are invasives bigger? A global study of seed size variation in two invasive shrubs. *Ecology,* **84,** 1434-1440.

Buechling, A., Martin, P. H., Canham, C. D., Shepperd, W. D. & Battaglia, M. A. (2016) Climate drivers of seed production in *Picea engelmannii* and response to warming temperatures in the southern Rocky Mountains. *Journal of Ecology,* **104,** 1051-1062.

Bureau of Meteorology (2018) Bureau of Meteorology weather stations – Edi Upper, Mount Hotham. Climate statistics for Australian locations. Viewed 7 November 2018.

Bureau of Meteorology (2018) Climate statistics for Australian locations.

Burnham, K. P. & Anderson, D. R. (2002) *Model selection and multimodel inference.* Springer, New York.

Burns, J. H., Pardini, E. A., Schutzenhofer, M. R., Chung, Y. A., Seidler, K. J. & Knight, T. M. (2013) Greater sexual reproduction contributes to differences in demography of invasive plants and their noninvasive relatives. *Ecology,* **94,** 995-1004.

Bush, E. R., Abernethy, K. A., Jeffery, K., Tutin, C., White, L., Dimoto, E., Dikangadissi, J.-T., Jump, A. S. & Bunnefeld, N. (2017) Fourier analysis to detect phenological cycles using long-term tropical field data and simulations. *Methods in Ecology and Evolution,* **8,** 530-540.

Cain, M. L., Bowman, W. D. & Hacker, S. D. (2008) *Ecology.* Sinauer Associates, Inc.

Campbell, D. R. & Motten, A. F. (1985) The mechanism of competition for pollination between two forest herbs. *Ecology,* **66,** 554-563.

Campbell, N. A. & Reece, J. B. (2002) *Biology.* Benjamin Cummings, San Francisco, CA.

Caplat, P., Nathan, R. & Buckley, Y. M. (2012) Seed terminal velocity, wind turbulence, and demography drive the spread of an invasive tree in an analytical model. *Ecology,* **93,** 368-377.

Carleton, R. B. (1949) The Willows: Helpers of Man. *The Scientific Monthly,* **69,** 48-55.

Caron, V. (2011) *Ecology and evolution of the invasive willow sawfly* Nematus oligospilus Forster. Degree of Doctor of Philosophy, Monash University.

Carr, G. W. (1996) Flora of Victoria. (eds N. G. Walsh & T. J. Entwistle), pp. 387-398.

Cha, D. H., Hochwender, C. G., Bosecker, E. M., Tucker, R. E., Kaufman, A. D., Fritz, R. S. & Smyth, R. R. (2009) Do exotic generalist predators alter host plant preference of a native willow beetle? *Agricultural and Forest Entomology,* **11,** 175-184.

Chapman, C. A., Wrangham, R. W., Chapman, L. J., Kennard, D. K. & Zanne, A. E. (1999) Fruit and flower phenology at two sites in Kibale National Park, Uganda. *Journal of Tropical Ecology,* **15,** 189-211.

Chen, X. Q., Hu, B. & Yu, R. (2005) Spatial and temporal variation of phenological growing season and climate change impacts in temperate eastern China. *Global Change Biology,* **11,** 1118-1130.

Chrtek, J., Hartmann, M., Mrazova, V., Zdvorak, P., Stefanek, M. & Mraz, P. (2018) Seed traits, terminal velocity and germination in sexual diploid and apomictic triploid *Hieracium alpinum* (Asteraceae): Are apomicts better dispersers? *Flora,* **240,** 76-81.

Chuine, I., Rehfeldt, G. E. & Aitken, S. N. (2006) Height growth determinants and adaptation to temperature in pines: a case study of *Pinus contorta* and *Pinus monticola*. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 36, 1059-1066.

Chuine, I. (2010) Why does phenology drive species distribution? *Philosophical Transactions of the Royal Society B-Biological Sciences,* **365,** 3149-3160.

Cipollini, D. (2005) Interactive effects of lateral shading and jasmonic acid on morphology, phenology, seed production, and defense traits in *Arabidopsis thaliana*. *International Journal of Plant Sciences,* **166,** 955-959.

Clark, J. S., Lewis, M., McLachlan, J. S. & HilleRisLambers, J. (2003) Estimating population spread: What can we forecast and how well? *Ecology,* **84,** 1979-1988.

Cleland, E. E., Allen, J. M., Crimmins, T. M., Dunne, J. A., Pau, S., Travers, S. E., Zavaleta, E. S. & Wolkovich, E. M. (2012) Phenological tracking enables positive species responses to climate change. *Ecology,* **93,** 1765-1771.

Cleland, E. E., Chuine, I., Menzel, A., Mooney, H. A. & Schwartz, M. D. (2007) Shifting plant phenology in response to global change. *Trends in Ecology & Evolution,* **22,** 357-365.

Colautti, R. I. & Barrett, S. C. H. (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science,* **342,** 364-366.

Cornelius, C., Petermeier, H., Estrella, N. & Menzel, A. (2011) A comparison of methods to estimate seasonal phenological development from BBCH scale recording. *International Journal of Biometeorology,* **55,** 867-877.

Coumou, D. & Rahmstorf, S. (2012) A decade of weather extremes. *Nature Climate Change,* **2,** 491-496.

Coutts, S. R., van Klinken, R. D., Yokomizo, H. & Buckley, Y. M. (2011) What are the key drivers of spread in invasive plants: Dispersal, demography or landscape: And how can we use this knowledge to aid management? *Biological Invasions,* **13,** 1649-1661.

Crawley, M. J. (2002) *Statistical computing: An introduction to data analysis using S-Plus.* Wiley, West Sussex, England.

Cremer, K. (1999) Willow management for Australian rivers. *Natural Resource Management,* **2**.

Cremer, K. W. (2003) Introduced willows can become invasive pests in Australia. *Biodiversity,* **4,** 17-24.

Crimmins, T. M., Crimmins, M. A. & Bertelsen, C. D. (2010) Complex responses to climate drivers in onset of spring flowering across a semi-arid elevation gradient. *Journal of Ecology,* **98,** 1042-1051.

Dafni, A. & Firmage, D. (2000) Pollen viability and longevity: Practical, ecological and evolutionary implications. *Plant Systematics and Evolution,* **222,** 113-132.

Davis, H. G., Taylor, C. M., Civille, J. C. & Strong, D. R. (2004) An Allee effect at the front of a plant invasion: *Spartina* in a Pacific estuary. *Journal of Ecology,* **92,** 321-327.

Diez, J. M., D'Antonio, C. M., Dukes, J. S., Grosholz, E. D., Olden, J. D., Sorte, C. J. B., Blumenthal, D. M., Bradley, B. A., Early, R., Ibanez, I., Jones, S. J., Lawler, J. J. & Miller, L. P. (2012) Will extreme climatic events facilitate biological invasions? *Frontiers in Ecology and the Environment,* **10,** 249-257.

Dlugos, D. M., Collins, H., Bartelme, E. M. & Drenovsky, R. E. (2015) The non-native plant *Rosa multiflora* expresses shade avoidance traits under low light availability. *American Journal of Botany,* **102,** 1323-1331.

Dukes, J. S. & Mooney, H. A. (1999) Does global change increase the success of biological invaders? *Trends in Ecology & Evolution,* **14,** 135-139.

Early, R. & Sax, D. F. (2014) Climatic niche shifts between species' native and naturalized ranges raise concern for ecological forecasts during invasions and climate change. *Global Ecology and Biogeography,* **23,** 1356-1365.

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

Espirito-Santo, M. M., Madeira, B. G., Neves, F. S., Faria, M. L., Fagundes, M. & Fernandes, G. W. (2003) Sexual differences in reproductive phenology and their consequences for the demography of *Baccharis dracunculifolia* (Asteraceae), a dioecious tropical shrub. *Annals of Botany,* **91,** 13-19.

Fabbro, T. & Korner, C. (2004) Altitudinal differences in flower traits and reproductive allocation. *Flora,* **199,** 70-81.

Fernandez-Martinez, M., Belmonte, J. & Maria Espelta, J. (2012) Masting in oaks: Disentangling the effect of flowering phenology, airborne pollen load and drought. *Acta Oecologica-International Journal of Ecology,* **43,** 51-59.

Fick, S. E. & Hijmans, R. J. (2017) Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*.

Firn, J., Moore, J. L., MacDougall, A. S., Borer, E. T., Seabloom, E. W., HilleRisLambers, J., Harpole, W. S., Cleland, E. E., Brown, C. S., Knops, J. M. H., Prober, S. M., Pyke, D. A., Farrell, K. A., Bakker, J. D., O'Halloran, L. R., Adler, P. B., Collins, S. L., D'Antonio, C. M., Crawley, M. J., Wolkovich, E. M., La Pierre, K. J., Melbourne, B. A., Hautier, Y., Morgan, J. W., Leakey, A. D. B., Kay, A., McCulley, R., Davies, K. F., Stevens, C. J., Chu, C. J., Holl, K. D., Klein, J. A., Fay, P. A., Hagenah, N., Kirkman, K. P. & Buckley, Y. M. (2011) Abundance of introduced species at home predicts abundance away in herbaceous communities. *Ecology Letters,* **14,** 274-281.

Fox, J. F. (1992) Pollen limitation of reproductive effort in willows. *Oecologia,* **90,** 283-287.

GBIF.org (25 October 2018) GBIF Occurrence Download https://doi.org/10.15468/dl.nwu8pu

Gage, E. A. & Cooper, D. J. (2005) Patterns of willow seed dispersal, seed entrapment, and seedling establishment in a heavily browsed montane riparian ecosystem. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **83,** 678-687.

Garcia-Camacho, R., Maria Iriondo, J. & Escudero, A. (2010) Seedling dynamics at elevation limits: Complex interactions beyond seed and microsite limitations. *American Journal of Botany,* **97,** 1791-1797.

Giljohann, K. M., Hauser, C. E., Williams, N. S. G. & Moore, J. L. (2011) Optimizing invasive species control across space: Willow invasion management in the Australian Alps. *Journal of Applied Ecology,* **48,** 1286-1294.

Glen, A. S., Pech, R. P. & Byrom, A. E. (2013) Connectivity and invasive species management: towards an integrated landscape approach. *Biological Invasions,* **15,** 2127-2138.

Gonzalez-Rodriguez, V., Villar, R. & Navarro-Cerrillo, R. M. (2011) Maternal influences on seed mass effect and initial seedling growth in four *Quercus* species. *Acta Oecologica-International Journal of Ecology,* **37,** 1-9.

Gordienko, N. S. & Sokolov, L. V. (2009) Analysis of long-term changes in the dates of seasonal phenomena in plants and insects of the Il'men Reserve as related to climatic factors. *Russian Journal of Ecology,* **40,** 86-92.

Greene, D. F. & Johnson, E. A. (1993) Seed mass and dispersal capacity in wind-dispersed diaspores. *Oikos,* **67,** 69-74.

Greene, D. F. & Quesada, M. (2005) Seed size, dispersal, and aerodynamic constraints within the Bombacaceae. *American Journal of Botany,* **92,** 998-1005.

Guo, H., Mazer, S. J. & Du, G. Z. (2010) Geographic variation in seed mass within and among nine species of *Pedicularis* (Orobanchaceae): effects of elevation, plant size and seed number per fruit. *Journal of Ecology,* **98,** 1232-1242.

Hahn, M. A., Lanz, T., Fasel, D. & Muller-Scharer, H. (2013) Increased seed survival and seedling emergence in a polyploid plant invader. *American Journal of Botany,* **100,** 1555-1561.

Haig, D. & Westoby, M. (1988) On limits to seed production. *American Naturalist,* **131,** 757-759.

Halpern, S. L. (2005) Sources and consequences of seed size variation in *Lupinus perennis* (Fabaceae): Adaptive and non-adaptive hypotheses'. *American Journal of Botany,* **92,** 205-213.

Hampe, A. (2011) Plants on the move: The role of seed dispersal and initial population establishment for climate-driven range expansions. *Acta Oecologica-International Journal of Ecology,* **37,** 666-673.

Harrison, X. A. (2014) Using observation-level random effects to model overdispersion in count data in ecology and evolution. *Peerj,* **2**.

Hauser, C. E. & McCarthy, M. A. (2009) Streamlining 'search and destroy': cost-effective surveillance for invasive species management. *Ecology Letters,* **12,** 683-692.

Hedhly, A., Hormaza, J. I. & Herrero, M. (2003) The effect of temperature on stigmatic receptivity in sweet cherry (*Prunus avium L*.). *Plant Cell and Environment,* **26,** 1673-1680.

Hedhly, A., Hormaza, J. I. & Herrero, M. (2005) The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. *Plant Biology,* **7,** 476-483.

Hedhly, A., Hormaza, J. I. & Herrero, M. (2009) Global warming and sexual plant reproduction. *Trends in Plant Science,* **14,** 30-36.

Heino, M. & Kaitala, V. (1999) Evolution of resource allocation between growth and reproduction in animals with indeterminate growth. Journal of Evolutionary Biology, 12, 423-429.

Hensher, D. A. & Stopher, P. R. (1979) Behavioural travel modelling. Taylor & Francis.

Herrero, M. (2003) Male and female synchrony and the regulation of mating in flowering plants. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* **358,** 1019-1024.

Heydel, F., Cunze, S., Bernhardt-Roemermann, M. & Tackenberg, O. (2015) Seasonal synchronization of seed release phenology promotes long-distance seed dispersal by wind for tree species with medium wind dispersal potential. *Journal of Vegetation Science,* **26,** 1090-1101.

Hickman, J.C., Editor. (1993) *The Jepson Manual: Higher plants of California*. University of California Press, Berkeley, CA.

Hill, J. K. & Hodkinson, I. D. (1995) Effects of temperature on phenological synchrony and altitudinal distribution of jumping plant lice (*Hemiptera, Psylloidea*) on dwarf willow (*Salix lapponum*) in Norway. *Ecological Entomology,* **20,** 237-244.

Holland-Clift, S. (2004) Willow identification: An essential skill for successful willow management. Weeds of National Significance. State Government of Victoria.

Holland Clift, S. & Davies, J. (2007) Willows national management guide: current management and control option for willows (*Salix spp.*) in Australia. (ed. Victorian Dep. of Primary Industries). Geelong, Australia.

Hopley, T. (2011) *Reproductive ecology and dispersal dynamics of the invasive willow,* Salix cinerea*, in south-eastern Australia*. Doctor of Philosophy, The Australian National University.

Hopley, T. & Young, A. C. (2015) Knowledge of the reproductive ecology of the invasive *Salix cinerea*, in its invaded range, assists in more targeted management strategies. *Australian Journal of Botany*.

Hopley, T., Zwart, A. B. & Young, A. G. (2015) Among-population pollen movement and skewed male fitness in a dioecious weed. *Biological Invasions,* **17,** 2147-2161.

Hou, Q.-Q., Chen, B.-M., Peng, S.-L. & Chen, L.-Y. (2014) Effects of extreme temperature on seedling establishment of nonnative invasive plants. *Biological Invasions,* **16,** 2049-2061.

Houle, G. (1999) Mast seeding in *Abies balsamea, Acer saccharum*, and *Betula alleghaniensis* in an old growth, cold temperate forest of north-eastern North America. *Journal of Ecology,* **87,** 413-422.

Howe, H. F. & Smallwood, J. (1982) Ecology of seed dispersal. *Annual Review of Ecology and Systematics,* **13,** 201-228.

Hudson, I. L. & Keatley, M. R. (2013) Scoping the budding and climate impacts on Eucalypt flowering: Nonlinear time series decomposition modelling. *20th International Congress on Modelling and Simulation (Modsim2013)***,** 1582-1588.

Inouye, D. W. (2008) Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology,* **89,** 353-362.

Inouye, D. W., Saavedra, F. & Lee-Yang, W. (2003) Environmental influences on the phenology and abundance of flowering by *Androsace septentrionalis* (Primulaceae). *American Journal of Botany,* **90,** 905-910.

Isebrands, J. G. & Richardson, J. (2014) *Poplars and willows: Trees for society and the environment.* Boston, MA : CABI ; Rome : FAO, [2014] ©2014.

Jakobsson, A. & Eriksson, O. (2003) Trade-offs between dispersal and competitive ability: a comparative study of wind-dispersed Asteraceae forbs. *Evolutionary Ecology,* **17,** 233-246.

Jarvis, J. C. & Moore, K. A. (2008) Influence of environmental factors on *Vallisneria americana* seed germination. *Aquatic Botany,* **88,** 283-294.

Jelbert, K., Stott, I., McDonald, R. A. & Hodgson, D. (2015) Invasiveness of plants is predicted by size and fecundity in the native range. *Ecology and Evolution,* **5,** 1933-1943.

Jongejans, E., Pedatella, N., M., Shea, K., Skarpaas, O. & Auhl, R. (2007) Seed release by invasive thistles: The impact of plant and environmental factors. *The Royal Society Publishing,* **274,** 2457-2464.

Jump, A. S. & Penuelas, J. (2005) Running to stand still: Adaptation and the response of plants to rapid climate change. *Ecology Letters,* **8,** 1010-1020.

Kass, R. E. & Raftery, A. E. (1995) Bayes factors. *Journal of the American Statistical Association,* **90,** 773-795.

Kehl, A., Aas, G. & Rambold, G. (2008) Genotypical and multiple phenotypical traits discriminate *Salix x rubens Schrank* clearly from its parent species. *Plant Systematics and Evolution,* **275,** 169-179.

Kluth, C. & Bruelheide, H. (2005) Effects of range position, inter-annual variation and density on demographic transition rates of *Hornungia petraea* populations. *Oecologia,* **145,** 382-393.

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J. & Ashman, T. L. (2005) Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology Evolution and Systematics,* **36,** 467-497.

Knops, J. M. H., Koenig, W. D. & Carmen, W. J. (2007) Negative correlation does not imply a tradeoff between growth and reproduction in California oaks. Proceedings of the National Academy of Sciences of the United States of America, 104, 16982-16985.

Koch, E., Bruns, E., Chmielewksi, F. M., Defila, C., Lipa, W. & Menzel, A. (2007) Guidelines for plant phenological observations (ed W. C. D. a. M. Programme). World Meteorological Organization.

Kollmann, J. & Banuelos, M. J. (2004) Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Diversity and Distributions,* **10,** 377-385.

Korner, C. & Basler, D. (2010) Phenology under global warming. *Science,* **327,** 1461-1462.

Kozlowski, T. T. & Pallardy, S. G. (1997) *Physiology of Woody Plants.* Academic Press, Inc, San Diego, California.

Kudo, G. & Ida, T. Y. (2013) Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology,* **94,** 2311-2320.

Kushwaha, C. P., Tripathi, S. K. & Singh, K. P. (2011) Tree specific traits affect flowering time in Indian dry tropical forest. *Plant Ecology,* **212,** 985-998.

Ladson, A. R. (1997) Willows along Victorian waterways: Towards a willow management strategy. (ed D. o. N. R. a. Environment). Victoria.

Lauron-Moreau, A., Pitre, F. E., Argus, G. W., Labrecque, M. & Brouillet, L. (2015) Phylogenetic relationships of American willows (*Salix L.,* Salicaceae). *Plos One,* **10**.

Lechowicz, M. J. (1995) Seasonality of flowering and fruiting in temperate forest trees. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **73,** 175-182.

Lediuk, K. D., Damascos, M. A., Puntieri, J. G. & Svriz, M. (2014) Differences in phenology and fruit characteristic between invasive and native woody species favor exotic species invasiveness. *Plant Ecology,* **215,** 1455-1467.

Lehtila, K. & Ehrlen, J. (2005) Seed size as an indicator of seed quality: A case study of *Primula veris*. *Acta Oecologica-International Journal of Ecology,* **28,** 207-212.

Leiblein-Wild, M. C., Kaviani, R. & Tackenberg, O. (2014) Germination and seedling frost tolerance differ between the native and invasive range in common ragweed. *Oecologia,* **174,** 739-750.

Levins, R. & Culver, D. (1971) Regional coexistence of species and competition between rare species. *Proceedings of the National Academy of Sciences of the United States of America,* **68,** 1246-&.

Litt, A. R., Cord, E. E., Fulbright, T. E. & Schuster, G. L. (2014) Effects of invasive plants on arthropods. *Conservation Biology,* **28,** 1532-1549.

Liu, H., Zhang, D., Yang, X., Huang, Z., Duan, S., & Wang, X. (2014) Seed dispersal and germination traits of 70 plant species inhabiting the Gurbantunggut Desert in Northwest China. *The Scientific World Journal*.

Lonnberg, K. & Eriksson, O. (2013) Relationships between intra-specific variation in seed size and recruitment in four species in two contrasting habitats. *Plant Biology,* **15,** 601-606.

Lord, J. M. (1994) Variation in *Festuca novae-zelandiae* (hack) Cockayne germination behavior with altitude of seed source. *New Zealand Journal of Botany,* **32,** 227-235.

Mack, M. C. & D'Antonio, C. M. (1998) Impacts of biological invasions on disturbance regimes. *Trends in Ecology & Evolution,* **13,** 195-198.

Maron, J. L., Vila, M., Bommarco, R., Elmendorf, S. & Beardsley, P. (2004) Rapid evolution of an invasive plant. *Ecological Monographs,* **74,** 261-280.

Mauseth, J. (2008) Botany: An introduction to plant biology. Jones & Bartlett Learning.

McDougall, K. L. (2007) Grazing and fire in two subalpine peatlands. *Australian Journal of Botany,* **55,** 42-47.

McDougall, K. L., Morgan, J. W., Walsh, N. G. & Williams, R. J. (2005) Plant invasions in treeless vegetation of the Australian alps. *Perspectives in Plant Ecology Evolution and Systematics,* **7,** 159-171.

McMahon, S. M., Parker, G. G. & Miller, D. R. (2010) Evidence for a recent increase in forest growth. *Proceedings of the National Academy of Sciences of the United States of America,* **107,** 3611-3615.

McMaster, G. S. & Wilhelm, W. W. (1997) Growing degree-days: One equation, two interpretations. *Agricultural and Forest Meteorology,* **87,** 291-300.

Meier, U. (1997) Growth stages of mono- and dicotyledonous plants. (ed F. B. R. C. f. A. a. Forestry). BBCH Monograph.

Merow, C., Bois, S. T., Allen, J. M., Xie, Y. Y. & Silander, J. A. (2017) Climate change both facilitates and inhibits invasive plant ranges in New England. *Proceedings of the National Academy of Sciences of the United States of America,* **114,** E3276-E3284.

Michaels, H. J., Benner, B., Hartgerink, A. P., Lee, T. D., Rice, S., Willson, M. F. & Bertin, R. I. (1988) Seed size variation: Magnitude, distribution, and ecological correlates. *Evolutionary Ecology,* **2,** 157-166.

Miller-Rushing, A. J., Hoye, T. T., Inouye, D. W. & Post, E. (2010) The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society B-Biological Sciences,* **365,** 3177-3186.

Miyamoto, Y., Griesbauer, H. P. & Green, D. S. (2010) Growth responses of three coexisting conifer species to climate across wide geographic and climate ranges in Yukon and British Columbia. Forest Ecology and Management, 259, 514-523.

Moody, M. E. & Mack, R. N. (1988) Controlling the spread of plant invasions - The importance of nascent foci. *Journal of Applied Ecology,* **25,** 1009-1021.

Mooney, H. A. & Gulmon, S. L. (1982) Constraints on leaf structure and function in reference to herbivory. *Bioscience,* **32,** 198-206.

Moore, J. L., Pascoe, C., Thomas, E. & Keatley, M. R. (2017) Implementing decision analysis tools for invasive species management. *Decision-making in*

conservation and natural resource management: Models for interdisciplinary approaches (eds N. Bunnefeld, E. Nicholson & E. J. Milner-Gulland). Cambridge University Press, Cambridge, UK.

Moore, J. L. & Runge, M. C. (2012) Combining structured decision making and value-of-information analyses to identify robust management strategies. *Conservation Biology,* **26,** 810-820.

Moot, D. J., Henderson, A. L., Porter, J. R. & Semenov, M. A. (1996) Temperature, CO2 and the growth and development of wheat: Changes in the mean and variability of growing conditions. *Climatic Change,* **33,** 351-368.

Mortlock, W. (1999) *Basic germination and viability tests for native plant seed.* FloraBank, Yarralumla, ACT.

Mosseler, A. (1989) Interspecific pollen-pistil incongruity in *Salix*. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* **19,** 1161-1168.

Mosseler, A. & Papadopol, C. S. (1989) Seasonal isolation as a reproductive barrier among sympatric *Salix* species. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **67,** 2563-2570.

Munguia-Rosas, M. A., Parra-Tabla, V. & Montiel, S. (2013) Extreme variation in the reproductive phenology of the weed *Ruellia nudiflora*. *Weed Research,* **53,** 328-336.

Myneni, R. B., Keeling, C. D., Tucker, C. J., Asrar, G. & Nemani, R. R. (1997) Increased plant growth in the northern high latitudes from 1981 to 1991. *Nature,* **386,** 698-702.

Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution,* **4,** 133-142.

Nathan, R., Katul, G. G., Bohrer, G., Kuparinen, A., Soons, M. B., Thompson, S. E., Trakhtenbrot, A. & Horn, H. S. (2011) Mechanistic models of seed dispersal by wind. *Theoretical Ecology,* **4,** 113-132.

Nathan, R., Katul, G. G., Horn, H. S., Thomas, S. M., Oren, R., Avissar, R., Pacala, S. W. & Levin, S. A. (2002) Mechanisms of long-distance dispersal of seeds by wind. *Nature,* **418,** 409-413.

O'Donnell, M. S. & Ignizio, D. A. (2012) Bioclimatic predictors for supporting ecological applications in the conterminous United States. (ed U.S. Geological Survey), pp. 10.

Oleksyn, J., Modrzynski, J., Tjoelker, M. G., Zytkowiak, R., Reich, P. B. & Karolewski, P. (1998) Growth and physiology of *Picea abies* populations from elevational transects: Common garden evidence for altitudinal ecotypes and cold adaptation. *Functional Ecology,* **12,** 573-590.

Olesen, T. (2005) The timing of flush development affects the flowering of avocado (*Persea americana*) and macadamia (*Macadamia integrifolia x tetraphylla*). *Australian Journal of Agricultural Research,* **56,** 723-729.

Ollerton, J. & Lack, A. (1998) Relationships between flowering phenology, plant size and reproductive success in *Lotus corniculatus* (Fabaceae). *Plant Ecology,* **139,** 35-47.

Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, W. Pearse. 2013. Caper: Comparative analyses of phylogenetics and evolution in R. R package version 0.5.2. http://CRAN.R-project.org/package=caper.

Orsenigo, S., Mondoni, A., Rossi, G. & Abeli, T. (2014) Some like it hot and some like it cold, but not too much: Plant responses to climate extremes. *Plant Ecology,* **215,** 677-688.

Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature,* **401,** 877-884.

Pannell, J. (1997) The maintenance of gynodioecy and androdioecy in a metapopulation. *Evolution,* **51,** 10-20.

Parker, I. M. (1997) Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive exotic shrub. *Ecology,* **78,** 1457-1470.

Parker, J. D., Torchin, M. E., Hufbauer, R. A., Lemoine, N. P., Alba, C., Blumenthal, D. M., Bossdorf, O., Byers, J. E., Dunn, A. M., Heckman, R. W., Hejda, M., Jarosik, V., Kanarek, A. R., Martin, L. B., Perkins, S. E., Pysek, P., Schierenbeck, K., Schloder, C., van Klinken, R., Vaughn, K. J., Williams, W. & Wolfe, L. M. (2013) Do invasive species perform better in their new ranges? *Ecology,* **94,** 985-994.

Piao, S. L., Cui, M. D., Chen, A. P., Wang, X. H., Ciais, P., Liu, J. & Tang, Y. H. (2011) Altitude and temperature dependence of change in the spring

vegetation green-up date from 1982 to 2006 in the Qinghai-Xizang Plateau. *Agricultural and Forest Meteorology,* **151,** 1599-1608.

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

Pogorzelec, M., Parzymies, M., Bronowicka-Mielniczuk, U., Banach, B. & Serafin, A. (2015) Pollen viability and tissue culture initiation of Salix lapponum, an endangered species in Poland. Acta Scientiarum Polonorum-Hortorum Cultus, 14, 151-161.

Pollnac, F. W., Maxwell, B. D., Taper, M. L. & Rew, L. J. (2014) The demography of native and non-native plant species in mountain systems: Examples in the Greater Yellowstone Ecosystem. *Population Ecology,* **56,** 81-95.

Porter, J. R. & Semenov, M. A. (2005) Crop responses to climatic variation. *Philosophical Transactions of the Royal Society B-Biological Sciences,* **360,** 2021-2035.

Post, E. S., Pedersen, C., Wilmers, C. C. & Forchhammer, M. C. (2008) Phenological sequences reveal aggregate life history response to climatic warming. *Ecology,* **89,** 363-370.

Prentis, P. J., Wilson, J. R. U., Dormontt, E. E., Richardson, D. M. & Lowe, A. J. (2008) Adaptive evolution in invasive species. *Trends in Plant Science,* **13,** 288-294.

Primack, R. B. (1987) Relationships among flowers, fruits, and seeds. *Annual Review of Ecology and Systematics,* **18,** 409-430.

Ranjitkar, S., Luedeling, E., Shrestha, K. K., Guan, K. & Xu, J. (2013) Flowering phenology of tree *Rhododendron* along an elevation gradient in two sites in the Eastern Himalayas. *International Journal of Biometeorology,* **57,** 225-240.

Rathcke, B. & Lacey, E. P. (1985) Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics,* **16,** 179-214.

Reyer, C. P. O., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R. P., Bonfante, A., de Lorenzi, F., Dury, M., Gloning, P., Abou Jaoude, R., Klein, T., Kuster, T. M., Martins, M., Niedrist, G., Riccardi, M., Wohlfahrt, G., de Angelis, P., de Dato, G., Francois, L., Menzel, A. & Pereira, M. (2013) A plant's perspective of extremes: Terrestrial plant responses to changing climatic variability. *Global Change Biology,* **19,** 75-89.

Riha, S. J., Wilks, D. S. & Simoens, P. (1996) Impact of temperature and precipitation variability on crop model predictions. *Climatic Change,* **32,** 293-311.

Rivera, G. & Borchert, R. (2001) Induction of flowering in tropical trees by a 30-min reduction in photoperiod: Evidence from field observations and herbarium specimens. *Tree Physiology,* **21,** 201-212.

Roland, C. A., Schmidt, J. H. & Johnstone, J. F. (2014) Climate sensitivity of reproduction in a mast-seeding boreal conifer across its distributional range from lowland to treeline forests. *Oecologia,* **174,** 665-677.

Ronnberg-Wastljung, A. C. & Gullberg, U. (1999) Genetics of breeding characters with possible effects on biomass production in *Salix viminalis (L.)*. *Theoretical and Applied Genetics,* **98,** 531-540.

Ruml, M., Vukovic, A. & Milatovic, D. (2010) Evaluation of different methods for determining growing degree-day thresholds in apricot cultivars. *International Journal of Biometeorology,* **54,** 411-422.

Sacchi, C. F. & Price, P. W. (1992) The relative roles of abiotic and biotic factors in seedling demography of arroyo willow (*Salix lasiolepis*, Salicaceae). *American Journal of Botany,* **79,** 395-405.

Santinelo Pereira, R. A., Rodrigues, E. & Menezes, A. d. O., Jr. (2007) Phenological patterns of *Ficus citrifolia* (Moraceae) in a seasonal humid-subtropical region in Southern Brazil. *Plant Ecology,* **188,** 265-275.

Sanzol, J., Rallo, P. & Herrero, M. (2003) Asynchronous development of stigmatic receptivity in the pear (*Pyrus communis*, Rosaceae) flower. *American Journal of Botany,* **90,** 78-84.

Saska, M. M. & Kuzovkina, Y. A. (2010) Phenological stages of willow (*Salix*). *Annals of Applied Biology,* **156,** 431-437.

Scheepens, J. F., Deng, Y. & Bossdorf, O. (2018) Phenotypic plasticity in response to temperature fluctuations is genetically variable, and relates to climatic variability of origin, in *Arabidopsis thaliana*. *Aob Plants,* **10**.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods,* **9,** 671-675.

Schoener, T. W. (1970) Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology,* **51,** 408-418.

Schwartz, M. D. (2013) *Phenology: An integrative environmental science.* Springer, Milwaukee, WI.

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pysek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jager, H., Kartesz, J., Kenis, M., Kreft, H., Kuhn, I., Lenzner, B., Liebhold, A., Mosena, A., Moser, D., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H. E., Scalera, R., Schindler, S., Stajerova, K., Tokarska-Guzik, B., van Kleunen, M., Walker, K., Weigelt, P., Yamanaka, T. & Essl, F. (2017) No saturation in the accumulation of alien species worldwide. *Nature Communications,* **8**.

Semenov, M. A. & Porter, J. R. (1995) Climatic variability and the modeling of crop yields. *Agricultural and Forest Meteorology,* **73,** 265-283.

Shafroth, P. B., Scott, M. L., Friedman, J. M. & Laven, R. D. (1994) Establishment, sex structure and breeding system of an exotic riparian willow, *Salix x rubens*. *American Midland Naturalist,* **132,** 159-172.

Sherry, R. A., Zhou, X., Gu, S., Arnone, J. A., III, Schimel, D. S., Verburg, P. S., Wallace, L. L. & Luo, Y. (2007) Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences of the United States of America,* **104,** 198-202.

Silvertown, J. W. (1981) Seed size, life-span, and germination date as co-adapted features of plant life-history. *American Naturalist,* **118,** 860-864.

Simberloff, D. (2009) The role of propagule pressure in biological invasions. *Annual Review of Ecology Evolution and Systematics,* **40,** 81-102.

Skov, F. & Svenning, J. C. (2004) Potential impact of climatic change on the distribution of forest herbs in Europe. *Ecography,* **27,** 366-380.

Smith, C. C. & Fretwell, S. D. (1974) Optimal balance between size and number of offspring. *American Naturalist,* **108,** 499-506.

Snyder, R. L. (1985) Hand calculating degree days. *Agricultural and Forest Meteorology,* **35,** 353-358.

Spano, D., Cesaraccio, C., Duce, P. & Snyder, R. L. (1999) Phenological stages of natural species and their use as climate indicators. *International Journal of Biometeorology,* **42,** 124-133.

Stevens, N., Seal, C. E., Archibald, S. & Bond, W. (2014) Increasing temperatures can improve seedling establishment in arid-adapted savanna trees. *Oecologia,* **175,** 1029-1040.

Strayer, D. L., Eviner, V. T., Jeschke, J. M. & Pace, M. L. (2006) Understanding the long-term effects of species invasions. *Trends in Ecology & Evolution,* **21,** 645-651.

Symonds, M. R. E., and S. P. Blomberg. 2014. A primer on phylogenetic generalised least squares. Chapter 5, pp. 105-130, in *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology* (Garamszegi, L. Z., ed.), Springer: Heidelberg.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

The Plant List (2013). Version 1.1. Published on the Internet; http://www.theplantlist.org/ Viewed 24 November 2018.

Theoharides, K. A. & Dukes, J. S. (2007) Plant invasion across space and time: Factors affecting nonindigenous species success during four stages of invasion. *New Phytologist,* **176,** 256-273.

Torimaru, T. & Tomaru, N. (2006) Relationships between flowering phenology, plant size, and female reproductive output in a dioecious shrub, *Ilex leucoclada* (Aquifoliaceae). *Canadian Journal of Botany-Revue Canadienne De Botanique,* **84,** 1860-1869.

Travis, J. M. J., Mustin, K., Barton, K. A., Benton, T. G., Clobert, J., Delgado, M. M., Dytham, C., Hovestadt, T., Palmer, S. C. F., Van Dyck, H. & Bonte, D. (2012) Modelling dispersal: An eco-evolutionary framework incorporating emigration, movement, settlement behaviour and the multiple costs involved. *Methods in Ecology and Evolution,* **3,** 628-641.

Treep, J., de Jager, M., Kuiper, L. S., Duman, T., Katul, G. G. & Soons, M. B. (2018) Costs and benefits of non-random seed release for long-distance dispersal in wind-dispersed plant species. *Oikos,* **127,** 1330-1343.

Turnbull, L. A., Coomes, D., Hector, A. & Rees, M. (2004) Seed mass and the competition/colonization trade-off: Competitive interactions and spatial patterns in a guild of annual plants. *Journal of Ecology,* **92,** 97-109.

Turnbull, L. A., Rees, M. & Crawley, M. J. (1999) Seed mass and the competition/colonization trade-off: A sowing experiment. *Journal of Ecology,* **87,** 899-912.

Valencia-Diaz, S. & Montana, C. (2005) Temporal variability in the maternal environment and its effect on seed size and seed quality in *Flourensia cernua DC*. (Asteraceae). *Journal of Arid Environments,* **63,** 686-695.

van Kleunen, M., Dawson, W., Schlaepfer, D., Jeschke, J. M. & Fischer, M. (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. *Ecology Letters,* **13,** 947-958.

van Kleunen, M., Weber, E. & Fischer, M. (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters,* **13,** 235-245.

Vaughton, G. & Ramsey, M. (1998) Sources and consequences of seed mass variation in *Banksia marginata* (Proteaceae). *Journal of Ecology,* **86,** 563-573.

Vellend, M., C. D. Brown, H. M. Kharouba, J. L. McCune, and I. H. Myers-Smith. 2013. Historical ecology: Using unconventional data sources to test for effects of global environmental change. American Journal of Botany 100:1294-1305.

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Springer, New York.

Vera, M. L. (1997) Effects of altitude and seed size on germination and seedling survival of heathland plants in North Spain. *Plant Ecology,* **133,** 101-106.

Visser, M. E. & Both, C. (2005) Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B-Biological Sciences,* **272,** 2561-2569.

Vitousek, P. M., Dantonio, C. M., Loope, L. L., Rejmanek, M. & Westbrooks, R. (1997) Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology,* **21,** 1-16.

Vitousek, P. M., Dantonio, C. M., Loope, L. L. & Westbrooks, R. (1996) Biological invasions as global environmental change. *American Scientist,* **84,** 468-478.

Vlachojannis, J., Magora, F. & Chrubasik, S. (2011) Willow species and aspirin: Different mechanism of actions. *Phytotherapy Research,* **25,** 1102-1104.

Wallwork, M. A. B., Jenner, C. F., Logue, S. J. & Sedgley, M. (1998) Effect of high temperature during grain-filling on the structure of developing and malted barley grains. *Annals of Botany,* **82,** 587-599.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J. M., Hoegh-Guldberg, O. & Bairlein, F. (2002) Ecological responses to recent climate change. *Nature,* **416,** 389-395.

Walther, G. R., Roques, A., Hulme, P. E., Sykes, M. T., Pysek, P., Kuhn, I., Zobel, M., Bacher, S., Botta-Dukat, Z., Bugmann, H., Czucz, B., Dauber, J., Hickler, T., Jarosik, V., Kenis, M., Klotz, S., Minchin, D., Moora, M., Nentwig, W., Ott, J., Panov, V. E., Reineking, B., Robinet, C., Semenchenko, V., Solarz, W., Thuiller, W., Vila, M., Vohland, K. & Settele, J. (2009) Alien species in a warmer world: Risks and opportunities. *Trends in Ecology & Evolution,* **24,** 686-693.

Warren, D. L., Glor, R. E. & Turelli, M. (2008) Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution,* **62,** 2868-2883.

Weed management guide for willow (*Salix spp*.). (2003) Department of Primary Industries, Water, and Environment. Tasmanian government.

Westoby, M., Leishman, M. & Lord, J. (1996) Comparative ecology of seed size and dispersal. *Philosophical Transactions of the Royal Society B-Biological Sciences,* **351,** 1309-1317.

Wheeler, H. C., Hoye, T. T., Schmidt, N. M., Svenning, J.-C. & Forchhammer, M. C. (2015) Phenological mismatch with abiotic conditions-implications for flowering in Arctic plants. *Ecology,* **96,** 775-787.

Wheeler, T. R., Craufurd, P. Q., Ellis, R. H., Porter, J. R. & Prasad, P. V. V. (2000) Temperature variability and the yield of annual crops. *Agriculture Ecosystems & Environment,* **82,** 159-167.

Wilke, B. J. & Irwin, R. E. (2010) Variation in the phenology and abundance of flowering by native and exotic plants in subalpine meadows. *Biological Invasions,* **12,** 2363-2372.

Wilkie, J. D., Sedgley, M. & Olesen, T. (2008) Regulation of floral initiation in horticultural trees. *Journal of Experimental Botany,* **59,** 3215-3228.

Wilson, W. G. & Harder, L. D. (2003) Reproductive uncertainty and the relative competitiveness of simultaneous hermaphroditism versus dioecy. *American Naturalist,* **162,** 220-241.

Wolkovich, E. M. & Cleland, E. E. (2011) The phenology of plant invasions: A community ecology perspective. *Frontiers in Ecology and the Environment,* **9,** 287-294.

Woodward, A., Silsbee, D. G., Schreiner, E. G. & Means, J. E. (1994) Influence of climate on radial growth and cone production in sub-alpine fir (*Abies lasiocarpa*) and mountain hemlock (*Tsuga mertensiana*). *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* **24,** 1133-1143.

Wulff, R. D. (1986) Seed size variation in Desmodium paniculatum: Factors affecting seed size. *Journal of Ecology,* **74,** 87-97.

Zinn, K. E., Tunc-Ozdemir, M. & Harper, J. F. (2010) Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany,* **61,** 1959-1968.

Zohner, C. M. & Renner, S. S. (2014) Common garden comparison of the leaf-out phenology of woody species from different native climates, combined with herbarium records, forecasts long-term change. *Ecology Letters,* **17,** 1016-1025.

Supplementary Materials

Chapter 2

Supplementary Materials

Duration, but not timing, of flowering phenology changes with elevation in an invasive shrub willow

Additional Methods

Salix identification

Salix spp. hybridize extensively making them difficult to identify in the field. In Australia, *S. cinerea* has two known subspecies: *S. cinerea ssp. cinerea* and *S. cinerea ssp. oleifolia,* and there are two common hybrids: *S. x reichardtii (S. cinerea x S. caprea)* and *S. calodendron (S. cinerea x S. caprea x S. viminalis)* (Cremer 2003). In this study we aimed to include *S. cinerea ssp. cinerea* only. We included only populations of willows with the following traits: generally <10 m tall, multi-stemmed shrub with no central trunk, reticulate, often slightly recurved, leaf shape spirally arranged with an entire margin, ribbed stem under grey-brown coloured bark (sometimes apparent in outer bark), male flowers dome-shaped about 3 cm long with bright yellow, occasionally orange pollen, and female flowers cylindrical in shape about 3-9 cm long at maturity (Holland Clift and Davies 2007).

Data collection

S. cinerea is multi-stemmed, so to avoid mistakenly double-counting a single individual, mature individuals were only included in a site if the stems of what was presumed to be one individual were located ≥ 1 m from the neighbouring individual. Low elevation sites were monitored from mid-August until late November when >90% of flowers had fallen from >90% of female individuals. High elevation sites were monitored until January, when >90% of their flowers had dropped. We chose to estimate phenophases visually, based on the amount of flowers present in each individual, because previous work suggests this is generally accurate to ±10% of shoot-count methods, and is considerably more time efficient (Maclean and Lidstone 1982).

October 2016 Flood

On October 4th, 2016 a severe flood occurred in the region of the low elevation sites. Water levels rose in the Ovens Catchment from a usual depth of 2-8 m to a maximum of 12.78 m, categorizing it as a major flood (Bureau of Meteorology 2018). Consequently, the month of October, along with the preceding winter months (May-September, 2016) were considerably wetter than average (between 15-90 mm more rainfall than average each month). The maximum monthly temperatures in 2016, however, were in line with long-term averages (www.bom.gov.au/climate/data, access date: 16-11-17). Damage to the low elevation study sites was considerable: several individuals were entirely uprooted and pulled downstream, while others were bent over. Additionally, many of the tags used to identify individuals and a temperature data logger were swept away in the river (see Phenological Monitoring section). Subsequently, only individuals that were

accessible for the duration of the study, and that could be identified with certainty were included in the data analysis (Table S2.1).

Study site climate

The climate at all study sites is temperate (sites are inland (115-165 km from the coast) and largely unaffected by oceanic and Tasman Sea temperatures). Temperatures are not cold enough for snow at low elevation, but snowfall does occur from May to September at high elevation. Average annual rainfall for all years at the closest low elevation weather station (Edi Upper, approximately 30-50 kms from low elevation sites), was 1054 mm (station open 1985-2017; latitude: 36.74° S, longitude: 146.47° E, 365 m elevation). The nearest high elevation weather station (Mount Hotham, approximately 10 kms from the high elevation site), had an annual precipitation, including rain and snowfall, of1454 mm (station open 1990-2017; latitude: 36.98° S, longitude: 147.13° E, 1849 m elevation). Coldest annual temperatures at these weather stations occurred in July with low elevation maximum temperatures reaching 11.9°C and minimum of 3.6°C, while high elevation temperatures reach a maximum of 1°C and a minimum of -3.7°C. Warmest temperatures occurred in January with a maximum of 30.2°C and minimum of 14.0°C at low elevation, and a maximum of 17.7°C and minimum of 7.8°C at high elevation (www.bom.gov.au/climate/data, access date: 16-11-17). The high elevation meteorological station is 209 m higher in altitude than the high altitude site, so actual temperatures at the Dinner Plain site are likely to be slightly warmer.

Tables

Table S2.1. Characteristics of study sites, including elevation, the number of individuals separated by sex, presence of a temperature data logger, and the number of visits to each site during each year of study. Individuals that were not observed to flower were classified as unknown.

Site	Location	Elevation (m)	Female (n)	Male (n)	Unknown sex (n)	Total (n)	Visits 2015/16	Visits 2016/17
1	Yarrabula Creek	302	10	16	2	28	8	16
2	Yarrabula Creek	292	13	11	0	24	8	15
3	Buckland River	410	14	10	1	25	7	15
4	Buckland River	384	10	11	1	22	7	14
5	Dinner Plain	1639	9	5	7	21	6	22
	Total		58	52	11	119		

Table S2.2. Location details of field site weather data compared to the SILO data drill. The SILO data drill function only supplies data to the degree minute, rounded to either 0 or 5. As such, Yarrabula Creek and Buckland River had only two SILO outputs for their four sites.

		Latitude	Longitude	Elevation (m)	Period of data available
Yarrabula Creek	Site 1	-36.75169	146.69054	302	31/8/2016-23/11/2016
	Site 2	-36.74851	146.68913	292	No logger
	SILO	-36.75	146.70	600	21/06/2016 - 21/06/2017
Buckland River	Site 3	-36.87483	146.87186	384	30/8/2016-27/9/2016
	Site 4	-36.84874	146.85474	410	No logger
	SILO	-36.85	146.85	449	21/06/2016 - 21/06/2017
Dinner Plain	Site 5	-37.02037	147.22842	1640	14-9-2016-3/1/2017
	SILO	-37.00	147.25	1559	21/06/2016 - 21/06/2017

Table S2.3. Linear regression models to compare field-collected data logger data and the SILO interpolated dataset. These values correspond to the lines plotted in Figure S2.1.

Parameter	Yarrabula		Buckland		Dinner Plain	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Intercept	2.659	0.687	-3.318	2.067	4.696	-1.313
Standard Error	0.869	0.416	3.586	1.13	0.529	0.225
Different from 0?	Yes t=3.062 p=0.002**	No t=1.653 p=0.102	No t= -0.925 p=0.363	No t=1.826 p=0.079	Yes t=8.872 p=1e-14***	Yes t= -5.842 p=5e-08***
Slope	1.080	0.808	1.306	0.592	1.013	0.852
Standard error	0.049	0.057	0.231	0.163	0.039	0.035
Different from 1?	No t=-1.618 p=0.109	Yes t=3.343 p=0.001**	No t=-1.323 p=0.197	Yes t=2.497 p=0.019*	No t=-0.329 p=0.743	Yes t=4.204 p=5e-05 ***
Multiple R^2	0.851	0.704	0.514	0.328	0.862	0.842
Sample size	85	85	29	29	112	112

Table S2.4. Generalised linear model output when GDD values were calculated using a threshold of 10 (THS=10), instead of 0.

Model	GLMM coefficient (SE)	*z*	p	Marginal R^2 (fixed factors)	Conditional R^2 (fixed factors + random effect)
Bud Break (female)					
Intercept	5.10 (0.59)	8.71	<0.001	0.89	0.91
GDD	5.82 (0.50)	11.74	<0.001		
Elevation	0.20 (0.86)	0.23	0.56		
Pollen Release					
Intercept	6.90 (0.98)	7.06	<0.001	0.97	0.98
GDD	11.99 (1.38)	8.66	<0.001		
Elevation	-9.20 (1.64)	-5.61	<0.001		
Seed Release					
Intercept	-8.53 (1.43)	-7.46	<0.001	0.97	0.98
GDD	12.19 (1.52)	8.00	<0.001		
Elevation	-8.86 (1.46)	-6.09	<0.001		

Table S2.5. 5a) Parameter estimates of generalized linear mixed models for the initial date of bud break (in females), pollen release, and seed release. Models include 2016 data only. All models were fit with binomial families with logit functions. Models with male data have a sample size of 848 observations, while female models have 878 observations. A nested random effect was included in the model for individual within site, but results are not presented in the main text because they accounted for little model variation.

5a) Growing degree days

Model	GLMM coefficient (SE)	*z*	p	Marginal R^2 (fixed factors)	Conditional R^2 (fixed factors + random effect)
Bud Break (female)					
Intercept	3.18 (0.39)	8.086	<0.001	0.90	0.91
GDD	6.71 (0.57)	11.79	<0.001		
Elevation	8.20 (0.83)	9.88	<0.001		
Pollen Release					
Intercept	4.75 (0.65)	7.36	<0.001	0.97	0.98
GDD	13.16 (1.49)	8.83	<0.001		
Elevation	15.34 (2.09)	7.35	<0.001		
Seed Release					
Intercept	-13.09 (1.73)	-7.58	<0.001	0.96	0.97
GDD	12.93 (1.58)	-7.58	<0.001		
Elevation	17.62 (2.59)	6.81	<0.001		

5b) Julian date

Model	GLMM coefficient (SE)	*z*	p	Marginal R^2 (fixed factors)	Conditional R^2 (fixed factors + random effect)
Bud Break (female)					
Intercept	4.92 (0.59)	8.29	<0.001	0.88	0.91
Julian date	5.55 (0.47)	11.73	<0.001		
Elevation	-0.14 (0.94)	-0.15	0.89		
Pollen Release					
Intercept	6.45 (0.99)	6.54	<0.001	0.96	0.97
Julian date	11.08 (1.26)	8.77	<0.001		
Elevation	-10.95 (1.91)	-5.74	<0.001		
Seed Release					
Intercept	-9.35 (1.25)	-7.51	<0.001	0.98	0.98
Julian date	14.14 (1.73)	8.20	<0.001		
Elevation	-13.85 (2.15)	-6.43	<0.001		

Table S2.6. Peak, start, and end Julian dates extracted from the OLR models. The peak date is back-calculated from the maximum probability of each phenophase occurring and its standard error was generated from all fitted value outputs generated over the 151 days considered by the model (days 50-200). The start and end dates are generated from the peak date and it's standard error to describe when 95% of individuals will have completed the phenophase. Bud break in males at high elevation, winter senescence, and dormancy are not included because we did not observe the entire phenophases, and therefore cannot accurately identify the peak dates.

	Peak date [SE]	Probability at peak date [CI]	Start date	End date
Males (low)				
Init. BB	77 [3.56]	0.74 [0.70, 0.78]	70	83
Full BB	87 [3.56]	0.13 [0.127, 0.138]	80	93
Init. PR	97 [3.56]	0.68 [0.64, 0.71]	90	103
Full PR	112 [3.56]	0.56 [0.54, 0.59]	105	119
Females (low)				
Init. BB	87	0.61 [0.58, 0.63]	80	93
Full BB	94	0.07 [0.068, 0.075]	87	101
Init. StRe	106	0.80 [0.76, 0.84]	90	104
StRe Anth.	125 [3.56]	0.70 [0.67, 0.73]	118	132
Init. SR	137 [3.56]	0.32 [0.30, 0.33]	130	144
Full SR	144 [3.56]	0.37 [0.35, 0.38]	137	151
Males (high)				
Full BB	107	0.85 [0.81, 0.89]	100	114
Init. PR	125	0.53 [0.51, 0.56]	118	132
Full PR	141	0.79 [0.75, 0.83]	134	148
Females (high)				
Init. BB	104	0.995 [0.93, 1]	97	111
Full BB	130	0.30 [0.29, 0.31]	123	137
Init. StRe	141	0.83 [0.79, 0.87]	134	148

StRe Anth.	159	0.83 [0.79, 0.87]	152	166
Init. SR	174	0.58 [0.56, 0.61]	167	181
Full SR	181	0.25 [0.24, 0.26]	174	188

Table S2.7. The average number of days since winter solstice (June 21, 2016) that each phenophase began and ended in *S. cinerea* for 2015-2016, as well as the average duration of reproductive events (in days). Standard errors are in parentheses beside the average number of days and stage durations.

Site	n	Mean start	Mean end	Duration (mean start-end)
Bud Break-male				
1 (low)	17	74.29 (1.98)	90.35 (2.77)	16.06
2 (low)	11	75.09 (2.59)	91.36 (1.14)	16.27
3 (low)	14	67.93 (2.72)	85.07 (1.72)	17.14
4 (low)	18	66.22 (1.44)	82.17 (0.95)	15.94
5 (high)	4	59	117.25 (3.25)	58.25
BBCH stages: Initial generative bud break (54) and full generative bud break (55)				
Bud Break-female				
1 (low)	9	86.56 (1.56)	92.78 (0.78)	6.22
2 (low)	10	87.50 (4.28)	99.50 (4.64)	12.00
3 (low)	13	74.08 (2.70)	87.38 (3.66)	13.31
4 (low)	16	74.31 (2.21)	86.75 (0.78)	12.44
5 (high)	9	83.67 (1.72)	127.89 (0.89)	44.22
BBCH stages: Initial generative bud break (54) and full generative bud break (55)				
Pollen Release				
1 (low)	17	92.94 (1.97)	114.59 (2.25)	21.65
2 (low)	11	98.82 (1.64)	119.00 (1.63)	19.18
3 (low)	15	92.27 (1.78)	114.67 (2.41)	22.40
4 (low)	18	87.72 (1.15)	106.39 (2.51)	18.67
5 (high)	4	125 (3.25)	149 (0)	23.75
BBCH stages: Initial pollen release (56) and anthesis (65)				

Seed Release				
1 (low)	11	132.45 (2.44)	145.18 (2.89)	12.73
2 (low)	12	135.58 (2.35)	142.67 (2.34)	7.08
3 (low)	15	132.40 (2.37)	143.80 (1.52)	11.40
4 (low)	9	126.11 (2.90)	137.00 (1.94)	10.89
5 (high)	9	168.67 (2.78)	183.67 (4.22)	15.00
BBCH stages: Initial seed release (56) and full seed release (65)				
Foliation				
1 (low)	26	111.52 (1.71)	329.96 (1.66)	218.45
2 (low)	24	114.30 (1.56)	328.57 (1.83)	215.28
3 (low)	24	111.47 (1.52)	328.74 (1.70)	217.27
4 (low)	21	105.47 (2.12)	321.42 (2.15)	215.95
5 (high)	14	138.9 (2.67)	304.6 (3.50)	165.7
BBCH stages: Leaf expansion (11) and dormancy (97)				

Table S2.8. a) Two-way ANOVA showing the difference in the duration (minimum number of days) of each phenophase across high and low elevation. b) One-way ANOVAs for each phenophase, comparing the duration at low vs. high elevation.

a)

Source of Variation	df	Mean sq.	F-value	p-value
Elevation	1	6179	66.39	**2.04e-14**
Phenophase	3	1416	15.22	**4.25e-09**
Elev:Phase	3	2028	21.79	**1.62e-12**
Residuals	240	93		

b)

Phenophase	Source of Variation	df	Mean sq.	F-value	p-value
BBm	Elevation	1	6594	103.80	**7.19e-15**
	Residuals	62	64		
BBf	Elevation	1	6667	63.20	**9.34e-11**
	Residuals	56	105		
PR	Elevation	1	7.32	0.06	0.80
	Residuals	65	117.29		
SR	Elevation	1	172.81	2.02	0.16
	Residuals	57	85.42		

Table S2.9. One-way ANOVA showing the difference in the duration of the growing season length (number of days) between high and low elevation. This includes data from both field seasons (2015 and 2016).

Source of Variation	df	Mean sq.	F-value	p-value
Elevation	1	42067	267.15	**< 2.2e-16**
Residuals	164	157		

Table S2.10. Variance explained by individual and site random effects for generalized linear mixed effects models explaining proportion of individuals in a given phenophase depending on growing degree days and elevation.

Phenophase	Model	Random effect variable	Variance	Standard error
Julian date	Bud break (female)	Site	1.53e-01	0.39
		Tree	2.68e-05	0.005
	Pollen Release	Site	6.99e-01	0.84
		Tree	5.69e-05	0.008
	Stigma Receptivity	Site	2.10e+00	1.45
		Tree	4.55e-06	0.002
	Seed Release	Site	1.25e+00	1.12
		Tree	3.43e-06	0.002
GDD	Bud break (female)	Site	5.81e-02	0.24
		Tree	2.68e-05	0.005
	Pollen Release	Site	3.79e-01	0.62
		Tree	5.33e-05	0.007
	Stigma receptivity	Site	8.46e-01	0.92
		Tree	1.63e-05	0.004
	Seed Release	Site	1.39e+00	1.18
		Tree	7.05e-06	0.003

Table S2.11. Coefficient tables (a-d) of ordinal logistic regressions for the probability of an individual exhibiting in a particular phenophase on a given date, separated by elevation and sex. Phenophase stage number can be interpreted in the main text, Table 2.2. The interpretation of the coefficients and intercepts can be described as such (for males at low elevation): as the date increases by one unit (day), the odds of observing one phenophase, compared to all other phenophases, decreases by 18% (exp(-(0.2))=0.82). Similarly, the odds of a willow being in phenophase 56 vs. any subsequent categories decreases by 42% each day (exp(17.24-17.78)=0.58).

McFadden's Pseudo R-Squared is reported for each of the four models compared to their intercept-only null model, followed by their degrees of freedom. McFadden's Pseudo R-Squared is based on the ratio of the deviance of the full model with the deviance of the null model. This metric ranges from 0-1, with a score of 1 indicating perfect predictive ability of the model. This measure of R-squared tends to have considerably lower values than basic R-squared measures, and as such all of these models appear to have outstanding fit relative to the null model (values of Pseudo R-squared=0.2-0.4 represent excellent fit (McFadden 1979)).

11a) Males at low elevation

	Stage	Value	Std. Error	T value	Residual Deviance	AIC	Psuedo-R^2
Coefficient	Day	0.20	0.01	20.56	954.66	966.66	0.61
Intercepts	53|54	13.43	0.69	19.41			
	54|55	17.24	0.85	20.33			
	55|56	17.78	0.86	20.56			
	56|65	21.07	1.03	20.52			
	65|69	23.65	1.17	20.22			

11b) Males at high elevation

Stage	Stage	Value	Std. Error	T value	Residual Deviance	AIC	Psuedo-R^2
Coefficient	Day	0.20	0.04	5.78	70.34	82.34	0.72
Intercepts	53|54	2.93	0.02	246.02			
	54|55	19.86	3.47	5.73			
	55|56	24.93	4.47	5.57			
	56|65	27.32	4.78	5.71			
	65|69	31.62	5.54	5.71			

11c) Females at low elevation

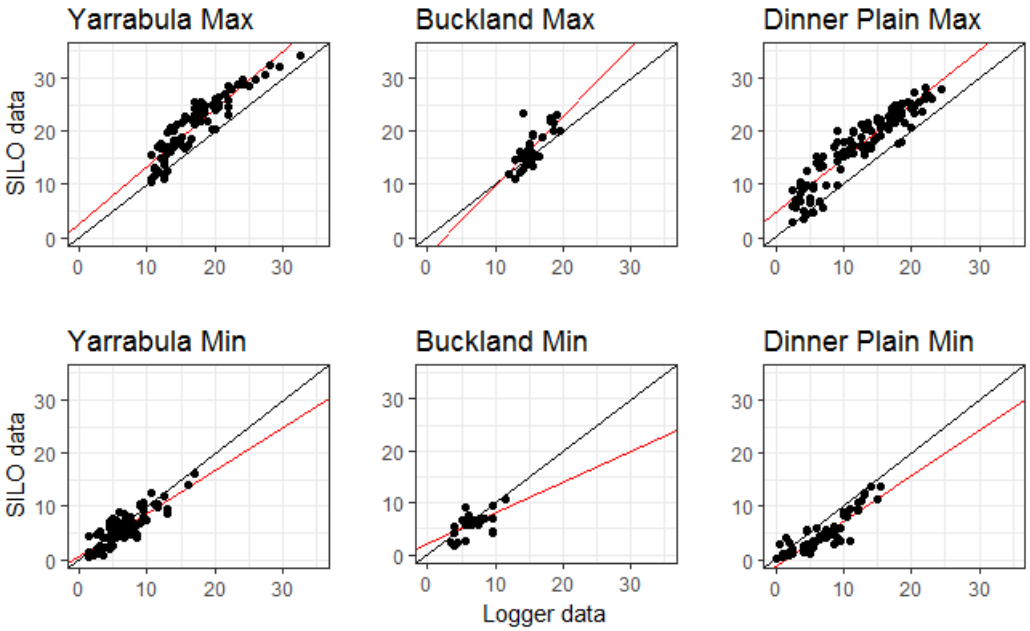
	Stage	Value	Std. Error	T value	Residual Deviance	AIC	Psuedo-R^2
Coefficient	Day	0.20	0.01	21.29	1225.53	1241.53	0.56
Intercepts	53|54	16.31	0.79	20.58			
	54|55	19.14	0.89	21.50			
	55|61	19.43	0.90	21.55			
	61|65	23.78	1.16	20.46			
	65|71	27.26	1.30	20.99			
	70|71	28.57	1.35	21.11			
	71|75	30.10	1.40	21.46			

11d) Females at high elevation

	Stage	Value	Std. Error	T value	Residual Deviance	AIC	Psuedo-R^2
Coefficient	Day	0.26	0.03	9.98	245.35	261.35	0.67
Intercepts	53|54	21.19	2.19	9.70			
	54|55	33.26	3.37	9.87			
	55|61	34.49	3.49	9.89			
	61|65	39.24	3.98	9.85			
	65|71	43.98	4.42	9.94			
	70|71	46.65	4.64	10.06			
	71|75	47.68	4.68	10.19			

Figures

Figure S2.1. Comparison of the temperature data collected from low elevation sites and the high elevation site with the in-field data loggers and the SILO data-drilled weather data. Sites 1 and 2 are found on the Yarrabula Creek, 3 and 4 on the Buckland River, and site 5 at Dinner Plain. Each point indicates one day's maximum or minimum temperature (slopes and intercepts can be found in Table S2.3). The red line shows the best-fit linear regression line for the data. The black line is for reference: x=y. Number of days sampled varies between the sites due to different logger implementation times and flood damage causing early removal of loggers. Number of days sampled at Yarrabula Creek: n=85, Buckland river: n=29, and Dinner Plain: n=112.



Chapter 3

Supplementary Materials

New methods reveal incompatibility of flowering phenology across elevation causes lowered seed output in an invasive, dioecious shrub

Additional methods

Description and effects of flood

On October 4th, 2016 an unusual flooding event occurred in the Ovens Catchment region of north-eastern Victoria, Australia. An additional 10 m of water was estimated flowing through the catchment, and stream-flows were in the 95th percentile from recent history. This flood resulted in a particularly wet month in October, 2016 compared to the previous 30 year average, though September and November 2016 were drier than the average. The temperature in 2016 remained roughly average during the course of the study. However, damage to the low elevation sites, particularly the two Buckland River sites (sites 3 and 4), was considerable: several individuals were entirely uprooted and pulled downstream and many were shifted from standing vertically to an approximately 45° angle. Additionally, many metal identification tags were swept away in the river. Sample sizes recorded for this study include only individuals that were present for the duration of the study and could be identified with certainty. The flood lowered our estimates of seed output in the low elevation sites, and as such the difference between high and low elevation is actually likely more pronounced in a normal spring season than we documented here.

Climate comparison

We aimed to address whether any variation found in phenological overlap identified between low and high elevation can be understood by comparing climatic conditions to those prevalent where the species evolved. We might expect that similar climatic conditions would produce similar degrees of overlap between pollen release and stigma receptivity. Specifically, high elevation Australian populations are expected to experience similar climatic conditions to the native European populations, and therefore have similar levels of phenological synchrony. Alternatively, low elevation Australian populations likely experience significantly warmer and drier climates, and may have shorter periods of phenological overlap, due to the sub-optimal climatic conditions. Specifically, we aimed to determine whether the climatic condition of the native range is more similar to *S. cinerea's* low or high elevation Australian distributions.

To investigate the overlap between the environmental conditions in *S. cinerea*'s Australian and European ranges, we implemented a climate niche overlap analysis as outlined by Broennimann et al. (2012) and based on the statistical tests developed by Warren et al. (2008). This analysis compares the climatic range between the native

European range and invasive Australian range based on the realised niche, and was created using presence-only modelling. Species presence data was collected from the Global Biodiversity Information Facility (GBIF), specifying *Salix cinerea subsp. cinerea* and returning 19,623 observations in 11 countries (GBIF.org, 25/10/18) (Figure S3.1). European countries (Belgium, Czech Republic, Denmark, Germany, Ireland, Isle of Man, Netherlands, Sweden, and the United Kingdom) were included to represent the native range of *S. cinerea* and were compared to the Australian data. In total, after the removal of individuals that were <0.05° from each other, there were 1712 occurrences from Europe and 41 occurrences from Australia. Climate data was collected from WorldClim at a resolution of 0.033° (about 4.8 kilometres), including 19 temperature and precipitation variables (Table S3.3) (Fick and Hijmans, 2017). For the four low elevation sites, WorldClim variables were extracted for one site from each river because the WorldClim resolution is low enough that both sites on each river fall within the same climate grid cell. The WorldClim data for the high elevation Dinner Plain site fell into a separate grid cell from the low elevation sites, thus its climate could be compared to low elevation.

To measure niche overlap, we used the Schoener (1970) D metric, which ranges from 0 (niche models have no overlap) to 1 (niche models are identical). We also calculated niche equivalency metrics to determine how similar the climatic niches are between Australia and Europe, and niche similarity metrics to determine if one region's niche model can predict the occurrences of the second region better than expected by chance (Broennimann et al., 2012). These metrics were derived from a PCA calibrated using the WorldClim variables associated with the occurrences of *S. cinerea* in its native (GBIF.org, 25/10/18) and invaded (field study site data) ranges. Models were calibrated using the occurrence data only (Figure S3.2), and then again on all the pixels of the native and invaded areas (Figure S3.3). Subsequent niche overlap figures were represented by the first two axes of the PCA. Simulated niche overlap, from which niche similarity and overlap are calculated, was iterated 1,000 times. These analyses were completed in R, using the packages dismo (version 1.1-4), rgdal (version 1.2-18), and ecospat (version 3.0).

For visualisation of variation between low elevation Australia, high elevation Australia, and the native European range of *S. cinerea*, we considered the WorldClim variables for mean annual temperature, maximum temperature in the warmest quarter, minimum temperature in the coldest quarter. These variables were chosen to represent average conditions, as well as average extreme conditions in each of the regions, since these conditions are likely to be highly influential to the locations where the individuals

grow. We focus on temperature here, rather than rainfall, because previous research has found that surrounding temperature is highly influential to the duration and hence timing of *S. cinerea*'s reproductive phenology (Chapter 2). To compare the climate in the Australian range with the climate in *S. cinerea*'s native range, we used the combined 19, 623 GBIF location records with the WorldClim data to extract the average, median, maximum, and minimum values for the following variables: mean annual temperature, maximum temperature in the warmest quarter, and minimum temperature in the coldest quarter.

The climatic niche overlap between *S. cinerea*'s native and invaded ranges was minimal in both the PCA completed with just occurrence data (D=0.06) and in the model with all of the pixels from the study area (D=0.043). The home range appeared to cover a broader climatic niche (Figure S3.4). However, their similarity was not significantly different for both the prediction of Europe to Australia and Australia to Europe (Figure S3.2, S3.3).

The average mean annual temperature in *S. cinerea*'s native European range was 8.01°C, while the mean annual temperature at Dinner Plain was 7.8°C, and is 10.4°C and 10.5°C at the low elevation waterways, Yarrabula Creek and Buckland River. The maximum mean annual temperature experienced in the native European range is 10.5°C. Low elevation Australia sites have mean annual temperatures and maximum temperatures in their warmest months that are right on the edge of the temperature range that *S. cinerea* is found in in its native European range. For both Yarrabula Creek and Buckland River, the maximum temperature in the warmest month was 25.1°C, while the absolute maximum temperature in the warmest month in the European range was 25.3°C. Dinner Plain's maximum temperature in the warmest month was 21.4°C, while the average maximum temperature of the warmest month in the European range was 21.66°C. The minimum temperature in the coldest month was -0.4°C for Yarrabula Creek, -0.3°C for Buckland River, -2.4°C for Dinner Plain, and -16.2°C for the European range (Table S3.4).

In comparing the native European and invasive Australian environmental niches we have shown that Australian climatic conditions are generally dissimilar to the European niche. Low elevation *S. cinerea* populations appear to be on the edge of the expected climatic envelope of the native populations, while high elevation *S. cinerea* populations are more representative of average conditions in the native range. Overall, it appears that approximately 20% of the Australian occurrence data have an overlapping climatic niche with the European range. As such, the majority of the Australian *S. cinerea* occurrences exist outside of the climatic niche experienced by the native populations. This is not an unusual result: previous research comparing the climatic distributions of 51 invasive plant species found that 22 species also had the majority of their climatic distributions outside of

their native climatic ranges (Early and Sax, 2014). However, the phenological overlap and seed output increase at low elevation Australia suggests that those warmer conditions actually enhance *S. cinerea* seed production compared to cooler native and high elevation Australian conditions. We speculate that this is because the current native range of *S. cinerea* in northern Europe may not be representative of the ideal conditions for growth of the species. In the native range of *S. cinerea* there are dozens of other *Salix* species which may be outcompeting *S. cinerea* for warm, lowland habitat at lower latitudes (Isebrands and Richardson, 2014). Because *S. cinerea* is fairly resilient to a variety of climatic conditions, but may not be strongly competitive against other members of the genus, the native distribution may have previously shifted up in elevation and latitude into cooler climates. As such, it is possible *S. cinerea* may be poorly adapted to its high-latitude native European range, which might explain the low phenological overlap and seed output at cool, high elevation Australian as well. Alternatively, variation in climate across elevation may have resulted in a growth-survival trade-off between the low and high elevation populations, where high elevation individuals have adapted to have higher survival and low elevation populations have adapted to have higher productivity. This is consistent with previous research which suggest that populations of *Pinus contorta* and *Pinus monticola* have evolved to allocate more resources towards survival in extreme (frost and drought) conditions than in comfortable conditions (Chuine et al., 2006).

Table S3.1. Phenophases included to create ordinal logistic regression models, and which categorical stages were included in each phenophase. Stages refer to those listed in Chapter 2 Table 2.1.

Phenophase	Abbreviation	Stages included
Bud break	BB	54, 55
Pollen release	PR	56, 65
Stigma receptivity	StRe	61, 65, 70
Seed release	SR	71
Senescence	Sen	69, 75

Table S3.2. Ordinal logistic regression coefficient outputs. Coefficient tables (a-b) of ordered logistic regressions for the probability of an individual exhibiting in a particular phenophase on a given date, separated by elevation and sex. McFadden's Pseudo R^2 is reported for each of the four models compared to their intercept-only null model, followed by their degrees of freedom. McFadden's Pseudo R^2 is based on the ratio of the deviance of the full model with the deviance of the null model. This metric ranges from 0-1, with a score of 1 indicating perfect predictive ability of the model, and a score of 0.4-0.6 indicates a good model fit (McFadden, 1979). Intercept values can be interpreted independently, where the odds of an individual appearing in any phenophase prior to that of interest is the natural log of the intercept. As the date increases by one, the odds of moving from one phenophase into any of the other phenophases increases by 0.13 (the date coefficient value).

a) Low elevation

	Stage	Value	Std. Error	t value	Residual Deviance	AIC	Psuedo-R^2
Coefficient	Date	0.13	0.005	27.02	1858.29	1868.29	0.46
Intercepts	54|56	11.66	0.46	25.61			
	56|70	13.71	0.52	26.41			
	70|71	16.56	0.63	26.48			
	71|75	17.02	0.63	26.85			

b) High elevation

	Stage	Value	Std. Error	t value	Residual Deviance	AIC	Psuedo-R^2
Coefficient	Date	0.14	0.01	10.44	318.44	328.44	0.54
Intercepts	54|56	18.11	1.79	10.12			
	56|70	19.64	1.91	10.28			
	70|71	24.36	2.33	10.45			
	71|75	24.76	2.34	10.57			

Table S3.3. Description of BioCLIM variables used in climatic niche analysis. Daily values were interpolated at 1 km resolution by Fick and Hijmans (2017).

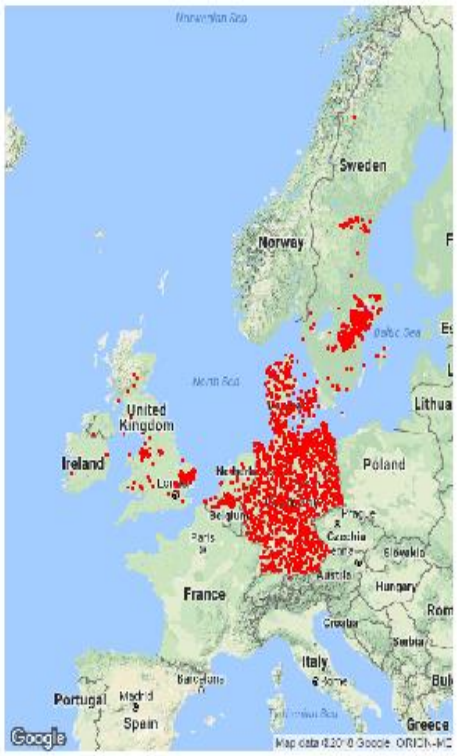
Code	Variable
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (*100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Table S3.4. Climate summary statistics for Australian field sites and 19,517 GBIF *S. cinerea* records from the native European range (GBIF.org, 25/10/18). Low elevation sites on Australian waterways have been combined to one raster grid cell for each waterway: Buckland River and Yarrabula Creek; high elevation site (Dinner Plain) is represented independently. For each of the three climate variables included (mean annual temperature, maximum temperature in warmest quarter, and minimum temperature in coldest quarter) the overall maximum, minimum, mean, and median are listed from the three Australian grid cells and the 19,517 European grid cells. This method aims to show how the Australian field sites compare to normal and extreme native European climate conditions. Climate data for all statistics were extracted from WorldClim (Fick and Hijmans, 2017).

Temp. source	Region	Mean Annual Temperature	Max. Temp. Warmest Month	Min. Temp. Coldest Month
Mean	Euro	8.01	21.66	-3.54
Median	Euro	8.2	21.7	-3.2
Max	Euro	10.5	25.3	3.2
Min	Euro	-0.9	14	-16.2
low-Yarra	Aus	10.4	25.1	-0.4
low-Buck	Aus	10.5	25.1	-0.3
high-DP	Aus	7.8	21.4	-2.4

Figure S3.1. GBIF data included in the models overlain on Google Maps images. Includes a) European data and b) Australian data for *S. cinerea subsp. cinerea*.

a)



b)

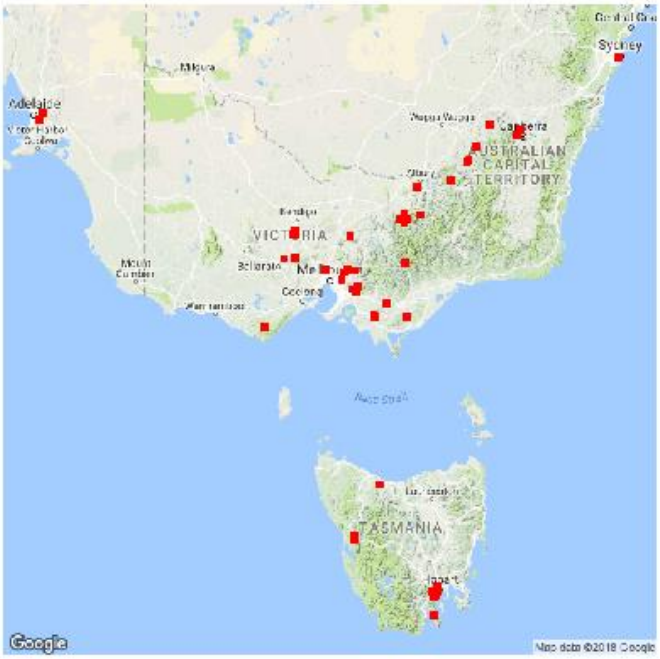


Figure S3.2. Measures of niche overlap between the European and Australian regions calibrated on **occurrence data only**. The top left and top right panels represent the niche of the species along the two first axes of the PCA in their home (EU) and away (AUS) ranges. Grey shading shows the density of the occurrences of the species by cell. The solid and dashed contour lines illustrate, respectively, 100% and 50% of the available (background) environment. (c) The contribution of the climatic variables on the two axes of the PCA and the percentage of inertia explained by the two axes. Histograms in the bottom right panel show the observed niche overlap D between the two ranges (bars with a diamond) and simulated niche overlaps (grey bars) on which tests of niche equivalency (bottom righ-top), niche similarity of AUS to EU (bottom right-bottom left), and niche similarity of EU to AUS (bottom right-bottom right) are calculated from 1000 iterations.

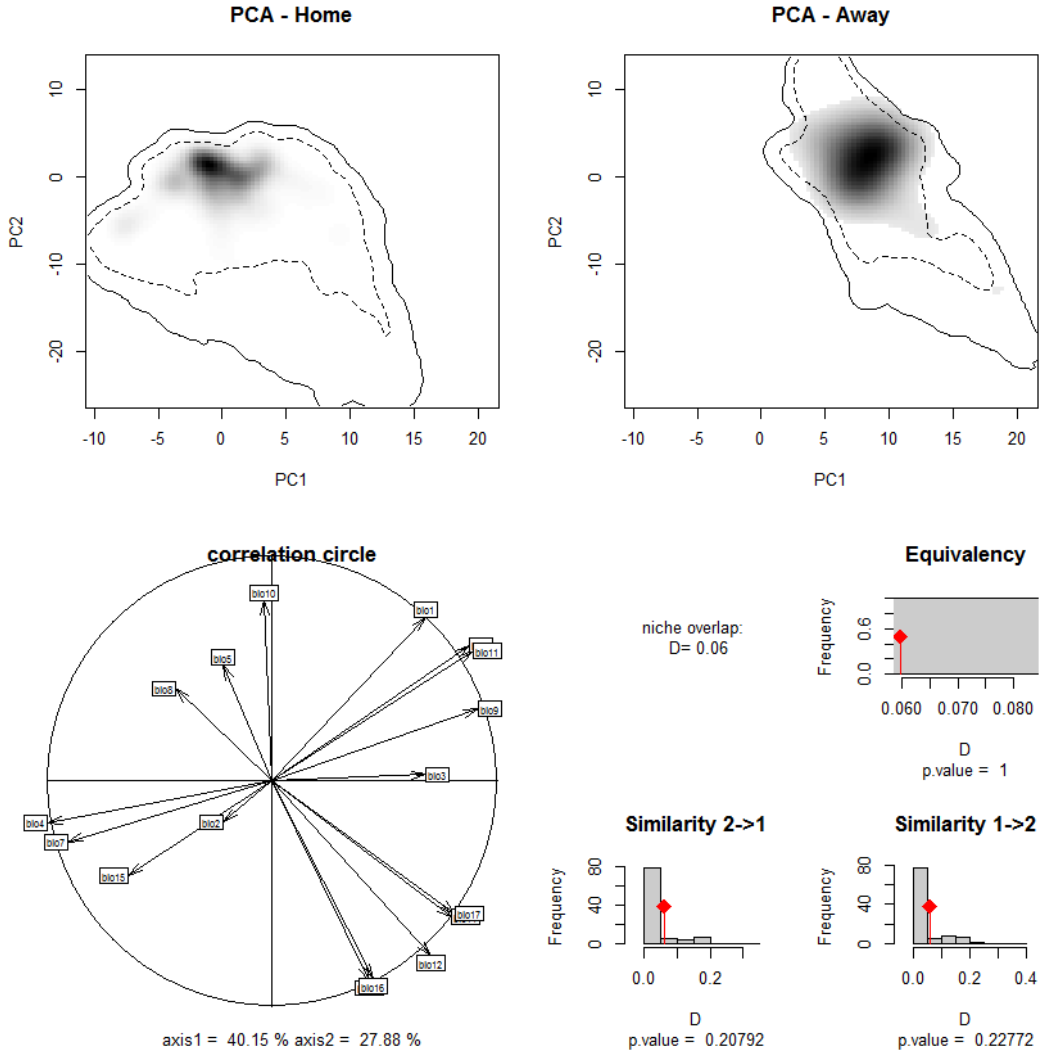


Figure S3.3. Measures niche overlap between the European and Australian regions calibrated on **all the pixels of the study area**. The top left and top right panels represent the niche of the species along the two first axes of the PCA for *S. cinerea* in its home (European) and away (Australian) range. Grey shading shows the density of the occurrences of the species by cell. The solid and dashed contour lines illustrate, respectively, 100% and 50% of the available (background) environment. The bottom left figure shows the contribution of the climatic variables on the two axes of the PCA and the percentage of inertia explained by the two axes. Histograms in the bottom right panel show the observed niche overlap D between the two ranges (bars with a diamond) and simulated niche overlaps (grey bars) on which tests of niche equivalency (bottom righ-top), niche similarity of AUS to EU (bottom right-bottom left), and niche similarity of EU to AUS (bottom right-bottom right) are calculated from 100 iterations.

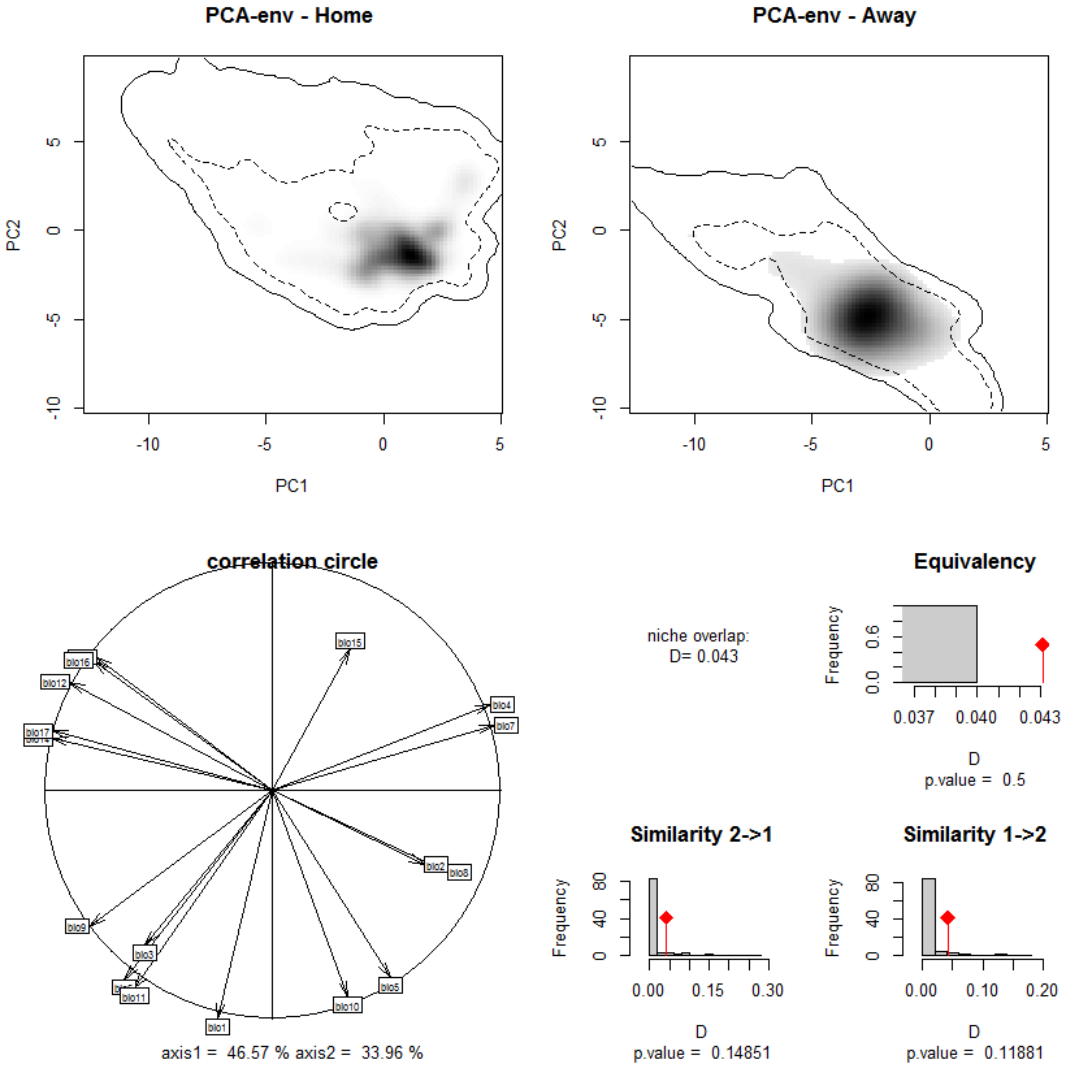
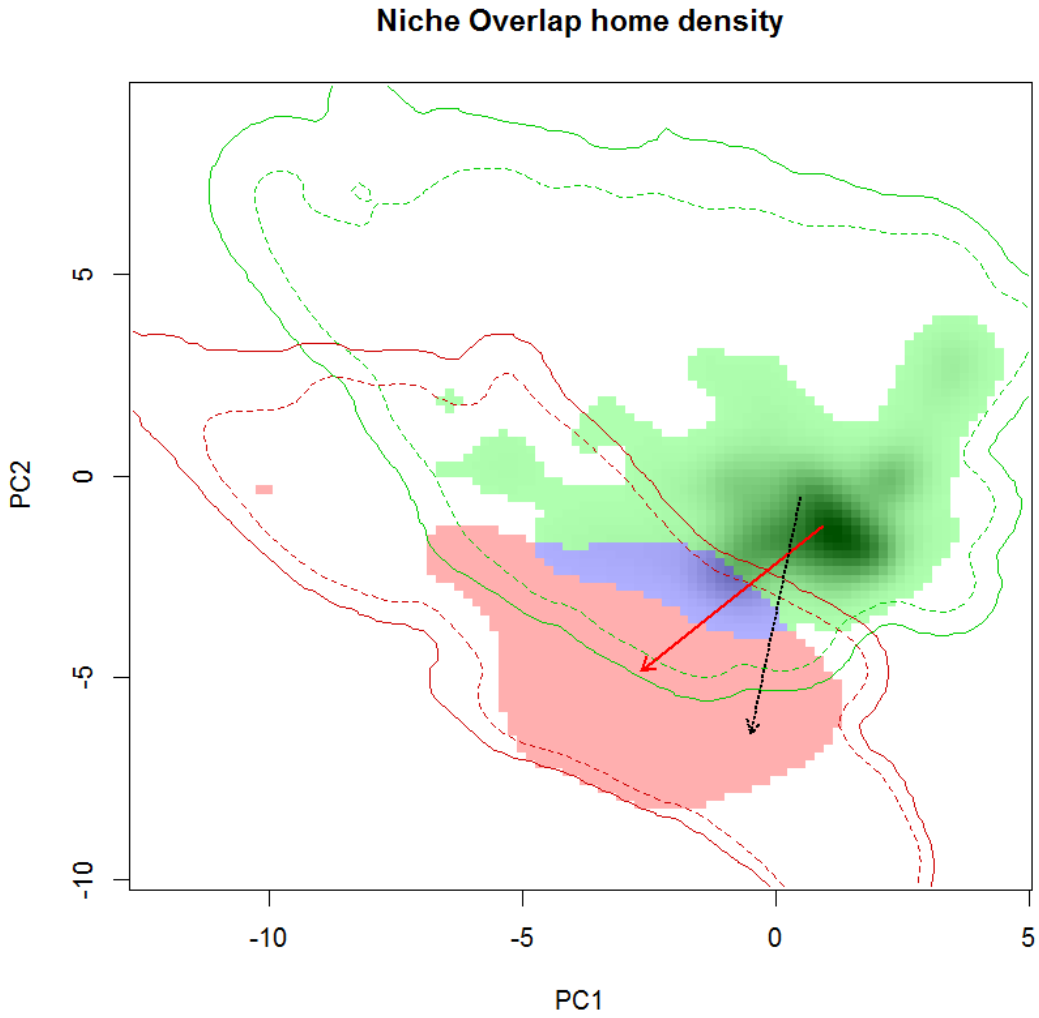


Figure S3.4. Niche overlap dynamics between the European and Australian regions are calibrated on all the pixels of the study area. These figures represent the niche of the species along the first two axes of the PCA. Green represents home (EU) range, while red represents away (AUS) range. The purple section shows the overlap of occurrences when accounting for environmental variation between the home and away ranges. Black shading shows the density of the occurrences of the species by cell in the home range. Contour lines show the climate space available in each region: solid lines, all available climate space; dashed lines, 75% of available climate space. Arrows represent how the centre of the niche has changed between Europe and Australia. The red arrow describes the center change when European data is overlain on Australian data and vice versa for the black arrow.



Chapter 4

Supplementary Materials

Effects of elevation and seed size on the competition-colonization trade off in a small-seeded invasive species

Table S4.1. Generalised linear mixed effects model to better understand the effects of seed size, CT cabinet germination temperatures, and maturation location (low or high elevation) on seed germinability. The marginal R^2, concerned only with the fixed effects in the model, equals 0.07. The intercept group for the model includes large seeds in the 16°C cabinet, matured at low elevation. There were 90 samples included in this model, representing the 90 petri plates of the germination study. Bolded *p-values* are significant when α=0.1. The observation-level random effect was included for each of the 90 samples to reduce overdispersion of the model (Harrison, 2014).

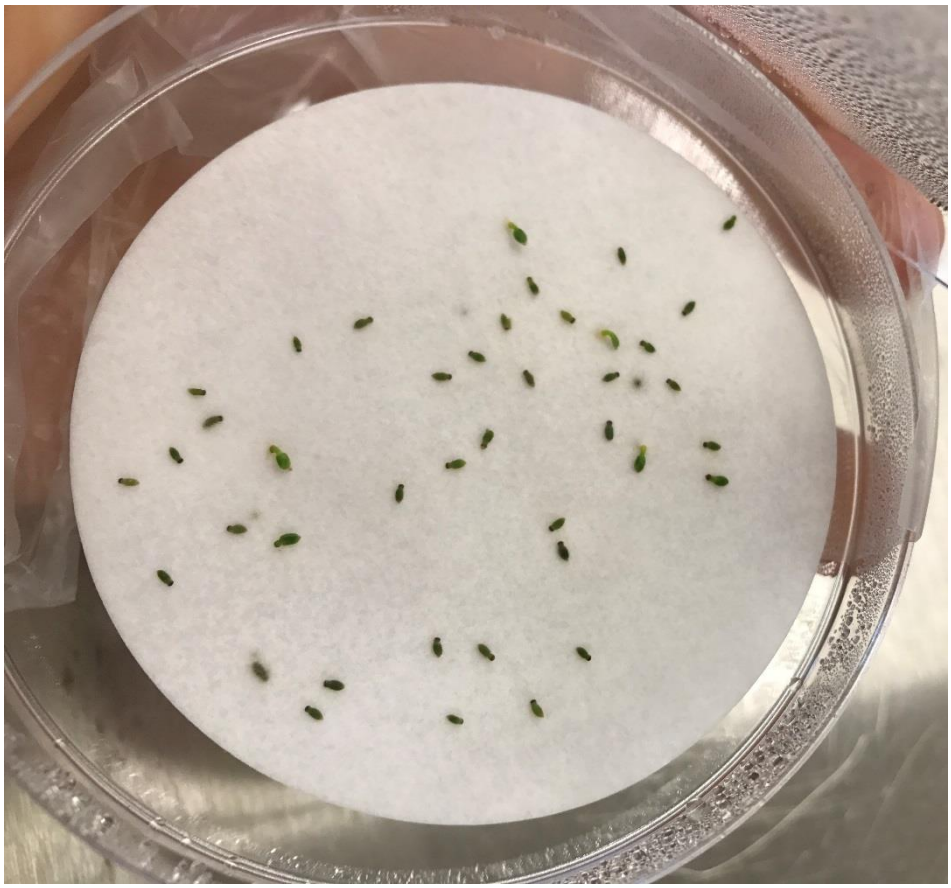
Fixed Effect Variables	Estimate	Std. Error	z-value	*p-value*
Intercept	-0.54	0.29	-1.88	**0.06**
Medium seeds	0.05	0.44	0.12	0.91
Small seeds	0.46	0.49	0.93	0.35
Cabinet 22°C	0.28	0.41	0.69	0.49
High elevation	0.42	0.53	0.80	0.43
Med. seeds: Cabinet 22°C	-0.39	0.63	-0.62	0.54
Small seeds: Cabinet 22°C	-0.37	0.70	-0.53	0.59
Med. seeds: High elev.	0.68	0.89	0.76	0.44
Small seeds: High elev.	-2.17	1.26	-1.7	**0.09**
Cabinet 22°C: High elev.	0.81	0.76	1.07	0.28
Med. seeds: Cabinet 22°C: High elev.	-0.45	1.27	-0.35	0.72
Small seeds: Cabinet 22°C: High elev.	1.15	1.65	0.70	0.49
Random effect variable	Var.	Std. Dev.		
Observation	1.02	1.01		

Table S4.2. Seed dispersal syndrome definitions (Liu, 2014).

Dispersal syndrome	Definition	Common plant genera
Anemochory	Seed dispersal by wind.	*Salix* (Salicaceae)- Willow *Taraxacum* (Asteraceae)- Dandelion *Eragrostis* (Poacae)
Barochory	Seed dispersal by gravity.	*Amaranthus* (Amaranthaceae)- Amaranth *Minuartia* (Asteraceae)
Endozoochory	Seed dispersal by animal ingestion and defecation.	*Musa* (Musaceae)- Banana
Epizoochory	Accidental seed dispersal by attachment to animals.	*Euclidium* (Brasscicaceae)
Hydrochory	Seed dispersal by water.	*Nymphaea* (Nymphaeaceae)- Water lilies *Rhizophora* (Rhizophoraceae)- Mangroves
Myrmechory	Seed dispersal by ants.	*Cecropia* (Urticaceae)

Figure S4.1. Photographs of two Petri dishes with seeds. Seed counts were completed using ImageJ software. Raw photographs were taken (a), then cropped in ImageJ to avoid counting non-seed objects as seeds (b). The software then highlighted and counted the seeds within the new set range.

a)



b)

Chapter 5

Supplementary Materials

Variation in fecundity across geographic and climatic gradients in the willow (*Salix*) genus

Figure S5.1. Phylogenetic tree of study genus (Lauron-Moreau et al., 2015). The *Salix* sub-genus is the tree willow group (9 species), and *Vetrix* is the shrub willow sub-genus (8 species).

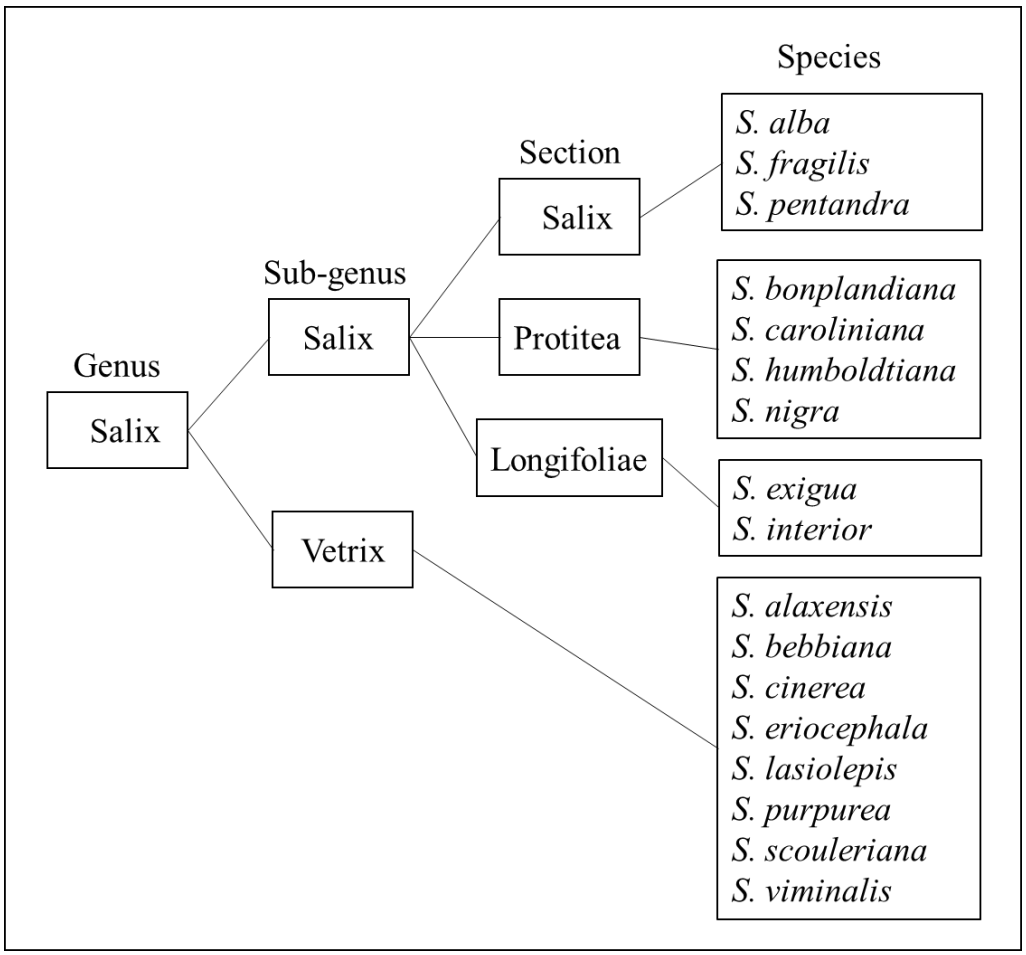


Figure S5.2. A linear regression model was completed to identify a significant positive correlation between catkin length and capsules per catkin (t=6.16, df=15, p<0.001). The correlation coefficient between the two variables was also strong (r=0.85). These data were collected from invasive *S. cinerea* individuals in the spring of 2016, from four low elevation populations (230-410m). Each point in the plot represents a single catkin (n=17).

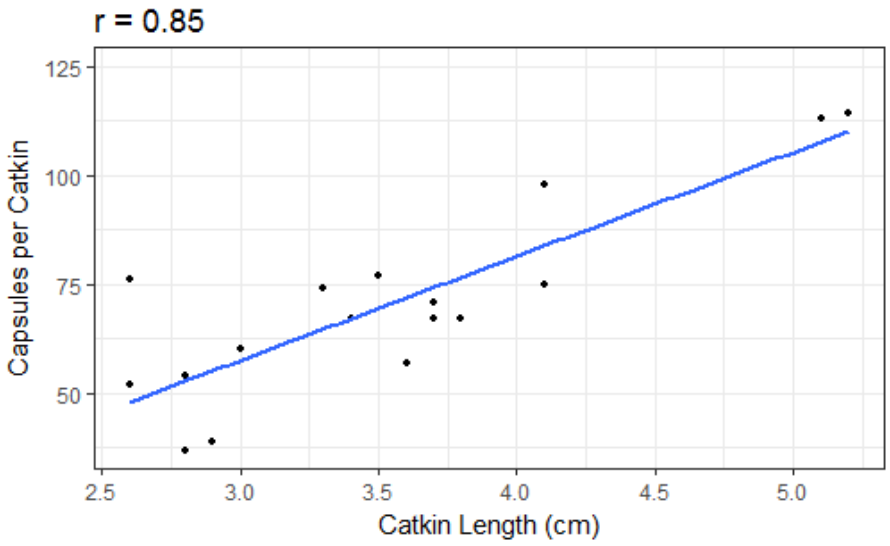


Figure S5.3. Average catkin length (left panel) for low elevation populations was approximately 4.5 cm, while low elevation catkins were only 1.5 cm long. Additionally, there were on average approximately twice as many catkins produced per crown at low elevation compared with high elevation (right panel). These data were collected from invasive *S. cinerea* individuals in the spring of 2016, from four low elevation populations (230-410m) and one high elevation population (1640m).

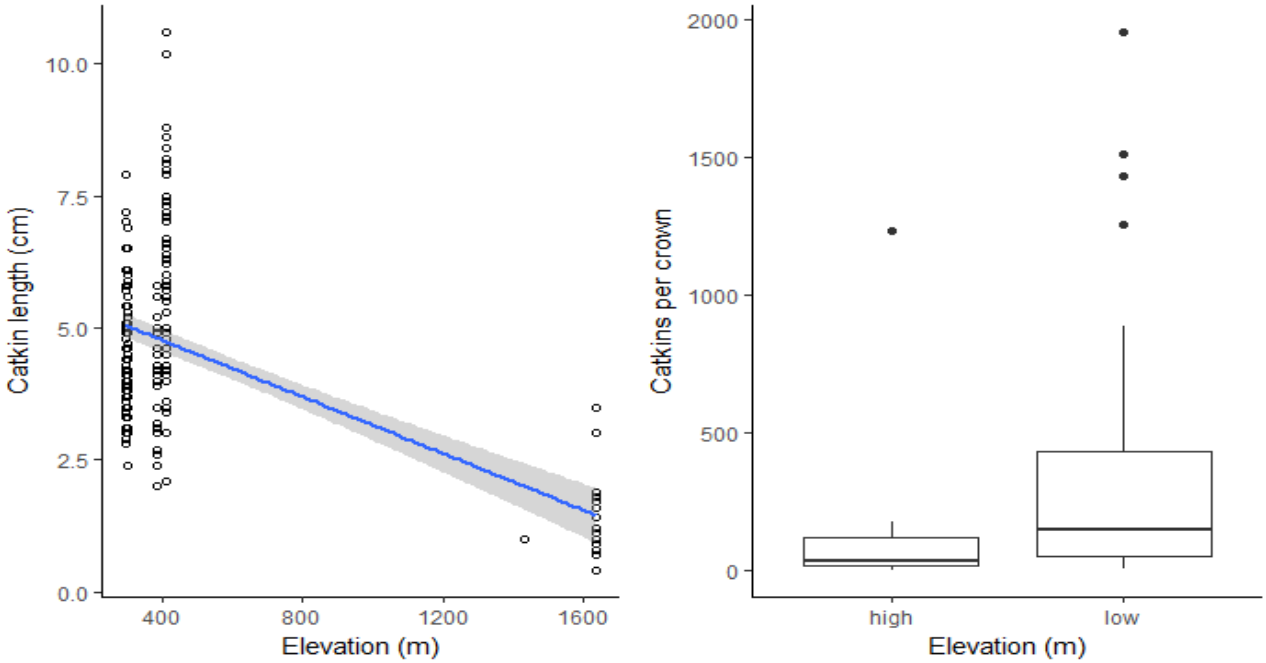


Table S5.1: Species list of 17 *Salix spp.* used in study, collected from 12 herbaria on three continents. Subgenus was distinguished according to the phylogeny described by Lauron-Moreau et al. (2015). Species that do not have established populations outside of their native range have an N/A listed under their introduced range. The section of the subgenus is listed in parentheses after the subgenus. Bolded species are those which were included in the home/away range analysis.

Species	Native range	Introduced range	Subgenus	Home range (n)	Away range (n)	Total samples
S. alaxensis	N. America	Europe	*Vetrix*	39	0	39
S. alba	Europe	Australia, N. America	*Salix (Salix)*	39	50	89
S. bebbiana	N. America	Europe	*Vetrix*	44	0	44
S. bonplandiana	N. America	N/A	*Salix (Protitea)*	39	0	39
S. caroliniana	N. America	N/A	*Salix (Protitea)*	30	0	30
S. cinerea	Europe	Australia, N. America	*Vetrix*	46	71	117
S. eriocephala	N. America	Europe	*Vetrix*	33	0	33
S. exigua	N. America	Australia	*Salix (Longifoliae)*	31	0	31
S. fragilis	Europe	Australia, N. America	*Salix (Salix)*	8	27	35
S. humboldtiana	N. and S. America	Australia	*Salix (Protitea)*	31	2	31
S. interior	N. America	Europe	*Salix (Longifoliae)*	29	0	29
S. lasiolepis	N. America	N/A	*Vetrix*	34	0	34
S. nigra	N. America	Australia	*Salix (Protitea)*	83	0	83
S. pentandra	Europe	N. America	*Salix (Salix)*	46	6	52
S. purpurea	Europe	Australia, N. America	*Vetrix*	33	15	48
S. scouleriana	N. America	N/A	*Vetrix*	30	0	30
S. viminalis	Europe	Australia, N. America	*Vetrix*	35	20	55
			Total	630	191	819

Table S5.2. Mixed effects model output for climatic and geographic variables on the catkin length at the genus-level, including a random intercept variable for species. All geographic and climatic variables were centered and scaled for analysis. Bolded p-values are those which are statistically significant when α=0.05. Table a) show the fixed effects and b) shows the species random intercept effect. Marginal R^2 values describe the variance explained by the fixed effects only, while the conditional R^2 also includes the variation explained by the random effect.

a)

Model	Variable	Coefficient	Std. Error	df	t-value	*p-value*	Marginal R^2	Conditional R^2
Geographic	**Intercept**	4.23	0.21	564	19.92	**<0.001**	0.03	0.34
	Latitude	-0.02	0.09	564	-0.21	0.86		
	Elevation	-0.25	0.06	564	-3.87	**<0.001**		
	Latitude: Elevation interaction	-0.006	0.06	564	-0.10	0.92		
Climatic	**Intercept**	5.02	0.45	563	11.11	**<0.001**	0.02	0.33
	Mean Annual Temperature	0.008	0.01	563	0.65	0.53		
	Diurnality	-0.07	0.03	563	-2.42	**0.02**		
	Precipitation Seasonality	-0.0003	0.003	563	-0.10	0.93		
	Annual Precipitation	-0.00007	0.0002	563	-0.34	0.74		

b)

Model	Random effect variable	Variance	Standard dev.
Geographic	Species	0.71	0.84
	Residual	1.54	1.24
Climatic	Species	0.72	0.85
	Residual	1.57	1.25

Table S5.3. AIC_c model selection outputs for the home range only. Candidate models included all univariate and bivariable combinations of predictior variables. Models were fitted using generalised least squares regression. ΔAIC_c is the difference in AIC_c scores from the top model. This table shows the AIC_c values for all candidate models. Corresponding R^2 values of the top models can be found in Table 5.1 of the main text.

Species	Model	Explanatory variable 1	Explanatory variable 2	AIC_c	ΔAIC_c	# Top Models
S. alaxensis	Geographic	null		171.11	0.00	3
		latitude		172.58	1.47	
		elevation		172.74	1.63	
		elevation	latitude	174.95	3.84	
	Climatic	null		171.11	0.00	2
		diurn		171.15	0.04	
		MAT		173.35	2.24	
		MAT	diurn	173.37	2.26	
		annual prec.		173.39	2.28	
		prec. seas.		173.43	2.32	
		annual prec.	diurn	173.46	2.35	
		prec. seas.	diurn	173.61	2.50	
		MAT	annual prec.	175.33	4.22	
		prec. seas.	annual prec.	175.69	4.58	
		prec. seas.	MAT	175.82	4.71	
S. alba	Geographic	null		92.01	0.00	2
		latitude		95.88	3.87	
		elevation		96.36	4.35	
		elevation	latitude	98.46	6.45	
	Climatic	null		92.01	0.00	1
		annual prec.		93.19	1.18	
		MAT	annual prec.	95.51	3.50	
		annual prec.	diurn	95.65	3.64	
		prec. seas.	annual prec.	95.79	3.78	
		diurn		95.90	3.89	
		prec. seas.		96.14	4.13	
		MAT		96.36	4.35	
		prec. seas.	diurn	97.88	5.87	
		MAT	diurn	98.53	6.52	
		prec. seas.	MAT	98.77	6.76	

Species	Model	Explanatory variable 1	Explanatory variable 2	AIC_c	ΔAIC_c	# Top Models
S. bebbiana	Geographic	latitude		133.50	0.00	2
		elevation	latitude	135.28	1.79	
		null		135.54	2.04	
		elevation		137.64	4.15	
	Climatic	annual prec.		132.85	0.00	3
		prec. seas.	annual prec.	133.35	0.50	
		MAT	diurn	134.53	1.69	
		MAT	annual prec.	134.98	2.13	
		annual prec.	diurn	135.22	2.37	
		MAT		135.54	2.69	
		null		135.54	2.69	
		diurn		135.76	2.92	
		prec. seas.		137.83	4.99	
		prec. seas.	MAT	137.95	5.11	
		prec. seas.	diurn	138.21	5.36	
S. bonplandiana	Geographic	latitude		135.63	0.00	2
		elevation		136.51	0.88	
		elevation	latitude	137.69	2.07	
		null		138.49	2.86	
	Climatic	null		138.49	0.00	8
		annual prec.	diurn	138.50	0.01	
		diurn		138.56	0.07	
		annual prec.		138.67	0.18	
		MAT	annual prec.	139.38	0.89	
		MAT		139.51	1.02	
		MAT	diurn	139.83	1.34	
		prec. seas.	diurn	139.89	1.40	
		prec. seas.		140.80	2.31	
		prec. seas.	annual prec.	141.11	2.62	
		prec. seas.	MAT	141.98	3.49	

Species	Model	Explanatory variable 1	Explanatory variable 2	AIC_c	ΔAIC_c	# Top Models
S. caroliniana	Geographic	elevation		95.28	0.00	1
		elevation	latitude	97.52	2.24	
		null		100.99	5.71	
		latitude		103.18	7.90	
	Climatic	MAT	annual prec.	94.93	0.00	3
		MAT		95.19	0.26	
		prec. seas.	MAT	95.22	0.29	
		MAT	diurn	97.27	2.34	
		prec. seas.		97.43	2.49	
		prec. seas.	annual prec.	99.47	4.54	
		prec. seas.	diurn	99.66	4.73	
		null		100.99	6.06	
		annual prec.		103.13	8.20	
		diurn		103.27	8.34	
		annual prec.	diurn	105.24	10.31	
S. cinerea	Geographic	null		161.06	0.00	2
		elevation		162.20	1.14	
		latitude		163.29	2.23	
		elevation	latitude	164.55	3.49	
	Climatic	null		161.06	0.00	4
		diurn		161.41	0.35	
		annual prec.		162.86	1.80	
		prec. seas.		162.88	1.82	
		annual prec.	diurn	163.09	2.03	
		MAT		163.34	2.28	
		prec. seas.	diurn	163.68	2.62	
		MAT	diurn	163.82	2.76	
		prec. seas.	annual prec.	164.72	3.66	
		MAT	annual prec.	165.25	4.19	
		prec. seas.	MAT	165.27	4.21	

Species	Model	Explanatory variable 1	Explanatory variable 2	AIC_c	ΔAIC_c	# Top Models
S. eriocephala	Geographic	null		85.14	0.00	2
		elevation		86.45	1.31	
		latitude		87.54	2.40	
		elevation	latitude	89.01	3.87	
	Climatic	prec. seas.	annual prec.	84.12	0.00	3
		null		85.14	1.02	
		prec. seas.		85.43	1.31	
		prec. seas.	diurn	86.37	2.25	
		MAT		87.07	2.96	
		annual prec.		87.38	3.26	
		diurn		87.42	3.31	
		prec. seas.	MAT	87.98	3.86	
		MAT	annual prec.	89.28	5.17	
		MAT	diurn	89.50	5.38	
		annual prec.	diurn	89.97	5.86	
S. exigua	Geographic	null		87.57	0.00	2
		latitude		87.64	0.07	
		elevation		89.68	2.11	
		elevation	latitude	90.29	2.72	
	Climatic	MAT	diurn	83.09	0.00	4
		annual prec.		83.71	0.62	
		annual prec.	diurn	83.94	0.85	
		diurn		85.08	1.99	
		prec. seas.	annual prec.	85.51	2.42	
		MAT	annual prec.	86.32	3.23	
		null		87.57	4.48	
		prec. seas.	diurn	87.64	4.56	
		MAT		88.60	5.51	
		prec. seas.		89.99	6.90	
		prec. seas.	MAT	91.01	7.92	

Species	Model	Explanatory variable 1	Explanatory variable 2	AIC_c	ΔAIC_c	# Top Models
S. fragilis	Geographic	null		27.68	0.00	1
		elevation		55.30	27.62	
		latitude		57.13	29.45	
		elevation	latitude	57.32	29.64	
	Climatic	null		27.68	0.00	1
		diurn		55.19	27.51	
		annual prec.		55.29	27.61	
		MAT		57.15	29.47	
		prec. seas.		57.36	29.68	
		prec. seas.	diurn	57.69	30.01	
		annual prec.	diurn	58.10	30.42	
		MAT	diurn	58.23	30.55	
		MAT	annual prec.	58.39	30.71	
		prec. seas.	annual prec.	58.64	30.96	
		prec. seas.	MAT	60.32	32.64	
S. humboldtiana	Geographic	elevation	latitude	93.31	0.00	2
		elevation		94.36	1.06	
		latitude		95.54	2.23	
		null		95.83	2.52	
	Climatic	MAT		95.40	0.00	3
		null		95.83	0.43	
		prec. seas.		97.20	1.80	
		prec. seas.	MAT	97.45	2.05	
		annual prec.		97.54	2.14	
		MAT	annual prec.	97.61	2.22	
		diurn		97.66	2.27	
		MAT	diurn	98.00	2.61	
		prec. seas.	diurn	99.79	4.39	
		prec. seas.	annual prec.	99.80	4.40	
		annual prec.	diurn	99.96	4.56	

Species	Model	Explanatory variable 1	Explanatory variable 2	AIC_c	ΔAIC_c	# Top Models
S. interior	Geographic	elevation	latitude	95.31	0.00	1
		null		97.35	2.04	
		latitude		98.85	3.54	
		elevation		99.22	3.91	
	Climatic	diurn		95.54	0.00	2
		null		97.35	1.81	
		prec. seas.	diurn	98.10	2.57	
		MAT	diurn	98.14	2.61	
		annual prec.	diurn	98.24	2.70	
		annual prec.		98.72	3.18	
		prec. seas.		99.17	3.63	
		MAT	annual prec.	99.62	4.08	
		MAT		99.70	4.16	
		prec. seas.	MAT	101.38	5.84	
		prec. seas.	annual prec.	101.42	5.88	
S. lasiolepis	Geographic	null		81.29	0.00	2
		latitude		81.97	0.68	
		elevation	latitude	83.36	2.07	
		elevation		83.60	2.31	
	Climatic	null		81.29	0.00	3
		prec. seas.		82.00	0.71	
		diurn		83.04	1.75	
		MAT		83.41	2.12	
		prec. seas.	annual prec.	83.64	2.35	
		annual prec.		83.72	2.43	
		prec. seas.	diurn	84.54	3.25	
		prec. seas.	MAT	84.59	3.30	
		MAT	diurn	85.33	4.04	
		annual prec.	diurn	85.46	4.17	
		MAT	annual prec.	86.01	4.72	

Species	Model	Explanatory variable 1	Explanatory variable 2	AIC_c	ΔAIC_c	# Top Models
S. nigra	Geographic	latitude		179.26	0.00	2
		null		180.70	1.44	
		elevation	latitude	181.32	2.06	
		elevation		182.52	3.27	
	Climatic	prec. seas.		176.46	0.00	2
		prec. seas.	diurn	178.42	1.95	
		prec. seas.	MAT	178.74	2.27	
		prec. seas.	annual prec.	178.80	2.33	
		null		180.70	4.24	
		MAT		181.78	5.31	
		MAT	annual prec.	181.79	5.33	
		annual prec.		182.67	6.21	
		diurn		182.84	6.37	
		MAT	diurn	184.10	7.64	
		annual prec.	diurn	184.76	8.30	
S. pentandra	Geographic	latitude		150.69	0.00	3
		null		150.79	0.10	
		elevation		152.67	1.98	
		elevation	latitude	153.07	2.38	
	Climatic	MAT		150.50	0.00	9
		annual prec.		150.53	0.03	
		diurn		150.74	0.24	
		null		150.79	0.29	
		prec. seas.	MAT	150.98	0.48	
		MAT	annual prec.	151.37	0.87	
		annual prec.	diurn	151.63	1.13	
		MAT	diurn	151.93	1.43	
		prec. seas.		152.30	1.80	
		prec. seas.	diurn	152.58	2.09	
		prec. seas.	annual prec.	152.93	2.43	

Species	Model	Explanatory variable 1	Explanatory variable 2	AIC_c	ΔAIC_c	# Top Models
S. purpurea	Geographic	null		82.40	0.00	1
		latitude		85.20	2.80	
		elevation		86.05	3.65	
		elevation	latitude	87.75	5.35	
	Climatic	null		82.40	0.00	2
		prec. seas.		83.67	1.27	
		annual prec.		84.55	2.15	
		diurn		85.16	2.76	
		prec. seas.	annual prec.	85.24	2.84	
		MAT		85.30	2.90	
		prec. seas.	MAT	85.36	2.96	
		prec. seas.	diurn	85.93	3.53	
		annual prec.	diurn	86.28	3.88	
		MAT	annual prec.	86.93	4.53	
		MAT	diurn	87.56	5.16	
S. scouleriana	Geographic	latitude		99.18	0.00	3
		elevation	latitude	99.53	0.35	
		null		100.17	0.99	
		elevation		102.49	3.31	
	Climatic	prec. seas.		97.34	0.00	2
		prec. seas.	diurn	99.13	1.80	
		prec. seas.	MAT	99.85	2.51	
		prec. seas.	annual prec.	99.87	2.53	
		null		100.17	2.83	
		MAT	diurn	101.25	3.91	
		MAT		101.39	4.05	
		diurn		101.85	4.52	
		annual prec.		102.38	5.04	
		MAT	annual prec.	103.98	6.64	
		annual prec.	diurn	104.52	7.18	

Species	Model	Explanatory variable 1	Explanatory variable 2	AIC_c	ΔAIC_c	# Top Models
S. viminalis	Geographic	null		121.60	0.00	3
		elevation		122.45	0.85	
		latitude		122.89	1.29	
		elevation	latitude	124.76	3.16	
	Climatic	null		121.60	0.00	3
		diurn		122.28	0.68	
		prec. seas.		122.97	1.37	
		MAT		123.82	2.22	
		annual prec.		123.98	2.38	
		prec. seas.	diurn	124.80	3.20	
		MAT	diurn	124.81	3.21	
		annual prec.	diurn	124.84	3.24	
		prec. seas.	MAT	125.48	3.88	
		prec. seas.	annual prec.	125.53	3.93	

Table S5.4. AIC_c model selection outputs for models containing the home/away range variable. All models contain the home/away range variables (except for the null model), so the range parameter is not included in the table. Interaction variables between each climatic or geographic variable and the home/away range variable were also considered as well. Tables are separated by the five species included in the analysis.

Salix alba

Model	Explanatory variable 1	Explanatory variable 2	Int. variable 1	Int. variable 2	AIC_c	ΔAIC_c	# Top Models
Geographic	**null**				282.75	0	1
	latitude				287.22	4.47	
	latitude	elevation			287.72	4.97	
	latitude		latitude		288.20	5.45	
	latitude	elevation	latitude		289.66	6.91	
	elevation	latitude	elevation		289.99	7.24	
	elevation				290.78	8.03	
	latitude	elevation	latitude	elevation	292.08	9.33	
	elevation		elevation		293.05	10.30	
Climatic	**null**				282.75	0.00	1
	MAT				288.16	5.41	
	MAT		MAT		289.62	6.87	
	prec. seas.	MAT			290.03	7.28	
	MAT	annual prec.			290.21	7.46	
	MAT	diurn.			290.44	7.69	
	annual prec.				290.58	7.83	
	prec. seas.				290.78	8.03	
	diurn.				290.90	8.15	
	MAT	prec. seas.	MAT		291.35	8.60	
	MAT	annual prec.	MAT		291.62	8.87	
	annual prec.		annual prec.		291.78	9.03	
	MAT	diurn.	MAT		291.97	9.22	
	annual prec.	MAT	annual prec.		291.98	9.23	
	prec. seas.	MAT	prec. seas.		292.37	9.62	

	prec. seas.	annual prec.			292.63	9.88	
	diurn.	MAT	diurn.		292.64	9.89	
	MAT	annual prec.	MAT	annual prec.	292.74	9.99	
	diurn.	annual prec.			292.83	10.08	
	prec. seas.	diurn.			292.92	10.17	
	diurn.		diurn.		293.03	10.28	
	prec. seas.		prec. seas.		293.06	10.31	
	prec. seas.	MAT	prec. seas.	MAT	293.76	11.01	
	annual prec.	prec. seas.	annual prec.		294.01	11.26	
	annual prec.	diurn.	annual prec.		294.12	11.37	
	MAT	diurn.	MAT	diurn.	294.13	11.38	
	prec. seas.	annual prec.	prec. seas.		294.98	12.23	
	diurn.	annual prec.	diurn.		295.05	12.30	
	diurn.	prec. seas.	diurn.		295.06	12.31	
	prec. seas.	diurn.	prec. seas.		295.25	12.50	
	prec. seas.	annual prec.	prec. seas.	annual prec.	296.43	13.68	
	diurn.	annual prec.	diurn.	annual prec.	296.51	13.76	

Salix cinerea

Model	Explanatory variable 1	Explanatory variable 2	Int. variable	Int. variable 2	AIC_c	ΔAIC_c	# Top Models
Geographic	**null**				393.11	0.00	1
	elevation				395.42	2.31	
	latitude				396.30	3.19	
	latitude		latitude		396.43	3.32	
	elevation		elevation		397.09	3.98	
	latitude	elevation	latitude		397.19	4.08	
	latitude	elevation			397.60	4.49	
	elevation	latitude	elevation		399.29	6.18	
	latitude	elevation	latitude	elevation	399.46	6.35	
Climatic	prec. seas.		prec. seas.		391.80	0.00	3
	prec. seas.	diurn.	prec. seas.		392.14	0.34	
	null				393.11	1.31	
	prec. seas.	diurn.	prec. seas.	diurn.	393.84	2.04	
	prec. seas.	annual prec.	prec. seas.		393.98	2.18	
	prec. seas.	MAT	prec. seas.		394.01	2.21	
	prec. seas.	annual prec.	prec. seas.	annual prec.	395.30	3.50	
	prec. seas.				395.32	3.53	
	MAT				395.82	4.03	
	diurn.				395.88	4.09	
	prec. seas.	MAT	prec. seas.	MAT	396.12	4.32	
	MAT		MAT		396.32	4.52	
	prec. seas.	diurn.			396.39	4.59	
	annual prec.				396.39	4.60	
	diurn.		diurn.		396.65	4.86	
	diurn.	prec. seas.	diurn.		396.89	5.09	
	prec. seas.	MAT			397.13	5.33	
	MAT	diurn.			397.26	5.47	
	prec. seas.	annual prec.			397.43	5.64	
	MAT	diurn.	MAT		397.62	5.82	
	annual prec.		annual prec.		397.73	5.93	

	diurn.	annual prec.			397.94	6.14	
	MAT	annual prec.			397.96	6.16	
	diurn.	MAT	diurn.		397.97	6.17	
	MAT	prec. seas.	MAT		398.14	6.34	
	MAT	annual prec.	MAT		398.52	6.73	
	MAT	diurn.	MAT	diurn.	398.69	6.89	
	diurn.	annual prec.	diurn.		398.83	7.03	
	annual prec.	prec. seas.	annual prec.		398.90	7.10	
	annual prec.	diurn.	annual prec.		399.12	7.32	
	annual prec.	MAT	annual prec.		399.36	7.57	
	diurn.	annual prec.	diurn.	annual prec.	399.96	8.16	
	MAT	annual prec.	MAT	annual prec.	400.02	8.23	

Salix fragilis

Model	Explanatory variable 1	Explanatory variable 2	Int. variable	Int. variable 2	AIC_c	ΔAIC_c	# Top Models
Geographic	elevation				96.40	0.00	3
	null				98.01	1.61	
	latitude	elevation			98.12	1.71	
	elevation		elevation		98.62	2.22	
	latitude				99.70	3.30	
	elevation	latitude	elevation		100.35	3.95	
	latitude	elevation	latitude		101.07	4.67	
	latitude		latitude		102.43	6.03	
	latitude	elevation	latitude	elevation	103.17	6.77	
Climatic	MAT	diurn.	MAT		91.31	0.00	2
	diurn.	MAT	diurn.		92.99	1.68	
	MAT	diurn.	MAT	diurn.	94.45	3.15	
	diurn.		diurn.		94.96	3.65	
	MAT	prec. seas.	MAT		94.97	3.67	
	diurn.				95.09	3.78	
	MAT		MAT		95.11	3.81	
	prec. seas.	MAT	prec. seas.	MAT	95.85	4.54	
	MAT	diurn.			96.87	5.56	
	MAT	annual prec.	MAT		97.04	5.74	
	diurn.	annual prec.			97.27	5.96	
	annual prec.				97.65	6.34	
	prec. seas.	diurn.			97.68	6.37	
	diurn.	annual prec.	diurn.		97.77	6.46	
	diurn.	prec. seas.	diurn.		97.85	6.54	
	null				98.01	6.70	
	prec. seas.	diurn.	prec. seas.		98.36	7.06	
	MAT	annual prec.			98.44	7.13	
	prec. seas.	diurn.	prec. seas.	diurn.	98.62	7.32	
	MAT	annual prec.	MAT	annual prec.	98.76	7.45	
	annual prec.	diurn.	annual prec.		98.85	7.55	

	prec. seas.	annual prec.	prec. seas.		99.50	8.20	
	annual prec.	MAT	annual prec.		99.94	8.64	
	prec. seas.	annual prec.			99.99	8.68	
	annual prec.		annual prec.		100.31	9.01	
	diurn.	annual prec.	diurn.	annual prec.	100.36	9.05	
	prec. seas.		prec. seas.		101.61	10.31	
	prec. seas.				101.78	10.48	
	MAT				101.90	10.59	
	prec. seas.	annual prec.	prec. seas.	annual prec.	102.27	10.97	
	prec. seas.	MAT	prec. seas.		102.35	11.04	
	annual prec.	prec. seas.	annual prec.		102.78	11.48	
	prec. seas.	MAT			103.22	11.91	

Salix purpurea

Model	Explanatory variable 1	Explanatory variable 2	Int. variable	Int. variable 2	AIC_c	ΔAIC_c	# Top Models
Geographic	elevation				121.18	0.00	1
	latitude	elevation			123.61	2.42	
	latitude				123.65	2.47	
	elevation		elevation		123.68	2.49	
	latitude	elevation	latitude	elevation	123.74	2.56	
	latitude		latitude		125.09	3.91	
	elevation	latitude	elevation		126.20	5.02	
	null				125.39	4.21	
	latitude	elevation	latitude	elevation	126.48	5.30	
Climatic	prec. seas.		prec. seas.		122.13	0.00	7
	annual prec.				122.47	0.34	
	prec. seas.	annual prec.	prec. seas.		122.58	0.45	
	prec. seas.	MAT	prec. seas.		122.73	0.60	
	diurn.		diurn.		123.42	1.29	
	MAT				123.71	1.59	
	prec. seas.				124.07	1.94	
	prec. seas.	diurn.	prec. seas.		124.19	2.06	
	diurn.	annual prec.	diurn.		124.30	2.18	
	diurn.				124.50	2.38	
	prec. seas.	annual prec.			124.69	2.56	
	annual prec.		annual prec.		124.76	2.63	
	MAT	annual prec.			124.96	2.83	
	diurn.	annual prec.			124.96	2.83	
	MAT		MAT		125.03	2.90	
	prec. seas.	annual prec.	prec. seas.	annual prec.	125.32	3.19	
	prec. seas.	MAT	prec. seas.	MAT	125.38	3.25	
	null				125.39	3.26	
	diurn.	MAT	diurn.		125.52	3.39	
	diurn.	prec. seas.	diurn.		125.79	3.67	
	prec. seas.	MAT			125.87	3.74	

	MAT	annual prec.	MAT		125.89	3.76	
	MAT	diurn.			126.21	4.08	
	prec. seas.	diurn.	prec. seas.	diurn.	126.43	4.31	
	prec. seas.	diurn.			126.57	4.44	
	diurn.	annual prec.	diurn.	annual prec.	127.05	4.92	
	MAT	prec. seas.	MAT		127.12	4.99	
	annual prec.	prec. seas.	annual prec.		127.16	5.04	
	annual prec.	MAT	annual prec.		127.37	5.25	
	annual prec.	diurn.	annual prec.		127.38	5.25	
	MAT	diurn.	MAT		127.65	5.52	
	MAT	diurn.	MAT	diurn.	128.14	6.01	
	MAT	annual prec.	MAT	annual prec.	128.45	6.32	

Salix viminalis

Model	Explanatory variable 1	Explanatory variable 2	Int. variable	Int. variable 2	AIC_c	ΔAIC_c	# Top Models
Geographic	**null**				191.39	0.00	2
	elevation				193.12	1.73	
	elevation		elevation		193.97	2.58	
	latitude				194.08	2.69	
	latitude	elevation			195.54	4.15	
	latitude		latitude		196.17	4.78	
	elevation	latitude	elevation		196.35	4.96	
	latitude	elevation	latitude		197.91	6.52	
	latitude	elevation	latitude	elevation	198.36	6.97	
Climatic	**null**				191.39	0.00	1
	MAT				193.89	2.50	
	annual prec.				193.96	2.57	
	prec. seas.				194.05	2.66	
	diurn.				194.27	2.88	
	diurn.	annual prec.			194.53	3.14	
	prec. seas.	annual prec.			195.06	3.67	
	MAT		MAT		195.34	3.95	
	MAT	annual prec.			195.41	4.02	
	annual prec.		annual prec.		195.76	4.37	
	MAT	diurn.			195.81	4.42	
	prec. seas.	MAT			195.89	4.50	
	diurn.		diurn.		195.99	4.60	
	prec. seas.	diurn.			196.22	4.83	
	prec. seas.		prec. seas.		196.45	5.06	
	annual prec.	diurn.	annual prec.		196.50	5.11	
	MAT	prec. seas.	MAT		196.61	5.22	
	MAT	diurn.	MAT	diurn.	196.88	5.49	
	diurn.	annual prec.	diurn.		196.92	5.53	
	diurn.	MAT	diurn.		197.16	5.77	
	annual prec.	prec. seas.	annual prec.		197.26	5.87	

	MAT	annual prec.	MAT		197.30	5.91	
	annual prec.	MAT	annual prec.		197.36	5.97	
	MAT	diurn.	MAT		197.48	6.09	
	prec. seas.	annual prec.	prec. seas.		197.58	6.19	
	prec. seas.	MAT	prec. seas.		198.29	6.90	
	diurn.	prec. seas.	diurn.		198.37	6.98	
	prec. seas.	diurn.	prec. seas.		198.59	7.20	
	diurn.	annual prec.	diurn.	annual prec.	199.11	7.72	
	prec. seas.	MAT	prec. seas.	MAT	199.20	7.81	
	MAT	annual prec.	MAT	annual prec.	199.53	8.14	
	prec. seas.	annual prec.	prec. seas.	annual prec.	199.88	8.49	
	prec. seas.	diurn.	prec. seas.	diurn.	200.88	9.49	

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