Clinal variation and phenology in two conspecifics of *Phragmites australis* (Cav.) Trin. Ex Steud in Northeastern North America: implications for current management and future climate change

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ABSTRACT

Within native and non-native plant species, the production of viable seeds can both promote range expansion and facilitate rapid climatic adaptations through the selection of adapted phenotypes. The success of sexual reproduction in many plant species has been linked to a critical level of accumulated growing degree-days (GDDs) throughout the season. However GDDs are often limited in northern climates. With climate change forecasted to cause temperature increases in many northern areas, GDDs are likely to increase and there exists the potential for some species currently limited by a lack of GDDs to undergo increases in sexual reproduction and ultimately range expansion. Field studies and in vitro germination trials were used to test the relationship between seasonal GDDs and development, height and sexual reproduction in an established invasive non-native species, *Phragmites australis* (Cav.) Trin. Ex Steud. (haplotype M) and its native conspecific, *P. australis* subsp. *americanus*. During the 2012 season, 29 populations were monitored within five isotherms along a1000-km long latitudinal gradient from New Jersey (USA) to Alma (Quebec). As seasonal GDDs decreased, non-native *P. australis* were on average shorter, required fewer GDDs to develop, and produced fewer viable seeds per floret. Within isotherms, native *P. australis* were shorter and developed earlier than non-native *P. australis*, and also generally produced more seeds per floret. Overall the response of native *P. australis* was not related to seasonal GDDs. Over the climate gradient of the study area it appears that a lack of GDDs is currently limiting the expansion of non-native *P. australis* through seed dispersal at the edge of its range. As local temperatures and ultimately GDDs increase with climate change, the adaptive response non-native *P. australis* has to climate will likely allow the species to rapidly expand its range to take advantage of newly available climatic niches.

RÉSUMÉ

La reproduction sexuée et la production subséquente de graines viables peuvent faciliter la dispersion d'espèces végétales indigènes ou non indigènes/envahissantes dans de nouvelles régions ainsi que possiblement une adaptation rapide à des conditions changeantes grâce à la sélection de phénotypes appropriés. Le succès de la reproduction sexuée pour de nombreuses espèces végétales a été lié à une accumulation critique de degrés-jours de croissance (DJCs) durant la saison. Cependant les DJCs sont souvent limités dans les climats nordiques. Avec les changements climatiques et les températures qui augmenteront particulièrement vers le nord, le DJCs sont susceptibles d'augmenter et des espèces actuellement limitées par un manque de DJCs pourraient voir une augmentation de leur reproduction sexuée et éventuellement une extension de leur aire de répartition. Un suivi sur le terrain et des essais de germination ont été utilisés pour tester la relation entre les DJCs saisonniers et le développement, la hauteur et la reproduction sexuée d'une espèce envahissante non indigène, *Phragmites australis* (Cav.) Trin. Ex Steud. (M haplotype) et sa congénère indigène, *P. australis* subsp. *americanus*. Durant la saison 2012, 29 populations ont été suivies dans cinq isothermes le long d'un gradient latitudinal de 1000 km du New Jersey (États-Unis) à Alma (Québec). Le *P. australis* non indigène était en moyenne plus court, requerrait moins de DJCs pour produire des inflorescences, et produisait moins de graines viables avec une diminution des DJCs saisonniers. Pour un même isotherme, le *P. australis* indigène était plus court et se développait plus tôt que le *P. australis* non indigène, et aussi généralement produisait plus de graines. Globalement, la réponse du *P. australis* indigène n'était pas liée au DJCs saisonniers. Le long du gradient climatique de la zone étudiée, il semble que le manque de DJCs limite actuellement l'expansion par dispersion de graines du *P. australis* non indigène, tout en ayant peu ou pas d'effet sur le *P. australis* indigène. Une production de graines accrue par les changements climatiques pourrait permettre au *P. australis* envahissant d'exploiter des niches climatiques nouvellement disponibles.

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PREFACE AND CONTRIBUTION OF AUTHORS

This thesis is written in a standard monograph style. Literature citations were formatted according to the journal Biological Invasions.

The idea for the thesis was proposed by Dr. Sylvie de Blois in the context of research on climate change and invasive species. Sampling design and data collection for this study were done primarily by the M.Sc. candidate, Christie Lovat, with additional help in field sampling by Lindsay Burkart and Michael Wadden. Statistical analysis was conducted by Christie Lovat under the guidance of Dr. Reto Schmucki and Dr. Sylvie de Blois. The first drafts of all chapters of this thesis were written by Christie Lovat and edited by Dr. Sylvie de Blois.

1. Introduction.

Numerous threats to natural ecosystems exist in today's global society. Chief among these are climate change and invasion by non-native species (Gurevitch and Padilla 2004; Ward and Masters 2007; Hellmann et al. 2008). Unfortunately, these two factors can also act in synergistic ways (Ward and Masters 2007; Brisson et al. 2008; Hellmann et al. 2008; Walther et al. 2009). One of the chief predictions of climate change calls for changing temperatures across the globe (IPCC 2007). In particular, temperate areas stand to experience considerable increases in temperature, with some areas seeing a rise in average temperature by as much as 4°C and an extension of the growing season by several weeks (Arft et al. 1999; IPCC 2007). In plants, temperature has a direct impact on sexual reproduction (Lyons et al. 1989). Small increases or decreases in temperature have been shown to have profound effects on pollen production, flower formation, length of flowering time, fungal infections of seeds, and ultimately the number of seeds produced and even the germination rate of those seeds (Lyons et al. 1989). It is likely that native plant species in these areas will experience a dramatic shift in climatic conditions and changes to sexual reproduction in the near future (Arft et al. 1999). However, sexual reproduction has also been identified as one of the most important biological factors contributing to naturalization success and invasiveness in non-native plant species (Callaway and Josselyn 1992; Honig et al. 1992; Perrins et al. 1993; Pyšek 1997; Lambrinos 2001; Daehler 2003; Sutherland 2004; Dukes and Mooney 1999; Walther et al. 2002; Colautti et al. 2006; Martínez-Ghersa and Ghersa 2006; Pyšek and Richardson 2007; Belzile et al. 2010; van Kleunen et al. 2010; Jenkins and Keller 2011). Plants which produce seeds have several colonization advantages over species which propagate mainly vegetatively, including the ability to disperse over longer distances, the ability to generate a greater propagule pressure, the ability to produce genetically diverse populations capable of overcoming adverse environmental conditions, and the ability to remain dormant in seed banks over long periods of time (Martínez-Ghersa and Ghersa 2006;

Pyšek and Richardson 2007; Speek et al. 2011). In this respect, temperature increases from climate change have the potential to increase the invasiveness of already established invasive species, or even produce entirely new ones from previously naturalized species (Arft et al. 1999; Forman and Kesseli 2003; Montague et al. 2007; Brisson et al. 2008).

Phragmites australis (Cav.) Trin. Ex Steud is a species of reed which has been present in North America since the Pleistocene (Hansen 1978; Meyerson et al. 2009). Within North America, *P. australis* (hereafter referred to as 'native *P. australis*') consists of several distinct haplotypes; it is a wetland obligate plant limited mainly to pristine wetlands (Meyerson et al. 2009; Haslam 2010; Plut et al. 2011). However in the late 19th century, a European cp DNA haplotype of *P. australis* (hereafter referred to as 'non-native *P. australis*'), was introduced to North America (Saltonstall 2002; Meyerson et al. 2009; Plut et al. 2011). Non-native *P. australis* has proven to be both phenotypically plastic and able to adapt to various abiotic conditions, such as flooding, high salinity levels, heavy metal contamination, nutrient loading, human disturbances, and severe winter temperatures (Mal and Narine 2004; Meyerson et al. 2009; Haslam 2010; Plut et al. 2011). From its initial introduction, non-native *P. australis* has spread to both wetlands and disturbed areas to become one of the most aggressive and harmful invasive plant species in North America, while native *P. australis* has been found to be disappearing throughout most of its range (Saltonstall 2002; Meyerson et al. 2009; Mozdzer and Zieman 2010).

Until recently it was believed that reproduction, propagation and invasion pathways in non-native *P. australis* were well understood (Brisson et al. 2008; Belzile et al. 2010). The plant forms dense strands using highly vigorous underground rhizomes and was known both for commonly invading areas recently disturbed by human activity and for being able to rapidly regenerate an entire plant from very small pieces of rhizome (Mal and Narine 2004; Belzile et al. 2010). This knowledge, in conjunction with a documented sporadic seed set,and

lack of documented seedlings in the field, led researchers to assume non-native *P. australis* propagated by vegetative means alone in the invaded areas of North America, and control methods were designed around this assumption (Mal and Narine 2004; Belzile et al. 2010). However in 2008 seedlings were documented in the field in Quebec, and it was suggested that perhaps increased local temperature from climate change may have contributed to the success of sexual reproduction in non-native *P. australis* (Brisson et al. 2008; Belzile et al. 2010). As well, modelling has recently identified growing degree-days (GDDs) as the most important climatic variable (out of six others) in determining the distribution of non-native *P. australis* in eastern North America, suggesting that local temperatures do indeed have a significant effect on growth in non-native *P. australis* (de Blois et al. in prep.). A single cane of non-native *P. australis* can produce upwards of 1000 florets capable of generating seed, and in North America populations typically reach densities of 200 canes per square meter (Bittmann 1953; Mal and Narine 2004). If populations of non-native *P. australis* are able to produce seeds successfully, and if this production was indeed positively affected by increasing local temperature, populations of non-native *P. australis* could have the potential for incredible levels of propagule production. As well, very little is known concerning the life history of the native *P. australis*, and it is uncertain if increasing temperatures from climate change will further hamper the native *P. australis* in competition with its non-native conspecific.

1.1. Objective and Hypotheses

It was the objective of this study to determine how vegetative growth, the timing of developmental events, sexual reproduction, and fungal contamination vary along a temperature gradient in both native and non-native *P. australis*. Specifically, this study investigated the relationship between the accumulation of GDDs throughout the growing season in the field and:

- GDDs required for inflorescence emergence and GDDs required for the initiation of flowering in non-native *P. australis.*
- Overall cane height in native and non-native *P. australis*.
- Viable seed production in native and non-native *P. australis*.
- The occurrence of *Claviceps* sclerotia within florets in both native and non-native *P. australis*.
- Levels of fungal contamination in viable seeds from both native and non-native *P. australis*.

As well, this study also investigated the effect of plant height on seed production in both conspecifics of *P. australis*. Finally, this study also sought to determine the differences between conspecific populations in height, seed production, *Claviceps* occurrence, and seed contamination during germination.

It was predicted that both native and non-native plants from isotherms with fewer GDDs throughout the growing season would be shown to develop earlier, reach shorter overall heights, produce fewer viable seeds, have higher levels of *Claviceps* occurrence, and have higher levels seed contamination during germination than plants from climates with a greater number of seasonal GDDs. As well, native plants would be shown to be poorer competitors than non-native plants by growing shorter, producing fewer viable seeds, and demonstrating greater levels of fungal parasitism within the same isotherm. As well, increasing plant height would be shown to have a positive correlation to overall seed production.

2. Literature Review

2.1. On the Origin of Invasive Species

2.1.1. The definition issue

Charles Sutherland Elton, considered by many to be the father of modern ecology, was also the first to identify that some species could have disproportionately negative effects on other species, ecosystem functions, and local economies (Elton 1958). He termed these problem organisms 'invasive species' (Elton 1958; Davis et al. 2001). However the prevalent use of the term 'invasive species' in both popular culture and across multiple scientific disciplines has resulted in many different, and even conflicting, definitions (Richardson et al. 2000; Davis et al. 2001). Some of the more popular definitions include both native and non-native aggressive species, benign non-native species only, non-native aggressive species only, population densities of undesirable species, and occasionally any undesirable species (Richardson et al. 2000; Davis et al. 2001; Colautti and MacIsaac 2004). For the purpose of this study, invasive species will be defined as 'a non-native species which has undergone an exponential population increase to the point that it has the potential to become harmful to the environment, the economy, or human health.' This definition is a combination of what is seen as the most beneficial aspects of numerous other published definitions of the term 'invasive species', with an emphasis placed on biological traits defining species' invasion (such as population growth) as opposed to a solely anthropocentric evaluation (Richardson et al. 2000). As well as invasive species, several other important terms in invasion ecology have been given multiple definitions over time, and it was deemed important to clearly outline which definitions were chosen to apply to this study (Richardson et al. 2000). Here naturalized species will be defined as a non-native species which has established a self-sustaining population, but only persists in low densities with low population growth rates and are not considered to be harmful either to the

environment, the economy, or human health. As well, non-native species will be considered to be any species not naturally found in the geographic area it is currently occurring in, and can be transient, naturalized or invasive.

2.1.2. The Process of Invasion

Invasion is not a single-step process. For a new species to invade successfully it must pass through several distinct population-defined steps separated by abiotic and biotic filters which may ultimately prevent the species from becoming a new invasive species (Goodwin et al. 1999; Theoharides and Duke 2007). The first step of invasion is the ability of the organism to successfully associate with a vector for travel and survive the trip to the novel recipient habitat (Goodwin et al. 1999; Theoharides and Duke 2007). When a species has survived transport, the next step is its ability to survive in the novel recipient ecosystem (Goodwin et al. 1999; Theoharides and Duke 2007). If a species is able to become established, the next phase is defined by an ability to form a self-sustaining reproductive population in a novel habitat (Goodwin et al. 1999; Theoharides and Duke 2007). It is at this stage that a species is considered to be 'naturalized' (Goodwin et al. 1999). Once the naturalized species begins to cause problems either economically or environmentally (typically associated with rapid population growth rate), the species has then reached the final step and is considered to be an invasive species (Goodwin et al. 1999; Theoharides and Duke 2007). Each non-native species must successfully survive these four steps in order to become invasive, and for each filter the number of species able to pass through is fewer than the filter before by an order of magnitude or more (Williamson and Fitter 1996; Goodwin et al. 1999). A rough estimation by Williamson and Fitter (1996), now widely cited as one of the most important studies quantifying invasion during the invasion process, suggests that for every 100 species introduced, 10 will become naturalized, and only 1 will become invasive. However it is important to note that this is a very rough estimation and serves more to demonstrate the relatively few introduced non-native species which are

able to achieve invasive status, than to provide a concrete number concerning invasion ability in introduced species (Williamson and Fitter 1996).

While the process of invasion is a vital component in our understanding of invasion ecology and ultimately controlling invasive species, an equally important component is our understanding of which species invade and why. A considerable amount of research has been done to determine which biological traits can be used to predict invasiveness in non-native species, and one which has been consistently identified as being important to successful invasion is sexual reproduction (Callaway and Josselyn 1992; Honig et al. 1992; Perrins et al. 1993; Rejmánek 1996; Rejmánek and Richardson 1996; Pyšek 1997; Rejmánek 2000; Lambrinos 2001; Daehler 2003; Sutherland 2004; Martínez-Ghersa and Ghersa 2006; Pyšek and Richardson 2007; Küster et al. 2008; Belzile et al. 2010; Küster et al. 2010; van Kleunen et al. 2010; Jenkins and Keller 2011). This may seem counterintuitive, as the popular ideal of an invasive plant typically calls up images of dense monocultures which are formed almost solely by the clonal growth characteristic of vegetative propagation (Martínez-Ghersa and Ghersa 2006). However, although clonal propagation may allow for aggressive growth and therefore increased competition at the local scale, it is nevertheless a very inefficient method for dispersal over long distances and therefore limiting for invasive behaviour at large scales, which is a defining characteristic of many of the worst invasive species (Martínez-Ghersa and Ghersa 2006; Pyšek and Richardson 2007; Speek et al. 2011). Several recent studies have also found patterns in the traits associated with invasiveness in sexual reproduction which support the inherent superiority of sexual reproduction in invading species. For instance in the Asteraceae family, species which are self-compatible have been found to be more invasive than species which are self-incompatible (Hao et al. 2011). This suggests that the greater seed production capability of small populations of self-compatible species as opposed to the limited seed production of small populations of self-incompatible species, may be giving self-compatible species a competitive advantage (Hao et al. 2011). Along this line of reasoning,

greater invasion success has been found to be corroborated with increased seed set in several studies (Moravcová et al. 2010; Closset-Kopp et al. 2011; Hao et al. 2011). Small seed size has also been positively correlated with invasiveness in numerous trait studies, which is most likely a function of the ability of small seeds to typically disperse much further than larger seeds (Cadotte and Lovett-Doust 2001; Hamilton et al. 2005; Pyšek and Richardson 2007; Dawson et al. 2009; Moravcová et al. 2010; Schmidt and Drake 2011). Invasive species have also been shown to have greater germination rates or germinate over a wider range of conditions, both compared to natives in their invaded ranges and to populations of the same species from their original native ranges (Pyšek and Richardson 2007; Perglová et al. 2009; Beckmann et al. 2011). Longer flowering periods and earlier or later flowering time than local natives has also been associated with successful invasion (Crawley et al. 1996; Goodwin et al. 1999; Cadotte and Lovett-Doust 2001; Pyšek et al. 2003; Lake and Leishman 2004; Lloret et al. 2005; Cadotte et al. 2006; Pyšek and Richardson 2007; Pyšek et al. 2009; Küster et al. 2010; Speek et al. 2011). Greater seedling growth rate, as well as greater seed dispersal and longer seed viability have also been identified with invasiveness, particularly in tree species (Pyšek and Richardson 2007; Bucharova and van Kleunen 2009; Perglová et al. 2009; Grotkopp et al. 2010).

There are still many questions to be answered concerning sexual reproduction in invasive species, and new research is continuously being performed. For instance, recent work has detected that there may be a difference in the importance of sexual reproduction to invasive capability between short-lived species and long-lived species (Fenesi and Botta-Dukát 2010). What is clear however, is that sexual reproduction plays a vital role in invasion ability in non-native species, and that any factor which can potentially affect sexual reproduction has the ability to affect competition and long term species survival for both native and non-native species (Foreman and Kesseli 2003; Brisson et al. 2008).

2.2. The Effect of Temperature on Sexual Reproduction.

Although temperature can have profound effects on all aspects of plant growth, it has some of its most dramatic effects on sexual reproduction (Lyons et al. 1989). Sexual reproduction in flowering plants consists of four distinct stages, flower formation, pollination, fertilization and seed maturation, and temperature can have a profound effect on each (Lyons et al. 1989). The flower formation stage consists of the development of the reproductive structures from vegetative material in the plant (Lyons et al. 1989; Raven 2005). Both excessively high and excessively low temperatures have been shown to interfere with the normal formation of reproductive structures, and already studies have shown that the temperature increase which has occurred in the last 100 years has caused some plant species to flower earlier in the season (Arft et al. 1999; Houle 2007). High temperatures have been associated with large numbers of aborted flowers or undeveloped flowers in tomato (*Lycopersicon esculentum* Mill.) (Sato et al. 2001). Temperature also seems to play a role in the sex determination of flowers in some species (Freeman et al. 1980). With both high temperatures and low temperatures, a greater number of male flowers have been observed to be produced in some species relative to female flowers (Thompson 1952; Heslop-Harrison 1957; Lange 1961; Frankel and Galun 1977; Freeman et al. 1980; Freeman et al. 1984). As well, stressful temperatures have been linked to dioecious plant species sporadically producing hermaphroditic flowers (Freeman et al. 1980).

The pollination stage of sexual reproduction is characterized by the transfer of male gametes to a receptive stigma (Lyons et al. 1989; Hartmann et al. 2002). Depending on the species being considered, this transfer can be characterized as occurring between flowers on the same plant, within the same flower on one plant, or between flowers on genetically distinct individuals of the same species or even across species (Hartmann et al. 2002). Pollen can be transferred through numerous abiotic and biotic factors, including wind, water,

dedicated pollinators, and generalist pollinators (Hartmann et al. 2002; Raven 2005). Temperature can affect both the ability of flowers to receive pollen and pollinator availability, and even prevent pollination completely by interfering with flower formation (Kudo et al. 2004; Hedhly et al. 2005; Zinn et al. 2010). Excessively warm temperatures have been shown to decrease the length of time stigmas are receptive to pollen in peach (*Prunus persica* L.) and sweet cherry (*Prunus avium* L.), while warmer temperatures can cause plants to flower before their pollinator is at a population peak and thus can miss pollination (Kudo et al. 2004; Hedhly et al. 2003; Hedhly et al. 2005). Both heat and cold stress can cause pollen sterility by interfering with nutritive tissues of the tapetum and disrupting sugar metabolism or causing tissue degradation (Saini et al. 1984; Sakata et al. 2000; Oliver et al. 2005; Zinn et al. 2010). Cold stress has also been shown to interrupt mitosis I and II, preventing the production of pollen grains in rice microspores (Sakata and Hayase 1970; Zinn et al. 2010).

The fertilization stage of the reproductive process is characterized by the germination of the pollen and the growth of the pollen tube down the stigma and style to reach the ovules, and ultimately the fusion of the male and female gamete for fertilization (Lyons et al. 1989; Hartmann et al. 2002). High temperature has been shown to impair pistal function in temperature sensitive chickpea cultivars (Srinivasan et al. 1999; Zinn et al 2010). As well, temperature stress can interfere with the carbohydrate deposition in the pollen grain, which can impair pollen tube growth (Jefferies et al. 1982; Herrero and Arbeloa 1989; Jakobsen and Martens 1994; Srinivasan et al. 1999; Jain et al. 2007; Zinn et al. 2010). Cold temperatures have been shown to slow pollen tube growth in plums (*Prunus domestica* L) and avocado (*Persea americana*), and interfere with pollination success in Asian sacred lotus (*Nelumbo nucifera*) (Sedgley and Annells 1981; Jefferies et al. 1982; Li and Huang 2009). As well at colder temperatures, ovules in avocado (*P. americana*) reported fewer successful penetrations by pollen tubes (Sedgley and Annells 1981). In several mango (*Mangifera indica* L) cultivars, pollen germination and tube growth was delayed both at high and low temperatures

(Sukhvibul et al. 2000). In sweet cherry (*P. avium*), high temperatures have been shown to reduce pollen germination and pollen adhesion (Hedhly et al. 2003).

The seed maturation phase involves the transformation of the fertilized egg cell through embryo development to the formation of a mature seed (Lyons et al. 1989; Hartmann et al. 2002). Temperature stress has been shown to cause ovary abnormalities in several plant species (Saini et al. 1984; Srinivasan et al. 1999; Whittle et al. 2009; Zinn et al. 2010). In *Arabidopsis* the total number of ovules was reduced and there was an increased incidence of ovule abortion, in chickpeas there was a reduction in ovule size, reduced ovule viability, and missing embryo sacs (Saini et al. 1984; Srinivasan et al. 1999; Whittle et al. 2009; Zinn et al. 2010). In avocado (*P. americana*) embryo development was observed to proceed very slowly at temperatures below 17°C day and 12°C night, and fruitlets were documented to absciss at 33°C day and 28°C night (Sedgley and Annells 1981). In tomatoes (*L. esculentum*), high temperature stress has been associated with a significant increase in the production of parthenocarpic fruit (Sato et al. 2001).

Available literature seems to suggest that there as a window of preferred temperature for successful sexual reproduction in many species (Paiaro et al. 2007). Climate change has the potential to shift this window geographically for many species, and it is clear that the temperature changes many populations will experience through climate change have the potential to cause significant changes in the reproductive success of numerous plant species (Dukes and Mooney 1999; Brisson et al. 2008).

2.3. *Claviceps* Fungi

The *Claviceps* genus is a group of parasitic fungi which specializes in infecting the florets of grasses, sedges and reeds (Pažoutová 2001). To date 45 species have been identified within the *Claviceps* genus, most of which have

monogeneric to polygeneric host ranges and range from paleoartic to tropical conditions (Pažoutová 2002). Typically *Claviceps* solely parasitize the ovary of their host, utilizing the resources of the plant to produce a sclerotium in the place of a seed (Pažoutová 2002), However *C. purpurea* has been shown to also produce sclerotia on the meristems of vegetative material, while the normal lifecycle of *C. phalaridis* infects the entire plant and results in all florets being utilized to produce sclerotia (Lewis 1956; Walker 1957; Walker 1970; Pažoutová 2002). *Claviceps* is also one of the few fungal parasites known to have successfully invaded new habitats through human activities (Pažoutová 2001).

Claviceps infections have been noted in non-native *P. australis* both in Europe and North America, although no studies have as of yet examined native *P. australis* for the presence of *Claviceps* parasitism (Gustafsson and Simak 1963; Mantle 1969; Gervais et al. 1993; Mazurkiewicz-Zapalowicz et al. 2005; Haslam 2010). Sclerotia found on *P. australis*, where identified, are typically found to be *C. purpurea* or *C. microcephala* (which is actually considered to be a variety of *C. purpurea*) (Gustafsson and Simak 1963; Mantle 1969; Gervais et al. 1993; Pažoutová et al. 2000; Mazurkiewicz-Zapalowicz et al. 2005; Haslam 2010). The lifecycle of *C. purpurea* involves an overwintering stage of sclerotia (McCrea 1931). In the spring, sclerotia which have overwintered on the soil surface or have been protected within plant material germinate with stromata which produce perithecia and ultimately the ascospores (McCrea 1931). Ascospores are transported by wind or insects to the open flowers of the host, and infect the ovary by germinating down the pollen tube as would a typical grain of pollen (Alderman et al.1999; McCrea 1931). The normal development of the ovary is halted with infection, and all resources are directed towards the production of conidia (McCrea 1931). During this period, a thick pinkish liquid containing conidia fills the floret and can be seen leaking to the exterior (McCrea 1931). This is called the 'honey dew' stage of infection, and is a feature of most *Claviceps* infections (McCrea 1931). This 'honey dew' gradually hardens and extends to form a sclerotium within the floret (McCrea 1931).

Despite *Claviceps* being known as an important pest species of grasses that both reduces seed production and renders grass crops inedible, very little investigation has been done into the *Claviceps* infection of *P. australis*. This is critical, as not only could *Claviceps* infection be reducing seed production in native *P. australis*, but non-native *P. australis* is commonly found beside farmers' fields in North America, providing a potential infection pool for grain crops. Knowledge of *Claviceps* in both conspecifics of *P. australis* will be vital to both conservation and economic management.

2.4. Study Species: Non-native *Phragmites australis* (Cav.) Trin. Ex Steud

2.4.1. Vegetative and Population Characteristics

Non-native *P. australis* (also referred to as haplotype M or *P. australis* subsp. *australis*) is a large herbaceous perennial graminoid species mainly found in moist and submerged soils, although it can be tolerant of drier upland conditions (Mal and Narine 2004; Meyerson et al. 2009). It is considered to be a C_3 plant, however four ecotypes from China were documented to have variations on their photosynthesis pathway which resembled C_4 photosynthesis and C_3-C_4 photosynthesis intermediates (Zheng et al. 2000; Mal and Narine 2004). Plants form annual tall, unbranching and upright cane-like stems each growing season (Hocking et al. 1983; Mal and Narine 2004). These stems are annual except in frost-free areas (such as Malta), where stems are biennial (Haslam 1972; Mal and Narine 2004). As well, although stems of non-native *P. australis* are characterized as unbranching, branching shoots have been documented being formed in response to herbivory, in hexaploid plants, or as part of the second-year of growth in biennial canes in frost free areas (Mal and Narine 2004). Canes have been documented to reach a height of 8 m and a diameter of up to 1 cm, and are typically light green in color (Hocking et al. 1983; Mal and Narine 2004; Haslam 2010). There are between 13 – 17 nodes on each cane with an intermodal length of 10 - 25 cm (Haslam 1972; Hocking et al. 1983; Mal and Narine 2004).

Internodes are hollow and have an intercalary meristem at each base (Hocking et al. 1983; Mal and Narine 2004). Growth occurs rapidly during the summer season, with canes in some western Canadian stands growing a documented 4 cm a day and some populations documented as growing steadily by 10% every year (Shay and Shay 1986; Rice et al. 2000; Mal and Narine 2004; Meyerson et al. 2009). Populations often consist of monocultures and have been reported to reach densities of 200 canes per m^2 (Small and Catling 2001; Osgood et al. 2003; Mal and Narine 2004; Meyerson et al. 2009). Leaves are narrow-lanceolate with smooth margins and large overlapping smooth sheaths at the leaf base (Haslam 1972; Hocking et al. 1983; Mal and Narine 2004). However it should be noted that several biotypes have been documented with broadly lanceolate leaves (Hocking et al. 1983; Catling et al. 2003; Mal and Narine 2004). Leaves are alternate, except in a single biotype noted in Western Ontario which has been observed to form leaves in a pinnate array (Haslam 1972; Hocking et al. 1983; Catling et al. 2003; Mal and Narine 2004). In late fall the leaves are released from the sheaths, so that only the aerial stem remains upright, and can persist for up to 2 years (Frankenberg 1997; Mal and Narine 2004). Ligules consist of a half membrane and half hairs between 1.5-3mm long (Mal and Narine 2004).

Spread in non-native *P. australis* occurs from an extensive rhizome system which consists of both horizontal and vertical rhizomes, and can be found up to 4m below the soil line (Mal and Narine 2004; Meyerson et al. 2009; Haslam 2010). Horizontal rhizomes allow for excessive local spread in non-native *P. australis* and have been observed to be dominant in colonizing populations, with a single rhizome capable of spreading over 15 m from the original source plant (Weaver 1960; Pallis 1961; Haslam 1969b; Hocking et al. 1983; Mal and Narine 2004). Vertical rhizomes give rise to the annual vegetative canes, and tend to be the dominant growth form in favourable habitats (Hocking et al. 1983; Mal and Narine 2004). Rhizomes of non-native *P. australis* can live an average of 3-7 years, with horizontal rhizomes typically surviving longer than vertical rhizomes (Halsman 1972; Hocking et al. 1998; Mal and Narine 2004). However this may be

an underestimation, as the oldest horizontal rhizomes ever recorded came from Japan and were documented as being an astonishing 13 years old (Kamio 1985; Mal and Narine 2004). Roots can grow both from rhizomes and any submerged shoot material (Mal and Narine 2004). The morphology of roots depends directly on the quality of the substrate (Mal and Narine 2004). Nutrient poor soils promote sparse root growth, while nutrient rich soils encourage dense root growth (Weaver and Himmer 1930; Mal and Narine 2004). As well, roots growing in submerged soils have been observed to be narrow, short, and branched, while roots from plants in drier mud soils were noted to be thicker, less branched, and up to 5 m long (Haslam 1969a). However published observations on root morphology remain scarce, and it is possible that the observed trends arose from biotype differences instead of a result of environmental influence. Non-native *P. australis* is also extremely tolerant of high levels of soil salinity and several heavy metals including Na, Ca, K, Mg, Fe, Cu, Zn, and Pb (Peverly et al. 1995; Ye et al. 1997; Mal and Narine 2004; Greenwood and MacFarlane 2006; Haslam 2010).

Non-native *P. australis* is an extremely well studied plant. However sexual reproduction in non-native *P. australis* is exceedingly complex and given its importance to this study, a separate section concerning our current understanding of processes involved and affecting sexual reproduction in non-native *P. australis* is warranted.

2.4.2. Sexual Reproduction

Inflorescences of non-native *P. australis* consist of one terminal panicle per cane, produced late in the growing season (Mal and Narine 2004). Inflorescences can be 30 cm long or more, and can range in color from yellow to a reddish purple (Mal and Narine 2004). Each inflorescence consists of several main branches, smooth except for unevenly distributed groups of long hairs (Haslam 1972). Each branch has several 10-18 mm long spikelets with hairy pedicels and rachillas with silky hairs (Haslam 1972; Mal and Narine 2004). At

the base of each spikelet are 2 lanceolate persistent glumes and 2-6 sessile florets (Mal and Narine 2004). Glume size varies by position, with lower glumes between 2.5 - 5 mm long and upper glumes 3.75 – 10 mm (Hocking et al. 1983). Lemmas have been noted to be between 9 – 13 mm long and tend to be twice as long as the upper glume on each spikelet (Mal and Narine 2004). Lemmas are lanceolate, smooth and membranous (Mal and Narine 2004). The number of stamens and sex of each floret differs based on its position on the spikelet (Mal and Narine 2004). Lower florets have between 1-3 stamens and are typically male, while the upper florets generally have 3 stamens only and are bisexual (Mal and Narine 2004). The ovary in bisexual flowers is glabrous (Mal and Narine 2004). The fruit formed after fertilization is a caryopsis which can reach a maximum of 2 mm long (Mal and Narine 2004). When released, the caryopsis is shed along with parts of the lemma, palea and the rachilla (Mal and Narine 2004).

The process of sexual reproduction itself seems to be limited in non-native *P. australis*. To begin with, although most canes within populations produce inflorescences and a single inflorescence can produce upwards of 1000 seeds, germination rates are often low and highly variable (Hürlimann 1951; Bittmann 1953; Gustafsson and Simak 1963; Curran 1969; Haslam 1970; Haslam 1972; Clucas and Ladiges 1980; Tucker 1990; Kraska et al. 1992; Gervais et al. 1993; McKee and Richards 1996; Ailstock et al. 2001; Ishii and Kadono 2002; Mal and Narine 2004). This has been suggested to be a result of self-incompatibility in the stands or lack of pollen viability (particularly in northern populations) (Curran 1969; Gustafsson and Simak 1963; Satyamurty and Seshavatharam 1984; Clevering and Lissner 1999; Small and Catling 2001; Ishii and Kadono 2002; Mal and Narine 2004). However recent evidence suggests that self-pollination is a viable and possibly often used method of sexual reproduction (Lambert and Casagrande 2007; Kettenring et al. 2010; Kettenring et al. 2011). Inflorescences are wind pollinated for a distance of up to 50 m, and seeds have been observed to be dispersed by wind, water, and animal activities (Haslam 1972; Mal and Narine 2004; McCormick et al. 2010a). In its native Europe, timing of flowering has been

noted to vary widely with latitude, with populations in more northern latitudes requiring fewer days to form flowers (Clevering et al. 2001, Haslam 2010). As well, cold-acclimated biotypes have been shown to form flowers in much colder conditions than more southerly populations (Clevering et al. 2001; Bastlova et al. 2004; Mal and Narine 2004; Haslam 2010). Seeds have a wide range of germination times, requiring between 4 days and 4 months for germination after dispersal, and some seeds can remain viable for up to 5 years (Hürlimann 1951; Clucas and Ladiges 1980). However it is the specific environmental requirements for successful germination and establishment which often limit germination and seedling recruitment in non-native *P. australis* (Washitani and Masuda 1990; Mal and Narine 2004). Despite the tolerance for standing water in adult non-native *P. australis*, seeds often only germinate in up to 1 cm of standing water (although germination under almost 1 m of water has been recorded) (Haslam 1972; Szczepanska 1977; Galinato and van der Valk 1986; Mauchamp et al. 2001; Mal and Narine 2004). As well, large diurnal temperature variations and full light have been shown to be essential to germination (van der Toorn 1972; Clucas and Ladiges 1980; Kraska et al 1992; Mal and Narine 2004). Seeds from cold-acclimated biotypes seem to require cold stratification for germination, while seeds from more southern populations seem to be able to germinate without acclimation (Galinato and van der Valk 1986; Wachitani and Masuda 1990; Ekstam et al. 1999; Mal and Narine 2004). Not surprisingly, seedlings have a very high first year mortality rate (Washitani and Masuda 1990; Mal and Narine 2004; Brisson et al. 2008). However once seedlings have passed this first year their survival rate increases considerably, and typically require 3 – 4 years to reach sexual maturity (Haslam 1972; Mal and Narine 2004).

Despite the lengthy and detailed work which has been done concerning the methods of sexual reproduction in non-native *P. australis*, the processes involved in its successful invasion and the contribution of sexual reproduction to that invasion success has been all but ignored in the literature until recently, and

current literature is struggling to understand what now seems to be a vital process in the invasion success of *P. australis* (Brisson et al. 2008).

2.4.3. The Contribution of Sexual Reproduction to Population Spread

The non-native conspecific of *P. australis* is believed to have been first introduced to North America from Europe in the early 19th century, with possible subsequent minor reintroductions occurring periodically over time (Brisson et al. 2010; Saltonstall et al. 2010; Hauber et al. 2011). However the species remained an innocuous naturalized invader for many decades until the late 19th century to early 20th century, when drastic increases in highway construction is believed to have facilitated its spread across North America (Saltonstall 2002; Brisson et al. 2010). Non-native *P. australis* has been in Quebec since at least 1916, where a sample was collected for herbarium records along the St. Lawrence River (Lelong et al. 2007; Brisson et al. 2010). For the first few decades of its presence in the province of Quebec, it remained restricted to the St. Lawrence River area (Lelong et al. 2007). However by the early 1980s, rapid road construction across the province from the late 60s to early 70s had led to the formation of vast stands of non-native *P. australis* along roadsides (Gervais et al. 1993; Lelong et al. 2007; Brisson et al. 2010). Currently it is believed that non-native *P. australis* takes advantage of disturbed roadside areas as habitat corridors to allow it to invade new areas (Catling and Carbyn 2006; Lelong et al. 2007; Maheu-Giroux and de Blois 2007; Brisson et al. 2010; LeBlanc et al. 2010). Often propagule material, whether consisting of rhizome fragments or seeds, is moved to these new areas through anthropocentric activities, particularly construction (Meyerson et al. 2009). Once established in a roadside area, it is very simple for populations to move vegetatively into vulnerable areas beside roads which might otherwise be more resistant to establishment (such as wetlands and farm fields), and this method of invasion has been documented in the field numerous times (Koppitz and Kühl 2000; Čurn et al. 2007; Maheu-Giroux and de Blois 2007; Saltonstall et al. 2010).

The method by which the non-native *P. australis* both maintains its populations and spreads to new locations has been a matter of debate within the field of invasion ecology (Brisson et al. 2008; Belzile et al. 2010). Since the earliest publications concerning non-native *P. australis* (and even some of the most recent ones), non-native *P. australis* was considered to spread and maintain its populations almost exclusively through vegetative propagation (Mal and Narine 2004; Belzile et al. 2010). This view is not necessarily unfounded, and can be supported by numerous diverse studies on non-native *P. australis* populations and its biological characteristics. For instance low viable seed set and seedling survival (even in its native Europe) coupled with vigorously spreading rhizomes seems to suggest that non-native *P. australis* evolved to be predisposed to spreading by vegetative means (Halsam 1972; Galinato and van der Walk 1986; Tucker 1990; Gervais et al. 1993; McKee and Richards 1996; Small and Catling 2001; Ishii and Kadono 2002; Alvarez et al. 2005; Brisson et al. 2008). This view was cemented in the literature by a lack of observed seedlings in the field in North America, coupled with several genetics and population growth studies which identified entire populations to be the origin of single clones (Hauber et al. 1991; Gervais et al. 1993; Koppitz 1999; Pellegrin and Hauber 1999; Keller 2000; Small and Catling 2001; Dianat et al. 2007; Brisson et al. 2008). However even during this time there were studies published indicating that some populations of non-native *P. australis* produced seeds with very high germination rates in the lab, even though overall the germination rates over meta-populations were still highly variable (Galinato and van der Valk 1986; Washitani and Masuda 1990; Gervais et al. 1993; McKee and Richards 1996; Ekstam et al. 1999; Ishii and Kadono 2002; Greenewood and MacFarlane 2006; Maheu-Giroux and de Blois 2007). At the same time, genetic and population dynamics studies were being published which identified more than one genotype per population and demonstrated population growth patterns which were not consistent with a single clonal origin (Koppitz 1999; Guo et al. 2003; Křiváčková-Suchá et al. 2007; Krzakowa et al. 2008). Then in 2008, Brisson et al. observed seedlings of non-native *P. australis*

in the field in Quebec, confirming that sexual reproduction was a viable option for population expansion and maintenance in the field in North America. Since then several studies have emerged linking sexual reproduction to population spread and maintenance in the non-native *P. australis* (Kettenring and Whigham 2009; Li et al. 2009; Belzile et al. 2010; Engloner et al. 2010; Kettenring et al. 2010; Krzakowa and Michalak 2010; McCormick et al. 2010a; McCormick et al. 2010b; Saltonstall et al. 2010; Kettenring et al. 2011; Kirk et al. 2011; Paul et al. 2011). Some have even tried to link sexual reproduction to particular steps in the invasion process or as a response to particular environmental characteristics (Alvarez et al. 2005; Brisson et al. 2008; Engloner et al. 2010; Kettenring et al. 2010; LeBlanc et al. 2010; Saltonstall et al. 2010; Kettenring et al. 2011). It has also been suggested that the sudden documentation of seedlings in the northern edge of its range in North America could be a consequence of climate change (Brisson et al. 2008). However as of yet, very little is understood concerning the potential environmental cues which may trigger or allow sexual reproduction to proceed in the non-native *P. australis* (Kettenring et al. 2010; Kettenring et al. 2011).

2.5. Study Species: Native *Phragmites australis* (Cav.) Trin. Ex Steud

For a considerable period of time there was no distinction made between native and non-native conspecifics of *P. australis* in North America (Saltonstall 2002). There were theories that the more aggressive populations of *P. australis* could be an introduced species, but no investigative study was done to determine if there were truly more than one species in North America, and most studies made no attempt to differentiate between the two in their work (Saltonstall 2002). However in 2002, Saltonstall examined the DNA in historical herbarium records and current populations of *P. australis,* and was able to demonstrate that the herbarium records and current populations differed in their mitochondrial DNA, making them distinct cpDNA haplotypes. Since then numerous studies have identified up to 11 different haplotypes of the native *P. australis* in North

America, and several morphological characters have been identified which can be used to differentiate between native and the non-native conspecifics in the field (Saltonstall 2003; Swearingen and Saltonstall 2010). Despite this progress, research into the native *P. australis* has been considerably less than the non-native *P. australis*, and those studies which have been published typically refer to the native *P. australis* only in its capacity to compete against the non-native *P. australis*. That being said, what research had been done is telling. There is evidence that native *P. australis* can spread and invade new wetlands where no non-native *P. australis* is present (Lynch and Saltonstall 2002). Unfortunately more and more, native and non-native *P. australis* inhabit the same environment, and this is a problem for native *P. australis*. In almost all biological characteristics, the native conspecific of *P. australis* seems to be weaker a competitor than non-native *P. australis*. Studies comparing growth between native and non-native populations at similar sites have shown that native plants produce less aboveground biomass, grow shorter, have fewer nodes with shorter intermodal distance, produce shorter leaves, have a later shoot emergence, and overall lower genetic diversity (League et al. 2006; Packett and Chambers 2006; Plut et al. 2011). However the competitive advantage demonstrated by the non-native *P. australis* may be due more to disturbance factors than to an inherent superiority. Several studies have suggested that the native *P. australis* may be a low-nutrient specialist, while the non-native *P. australis* thrives in more high nitrogen environments (Packett and Chambers 2006). Saltonstall and Stevenson (2007) noted that in low nutrient conditions seedlings of the native and non-native *P. australis* achieved similar levels of biomass, but in high nitrogen conditions the non-native plants grew taller, produce more shoots, and accumulate between 3-4 times more biomass than native plants. Mozdzer and Zieman (2010) have also noted that the native *P. australis* has a more efficient photosynthetic mechanism and a lower nitrogen demand than the non-native *P. australis*, and Packett and Chambers (2006) noted much higher levels of nitrogen in the leaves of non-native compared to nearly native *P. australis*. Unfortunately for the native *P. australis*, many of its wetland habitats are being continuously overfed with nitrogen runoff

from anthropogenic factors, which in the context of the most recent research presented here, has likely helped the non-native *P. australis* push the native out of its former habitat (Packett and Chambers 2006; Maheu-Giroux and de Blois 2007; Mozdzer and Zieman 2010).

Although studies have finally begun investigating the life history of native *P. australis*, much remains to be clarified. Research has begun to shine a light onto the local competition dynamics between native and non-native *P. australis*, but there is still relatively little understanding into how non-native *P. australis* had become a wide ranging invader while the native *P. australis* had been rapidly disappearing. Critically, it is not known how native *P. australis* reproduces in the field, or if it even produces seed successfully. The results of the work presented in this thesis accurately address this issue, and provide the first concrete look at how native and non-native *P. australis* are currently growing and reproducing in the field, and how this relationship is likely to change in the future with climate change.

3. Materials and Methods

3.1. Preliminary Studies

Much of the work covered in this project had little precedent in the literature, and it was deemed necessary to perform separate studies on several aspects of the methodology before they could be used confidently in a larger study. As such, several preliminary studies were conducted in 2011. Where the information obtained from this preliminary work was instrumental in determining methodological aspects of the 2012 study, or where results are shown to correspond with results obtained in the 2012 study, information is presented in this thesis and will be identified as belonging to the 2011 preliminary study (Appendix Table S.1). This information is provided for reference and comparison only.

3.2. Study Area

The study area covered a region spanning 1000 km from Secaucus (New Jersey, USA) to Alma (Quebec, Canada). This region encompasses five distinct isotherms (Figure 3.1), including the coldest known isotherm of non-native *P. australis* occurrence in North America. Native *P. australis* has been reported at even colder isotherms, but no populations could be found within the study area during the timeframe of this study (Mal and Narine 2004; Swearingen and Saltonstall 2010). The isotherms of this study range from 11.8ºC average annual temperature in the southern most isotherm to 2.3ºC in the northern most isotherm. Study locations were chosen in each isotherm within the regions of Secaucus (New Jersey), Catskill (New York), Montreal (Quebec), Quebec City (Quebec) and Alma (Quebec) (Table 3.1). Isotherms were determined based on temperature data from 52 meteorological stations across Eastern North America from 1971-2000 as generated by the World Climate Research Programme's (WCRP's) Coupled Model Intercomparison Project phase 3 (CMIP3) multi-model dataset (Figure 3.1).

Populations of non-native *P. australis* were found throughout the study area. Within each isotherm, four geographically separate study sites were selected to monitor non-native populations (Appendix Table S.2). Native populations were considerably rarer, and could only be found within Canadian isotherms during the timeframe of this study. Three native sites were selected within each of the three Canadian isotherms (Appendix Table S.2). Native and non-native populations were distinguished morphologically using the field guide compiled by Swearingen and Saltonstall (2010), work reported by Catling et al. (2007) and previous field experience in our laboratory (Taddeo and de Blois). Populations were considered either native or non-native if they demonstrated four or more distinctive morphological characters considered to be deterministic of their status (Swearingen and Saltonstall 2010). Sites were placed along paved roadways, as it

was determined that these areas contained similar disturbance regimes, wind conditions, soil composition (an artefact of road construction), and soil salinity levels; they are known to be a preferred habitat for *P. australis* (Catling and Carbyn 2006; Jodoin et al. 2008; Lelong et al. 2009; Lavoie et al. 2010). Sites were also placed a minimum of 15 km from adjacent sites to reduce the chance of cross pollination. As well, sites were only placed in populations which displayed signs of past sexual reproductive success through the presence of last year's canes and inflorescences. All sites were later found to have near 100 % inflorescence production among canes. Given the difficulty in locating native populations, two sites within the Montreal isotherm could not be placed adjacent to a roadway but were located on the shores of a freshwater island. Within each study site, three distinct 1 m^2 plots were placed on the edges of *P. australis* populations. Population edges were found primarily within ditches; therefore, plots were placed along the slopes of these ditches in order to incorporate the moisture gradient inherent in the slope.

Within each plot, the 10 tallest plants were flagged for study. As a result, 270 native plants and 600 non-native plants were included in this study. Given the objectives of this study, this was done to select individuals with the greatest reproductive potential to ensure that seeds could be sampled in the field, as previous research had documented a low and sporadic seed set in *P. australis* in North America (Gervais et al. 1993; Mal and Narine 2004).

Utilizing isotherms to distribute sites along a latitudinal gradient allowed us to ensure annual temperature differences across the study area. However in order to more accurately quantify differences in growing seasons between isotherms, growing degree days (GDDs) within isotherms were used as the main climate variable in our study. Recent work has also identified GDDs as the most important climatic variable in determining the distribution of *P. australis* in eastern North America (de Blois et al. in prep.). The number of GDDs for each isotherm was taken to be a combination of GDDs accumulated between March 1st

2012 and November 30th 2012, as this is the maximum growing season for *P. australis* within the study area (Wielgolaski 1999). This value will be termed 'seasonal GDDs' hereafter in order to distinguish between GDDs values calculated during the developmental study, and seasonal GDDs used to differentiate isotherms. GDDs used throughout this study were calculated using the standard equation (Eq 3.1), and according to the 'standard 1' interpretation of GDDs identified by McMaster and Wilhelm (1997). In estimating T_{base}, it has been shown than underestimating T_{base} has a limited effect on differences in developmental date estimations, while overestimating T_{base} can have a large effect (Durand et al. 1982; Bonhomme 2000). T_{base} has been shown to vary in perennial grasses from 4ºC to as much as 10ºC, however no temperature thresholds have been yet identified in either *P. australis* species (Bonhomme 2000; Haslam 2010; Moot 2010). As such, T_{base} was chosen to be 4ºC in order to minimize potential error. Although T_{max} is typically recommended to be capped at 30ºC, several studies have noted much higher temperature thresholds (Holt and Orcutt 1996; Bonhomme 2000). *P. australis* populations are found in many areas of the world where daytime temperatures often reach higher than 30ºC, and there is no evidence that growth stops at this temperature (Cross and Zuber 1972; McMaster and Wilhelm 1997). Therefore, given this knowledge of *P. australis'* biological needs, as well as the fact that a minority of days in the study area went above 30ºC, no T_{max} was set in this study. Where T_{min} fell below T_{base}, T_{min} was set to T_{base} (McMaster and Wilhelm 1997). When neither T_{max} nor T_{min} were greater than T_{base}, the number of GDDs set for the calendar date was 0 (McMaster and Wilhelm 1997). Daily local temperature data was obtained by taking an average of the three closest weather stations to all sites within each isotherm (Appendix Table S.3). For Canadian isotherms, temperature and precipitation data was collected from the National Climate Archive. For American isotherms, temperature and precipitation data was taken from the National Weather Service through Weather Underground.

$$\text{Growing Degree-Days} = \left(\frac{T_{max} + T_{min}}{2} \right) - T_{base} \qquad \text{(Eq. 3.1)}$$

3.2.1 Impacts of Hurricane Sandy

During the 2012 study season, sites within the Secaucus isotherm were exposed to a hurricane during a critical development period after pollination but before complete seed maturation. Hurricane Sandy made landfall in New Jersey on October 29th 2012. Wind speeds were estimated to be approximately 70 kt at landfall, although they may have been as high as 83 kt is select areas (Blake et al. 2012). Minimal central pressures at landfall were estimated to be approximately 945 mb (Blake et al. 2012). The Hudson county of New Jersey, in which Secaucus was located, also saw storm surges between 3 – 7 feet (Blake et al. 2012).

It was not possible to enter the study area while Hurricane Sandy was occurring, and even for several days after the storm it was not possible to travel to the city. Because of this, it is impossible to determine whether sites were exposed to high wind speeds and flooding. In the discussion section, results will be interpreted to take into consideration the possible impacts of Hurricane Sandy.

3.3. Data Collection

3.3.1. Developmental Timing

No precedent existed in the literature to determine the timing of developmental events in *P. australis* within the study area. Therefore during the 2011 season, a preliminary study was conducted to assess both the approximate initiation dates and physical changes involved in two important developmental events in non-native *P. australis*: emergence of the inflorescence from the cane and flowering (defined as the emergence of stamens). To track initiation dates of developmental events, populations at locations from Montreal, Quebec City and Alma were visited weekly to bi-weekly. Attempts were also made to track development in native *P. australis* at these locations, but in all cases it was found

that the native *P. australis* populations developed considerably earlier than non-native *P. australis* and the dates of initiation for native populations were almost always missed as a result. To study the physical changes and the length of time required to undergo developmental events, three invasive populations in Montreal were monitored daily starting from early July (prior to the emergence of the inflorescence) until the end of flowering in September. For inflorescence emergence, the length of inflorescence was recorded over time. During the flowering stage of development, a detailed description of physical changes (from the emergence of stamens, to the emergence of stigmas, to the senescence of stamens and finally the senescence of stigmas) was recoded over time. This information was used to design a sampling schedule in 2012 that would ensure the maximum number of useful observations could be made within the limitations of the season and traveling distances, and to ensure that the approximate dates of development could be estimated for each plant even if the direct observation of development events were missed between visits.

In 2012, each site was visited bi-weekly beginning in early July to estimate the initiation dates of two easily identifiable developmental events: the emergence of the inflorescence from the cane, and flowering (defined as the emergence of stamens). It was impossible to constantly monitor each individual study plant during this period considering the extent of the study area and the overlapping timing of developmental events across isotherms. A degree of measurement error was therefore unavoidable and was quantified in the most conservative manner possible. Initiation dates were determined both through direct observation, and through utilizing the knowledge and data collected in 2011 to estimate the most likely date of initiation of an event based on the physical state observed in the plant during the visit, the time elapsed since the last visit, and knowledge on the duration of a particular stage of development. For example an inflorescence observed to have undergone no flowering on one visit, was found to have florets with emerging stigmas and browning styles during a subsequent visit 2 weeks later. Data from the 2011 preliminary study has shown that a minimum of

7 calendar days are required for florets to develop browning stigmas and emerging styles. As well, it is known from the first visit 2 weeks prior that no development had occurred during the first visit. Therefore the maximum date for the initiation of flowering is the day after the first visit (the visit which found no development), and the minimum date for the initiation of flowering is the date predicted by the 2011 study. The dates for the initiation of development for each plant were taken to be an average of the minimum and maximum dates of development observed for that plant in the field. These dates were then transformed into GDDs to allow for comparison between isotherms (Eq. 3.1). In some cases individuals were damaged mechanically or destroyed over the course of the study. In these cases new plants were selected within the same plot, or if a plot was destroyed, a new plot was placed within the same site and plants were selected from within the new plot. When new plants were selected, they were chosen to be the remaining tallest within a plot.

3.3.2. Production of Viable Seeds

In 2011 a preliminary study was done to determine the optimal time of seed collection for *P. australis*. Given the wind-dispersed nature of the plant, there was concern that viable seeds may be the first to disperse and therefore when the inflorescences are collected, enough viable seeds may have already dispersed that the measured seed count could be under-representative of true levels of seed production. The placement of study plots and sites were very similar between this preliminary study and the larger study conducted in 2012. In the preliminary study, two sites were placed within each isotherm from Montreal to Alma, with 10 $1m^2$ plots within each isotherm and the 10 tallest plants flagged within each plot. A random sample of half of the 10 flagged plants within each plot had pantyhose placed over inflorescences at the end of the flowering season but before seed maturation. Pantyhose prevents the dispersal of seeds, while allowing air to naturally reach the inflorescences within the pantyhose. Plant height from the soil line to the base of the inflorescence was also measured at the

same time. However when plants were collected and examined in late November and early December, there was no difference in seed count between plants with and without pantyhose ($p = 0.2767$). Therefore it was determined that late November to early December was an acceptable time to harvest plants without losing a significant number of seeds to dispersal.

In 2012, all inflorescences from flagged plants followed during the developmental study were collected after cane senescence over a period of two weeks from late November to early December. At the same time, the height of each cane was measured from the soil-line to the base of the inflorescence. Where flagged plants had been damaged over the course of the season, new plants were selected from the remaining tallest plants within the plot. Inflorescences were dried in paper bags for one week, and then seeds were removed from panicles manually. For each cane, 50 dispersal units (defined as florets with the undamaged infertile lemnas and paleas) were examined for the presence of viable seeds, non-viable seeds and *Claviceps* sclerotia. In total, 43 500 dispersal units were examined over the course of this study. Viable seeds were defined according to the classical unimbibed seed crush test (Sawma and Mohler 2002). Seeds were required to be full and round, have a dark color which appeared amber in full light and to resist crushing (Sawma and Mohler 2002; Kettenring and Whigham 2009). Seeds which were deemed viable by the unimbibed seed crush test will be hereafter referred to as 'visually viable seeds' to distinguish them from seeds shown to be viable through germination. Non-viable seeds were defined as those seeds which were otherwise formed (successful fertilization took place) but appeared to be incapable of germination. Such seeds appeared shrunken, broken, very small or off-coloured, and could be crushed very easily (Sawma and Mohler 2002; Kettenring and Whigham 2009). Visually viable seed from individual plants were pooled into representative samples for each isotherm and conspecific combination, for a total of 8 treatments in all. Numbers of non-viable seed, viable seed, and sclerotia were recorded and transformed into percent values representative of the percentage of florets which would produce either non-viable

seed, visually viable seed, sclerotia, or empty seed dispersal units based on our results.

Viability was ultimately determined through a germination test with the visually viable seed identified by the classical unimbibed seed crush test. Stratification requirements are an issue of contention in *P. australis*, with studies both proving and disproving its use in increasing seed germination rates (Galinato and Van der Valk 1986; Kettenring and Whigham 2009). Preliminary studies done in 2011 with seeds from the Montreal and Quebec City populations showed no marked difference in germination rate between seeds which had been either stored at room temperature or cold moist stratified at 4ºC for one month ($p = 0.5079$). For the 2012 study, stratification was removed entirely. For each isotherm and conspecific treatment, three replicates of 100 seeds each were placed in a randomized block design to control for the effect of shelf position within the growth chamber. Visually viable seeds were placed on moist filter paper and sealed in a 5 cm diameter Petri dish. 10 seeds were placed in each dish. Petri dishes were kept in a growth chamber for 12 hour days at 30ºC/15ºC, as large temperature fluctuations are shown to be needed to break dormancy in *P. australis* (Ekstam et al. 1999). Seeds were observed for one month, and germination was considered successful when the radicle was observed to have emerged from the caryopsis (Kettenring and Whigham 2009). The occurrence of fungal contamination within seeds was also noted. Seeds determined to be viable according to both the unimbibed seed crush test and the germination test will be referred to hereafter as 'germination-capable seeds' to distinguish them from seeds deemed viable solely by the unimbibed seed crush test. Fungal growths found to have contaminated germinated seeds consisted of several species which proved difficult to differentiate. As such, these were not identified to species, and instead were quantified as a whole.

3.4. Statistical Analysis

To evaluate the effect of seasonal GDDs, as a measure of climate effect, on height, GDDs for developmental events, seed count, or *Claviceps* occurrence, mixed models were used. Site and plot were treated as random factors within the model, while seasonal GDDs was fixed (Bennington and Thayne 1994). Plot was nested within site and site was nested within seasonal GDDs. The same model was used to determine the effect of height on the production of visually viable seed, with height used as the independent variable in the place of seasonal GDDs. A mixed model was also used to evaluate the effect of the pantyhose treatment during the 2011 preliminary study where pantyhoses were placed over half of the plants within each plot. Pantyhose was treated as a fixed factor nested within plot. Restricted estimated maximum likelihood (REML) was used to estimate the coefficients of the model and a likelihood ratio test (LRT) was used to determine if seasonal GDDs contributed to explain the variance.

To evaluate the effect of seasonal GDDs on germination rate from the germination trial, a mixed model incorporating replicate and seasonal GDDs was used. Replicates were used to control for the effect of shelf position and were thus treated as random factors within the model, while seasonal GDDs was fixed (Bennington and Thayne 1994). Replicate was nested within seasonal GDDs. The effect of the stratification treatment of the 2011 preliminary study was also tested similarly with stratification nested within seasonal GDDs. REML was used to estimate the coefficients of the model and a LRT was used to determine if seasonal GDDs contributed to explain the variance. Statistical analysis was done with the open source statistical program, R version 2.15.3, and library package lmer4 version 0.999 (Bates et al. 2012; R Core Team 2012).

Table 3.1 Environmental averages of study locations[1]

Study Locations	Annual Average Temperature	Annual Average Precipitation	Seasonal Growing Degree-Days[1]
Secaucus	11.8 ºC	1201.36 mm	3829.33
Catskill	8.9 ºC	1139.47 mm	3155.67
Montreal	6.2 ºC	767.9 mm	2698.07
Quebec City	4.0 ºC	923.8 mm	2266.82
Alma	2.3 ºC	591.2 mm	2131.37

1. Seasonal growing degree-days were calculated according to equation 3.1.

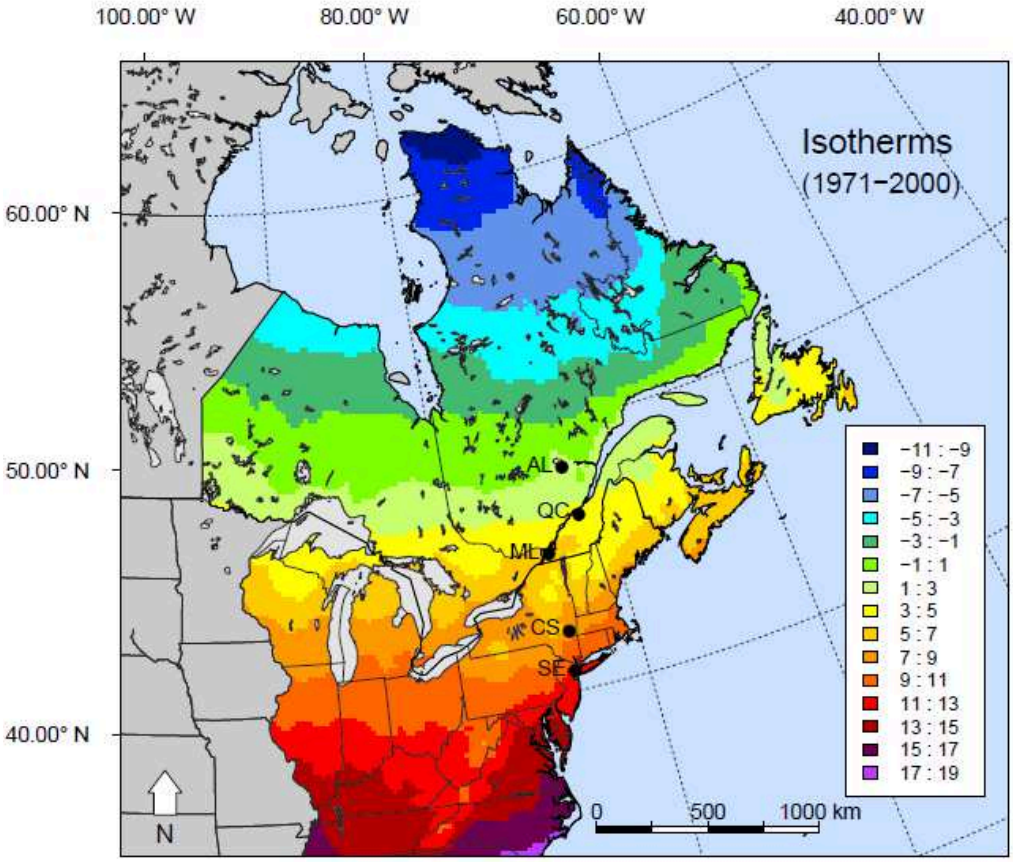

Fig. 3.1. Location of study sites within isotherms

4. Results

4.1. Developmental Timing

Although there was a degree of measurement error incurred during the developmental study, seasonal GDDs were shown to have an effect on the emergence of inflorescences ($p<0.0001$) and the initiation of flowering ($p<0.0001$) in non-native *P. australis* (Figure 4.1). As seasonal GDDs decreased across isotherms, the cumulative number of GDDs required to initiate both inflorescence emergence and flowering also decreased. As well, it was observed in the field that plants from isotherms with fewer seasonal GDDs seemed to undergo developmental events much quicker than plants from isotherms with a greater number of seasonal GDDs. Average inflorescence emergence and flowering times were very similar between the preliminary study conducted in 2011 and results obtained in 2012.

4.2 Height

The relationship between seasonal GDDs and height differs between conspecifics. Within non-native *P. australis*, results indicate that there is a direct relationship between seasonal GDDs and plant height ($p=0.0002$). Results seen in Figure 4.2 would suggest that within the study area as seasonal GDDs increases, overall plant height increases linearly. Preliminary studies in 2011 also noted a significant relationship between height and seasonal GDDs across populations from Alma to Montreal ($p = 0.0013$). Within native *P. australis*, there was no relationship between seasonal GDDs and plant height. Although height was found to be greatest in Montreal populations and decreased in the Alma populations, height was shortest overall in Quebec City populations (Figure 4.2). However, within each isotherm plants of native *P. australis* were consistently shorter than plants from the non-native conspecific. This occurred even when native and non-native populations were located at the same site or were in adjacent sites.

4.3. Seed Production

In native *P. australis*, likely factors other than seasonal GDDs affect the production of visually viable seed. Native plants within the isotherm with the fewest seasonal GDDs actually demonstrated the highest levels of production of visually viable seed in this study at 40.45% (Table 4.1). Additionally, in two out of the three isotherms native *P. australis* produced more seeds on average than its non-native conspecific within the same isotherm. Only native plants in the Quebec City isotherm performed worse than non-native plants. Within non-native *P. australis*, results indicate a relationship between seasonal GDDs and seed production ($p=0.0044$). As with plant height and developmental timing, the production of visually viable seed in non-native *P. australis* decreased as seasonal GDDs decreased (Table 4.1). Non-native plants in the northernmost populations in Alma produced seeds at a rate of 0.05%, while populations in Secaucus saw production rates of 19.57% overall. Preliminary studies in 2011 report similar results in seed production in non-native populations from Alma to Montreal ($p = 0.0349$).

As well, was no correlation was identified between plant height and the production of visually viable seed in either the native or non-native *P. australis*. This is consistent with data collected during 2011 preliminary study, which demonstrated no relationship between height and seed production in non-native populations from Montreal to Alma.

4.4. Germination test

There was no relationship between seasonal GDDs and the germination rate of visually viable seeds in either native or non-native *P. australis* (Table 4.1). Non-native *P. australis* maintained a similar germination rate between 11.33% and 23.00%, except populations from the Alma isotherm which did not produce

enough seeds to test statistically (Table 4.1). Germination rates during the 2011 preliminary study could not be compared statistically because of a lack of seeds from the Alma populations relative to the other two isotherms. Germination rates for visually viable seeds of native *P. australis* were found to range between 12.00% and 20.67% overall. As well, native *P. australis* had greater germination rates than non-native *P. australis* in two of the three isotherms. As with seed production, only native plants from the Quebec isotherm performed worse than their non-native conspecifics.

Although little relationship was found between seasonal GDDs and germination rate, when rates of visually viable seed production are taken into consideration with germination rates, a relationship with seasonal GDDs does emerge in non-native *P. australis* (Table 4.1). For instance, while in the Secaucus isotherm an average of 19.57% of florets were likely to produce visually viable seed, only 12.33% of visually viable seeds were found capable of germination during the germination trial. Therefore in reality, only an average of 2.41% florets within the Secaucus isotherm are capable of producing seed which will ultimately germinate (germination-capable seed), and this number represents the most accurate measure of propagule output within the isotherm (Table 4.1). Overall non-native populations in the southern two isotherms (Secaucus and Catskill) produce the most germination-capable seed, while the production of germination-capable seed then decreases as seasonal GDDs decrease. In native *P. australis*, seasonal GDDs have no effect on the production of germination-capable seeds.

4.5. Pathogen Occurrence

The relationship between seasonal GDDs and the occurrence of *Claviceps* sp. is different between conspecifics. Native *P. australis* populations show no relationship between *Claviceps* occurrence and seasonal GDDs. However, sclerotia occurrence was lower in native than non-native *P. australis* in all isotherms. In non-native *P. australis* populations, a relationship was found

between the occurrence of *Claviceps* sclerotia and seasonal GDDs ($p = 0.0316$). During the 2011 preliminary study with non-native *P. australis*, a relationship between seasonal GDDs and *Claviceps* sclerotia was also observed ($p = 0.0032$).

A complex relationship was found between fungal contamination rates in seeds during germination and seasonal GDDs. With non-native *P. australis*, there was no relationship between seasonal GDDs and contamination rates in germinating seeds in either the 2012 study or the 2011 preliminary study. However within the native *P. australis*, results indicate a relationship between seasonal GDDs and fungal contamination of seeds during germination ($p = 0.0018$). Seed contamination was lowest in the warmest isotherm and increased with decreasing seasonal GDDs, with plants from the coldest isotherm demonstrating the highest levels of contamination in germinating seed. There was also no clear relationship between seed contamination rates during germination across native and non-native conspecifics within the same isotherm.

Table 4.1 Viable seed and germination rates across isotherms and conspecifics

Isotherm	Conspecific	Visual Viable Seed[1]	Germination Rate[2]	Germination-Capable Seed [3]
Secaucus	Non-native	19.57 %	12.33 %	2.41%
Catskill	Non-native	21.32 %	18.33 %	3.91%
Montreal	Non-native	13.90 %	11.33 %	1.57%
	Native	23.53 %	13.33 %	5.41%
Quebec City	Non-native	2.95 %	23.00 %	0.39%
	Native	0.49 %	12.00 %	0.06%
Alma	Non-native	0.05 %	0.00 %[4]	0.00%[4]
	Native	40.45 %	20.67 %	8.36%

1. Percent of 50 intact seed casings found to have viable seed as determined by the classical unimbibed seed crush test. 2. Percent of viable seed which germinated during the germination trails. 3. The percentage of florets capable of producing germination-capable seeds. 4. So few seeds were found in Alma that germination rates could not be measured accurately.

Table 4.2 Rates of occurrence for *Claviceps* sclerotia and seed contamination during germination across conspecifics and isotherms

Isotherm	Conspecific	*Claviceps* sp. Occurrence[1]	Rates of Seed Contamination[2]
Secaucus	Non-native	13.21 %	18.66 %
Catskill	Non-native	6.20 %	19.66 %
Montreal	Non-native	8.20 %	34.33 %
	Native	0.64 %	27.00 %
Quebec City	Non-native	8.72 %	24.67 %
	Native	2.71 %	32.00 %
Alma	Non-native	3.84 %	0.00 %[1]
	Native	0.00 %	47.67 %

1. This number is a reflection of the very low number of seeds extracted from Alma populations and is not an accurate portrayal of true levels of seed contamination in Alma.

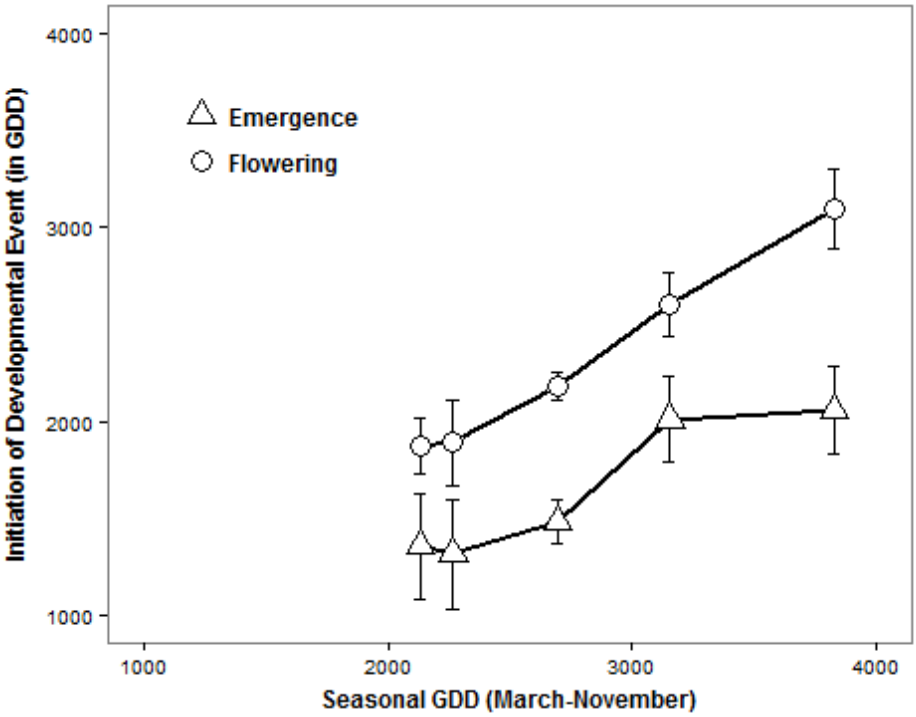

Fig. 4.1 Average cumulative GDDs with error bars representing measurement error required to initiate the emergence of inflorescences and flowering in non-native *P. australis* populations in relation to seasonal GDDs

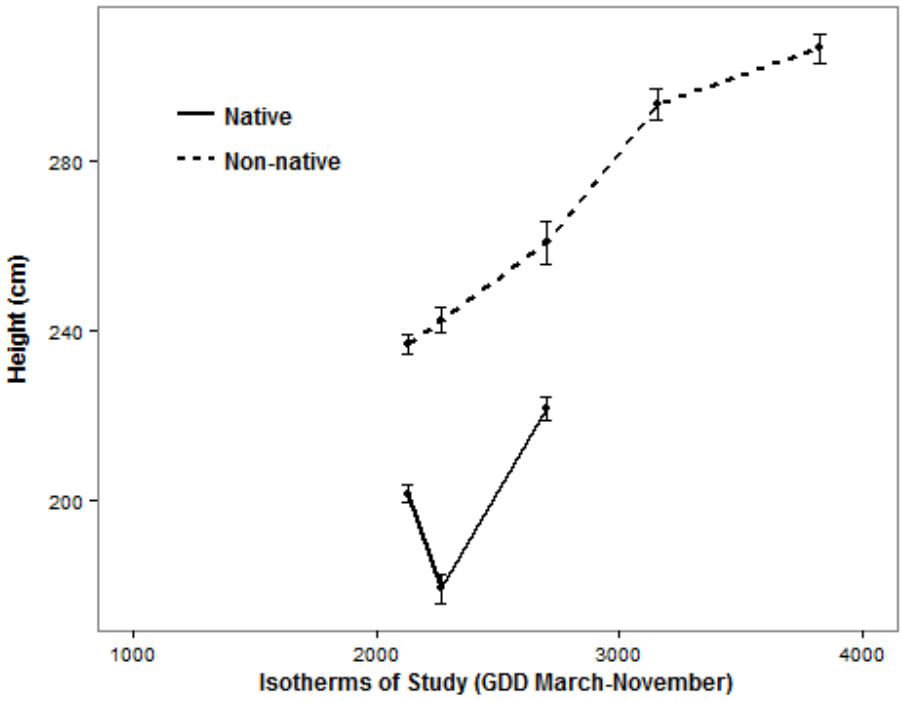

Fig. 4.2 Average height of native and non-native conspecifics of *P. australis* in relation to seasonal GDDs

5. Discussion

5.1. Developmental Timing

Seasonal GDDs have a significant effect on developmental timing in non-native *P. australis*. As seasonal GDDs decrease, plants develop earlier in the calendar year and require fewer GDDs to reach important developmental events. As well, differences in the length of developmental events were observed in the field, although could be not tested statistically due to measurement error. Plants within the southern-most isotherm in Secaucus were observed to require weeks to undergo developmental events, and at any one time several individuals were at many different stages of development within a single stand. Plants in the northern-most isotherm in Alma were observed to develop much quicker, taking at most a week for each site. As well, all plants within a stand were observed to develop almost synchronously, undergoing each developmental step at the same time. While available resources and differences in development timing between native and non-native *P. australis* did not allow for the detailed tracking of development in native *P. australis*, it was observed that within the same isotherm native *P. australis* developed much earlier than non-native *P. australis*, sometimes in the order of weeks. Studies have suggested that native and non-native *P. australis* may be capable of hybridizing (Meyerson et al. 2010; Paul et al. 2010; Saltonstall 2011). Meyerson et al. (2010) were able to produce hybrids from plants grown in greenhouse conditions in their study. They even noted an overlap in flowering time from native and non-native populations from the same geographic region of origin by an average of 5.25 days (Meyerson et al. 2010). However the native and non-native populations they crossed were not from the same sites (Meyerson et al. 2010). This study has found that populations from areas as close as Catskill and Secaucus (approximately 180 km) can have significantly different developmental timing despite their close geographic location. Therefore despite the positive results Meyerson et al. (2010) noted, it is uncertain if the plants they used in their study would truly have overlapping flowering times in the field. Paul et al. (2010)

reported finding natural hybrids in the field in Southern Ontario. As of yet this is the first and only study which has reported the presence of natural hybrids in the field, and it is uncertain if unique conditions in Southern Ontario have allowed these hybrids to form while preventing hybridization elsewhere. The differences in flowering timing observed in this study between native and non-native *P. australis* suggest that within the Canadian portion of the study area, natural hybridizing between native and non-native invasive *P. australis* would be difficult and this may help explain why very few hybrids have been found despite repeated testing (Saltonstall 2003; Meyerson et al. 2010; Saltonstall 2011).

Very few studies from North America have examined clinal variation and developmental timing in *P. australis*, and any that have are complicated by the fact that native and non-native *P. australis* were only distinguished as separate species in 2002 (Saltonstall 2002). One of the few was done by Gervais et al. (1993), and although investigating clinal variation was not a focus of their study, they did note that plants from the northern edge of their study area (Alma, QC) flowered much quicker than plants in more southern areas. Unfortunately Gervais et al. (1993) did not distinguish between native and non-native populations, so it is difficult to say if their study included solely non-native *P. australis* or was complicated by the inclusion of native populations. Studies from Europe are much more definitive concerning the relationship between temperature and development in non-native *P. australis*. However Europe-focused studies must be considered with caution. Despite the fact that the non-native *P. australis* in North America has likely been introduced directly from Europe, founder effects including potential genetic drift and local adaptation may have altered the non-native to the point where is may behave differently in North America compared to its native Europe (Broennimann et al. 2007). This has been recorded in the past with other introduced species (Broennimann et al. 2007). In the context of Eurasian studies, the Phragmites species referred to as non-native *P. australis* within North America will be referred to as 'Eurasian *P. australis*' to avoid confusion with studies focusing on North American native and non-native *P. australis*

populations. It is well documented in the European literature that with Eurasian *P. australis* populations, developmental timing is very closely related to local temperature, with plants from colder climates developing earlier and quicker than plants from warmer climates (Haslam 1969; Clevering et al. 2001; Bastlova et al. 2004; Bastlova et al. 2006; Hansen et al. 2007; Haslam 2010). For instance, plants from England are known to require 4-5 months to flower (Haslam 2010). This period of development is similar to what was observed in non-native *P. australis* in our study. However populations from the southern location of Malta can commonly reach 8 months of age before they flower (Haslam 2010). As well, several common garden studies have shown these climate adaptations to be fixed. For instance, Haslam (2010) noted that plants transplanted from Malta to England failed to finish their lifecycle within England's shorter growing season (Haslam 2010). As well, Clevering et al. (2001) noted that plants taken from locations varying as widely as Northern Sweden and Spain retained their original developmental timing when grown in a common garden in the Netherlands. Plants from Northern Sweden completed their life cycle very early compared to the local growing season, while plants transplanted from Spain actually failed to complete one growing cycle in the shorter growing season of the Netherlands (Clevering et al. 2001). Bastlova et al (2006) noted similar results in their study with plants taken from locations along a gradient from Sweden to Spain, and suggested a similar gradient may be found in non-native *P. australis* populations within North America

5.2. Height and Seed Production

Seasonal GDDs have a significant effect on both height and the production of visually viable seed in non-native *P. australis*. As seasonal GDDs increase, average cane height increases. With the production of visually viable seed, average seed production increases as seasonal GDDs increase until the Secaucus isotherm, where seed production numbers are similar to the Catskill isotherm. However within the Secaucus isotherm, Hurricane Sandy passed directly over

study sites when seeds were beginning to mature. Considering that *P. australis* seeds are wind dispersed, it's very possible that greater seed production would have been seen in the Secaucus isotherm had Hurricane Sandy not occurred at this particular stage of development. Within native *P. australis*, the relationship between seasonal GDDs and both height and seed production is more complicated. Results from this study would seem to suggest that there is no relationship at all between seasonal GDDs and both height and seed production. However in both respects, plants from the Quebec City isotherm vastly underperformed compared to native plants from the Alma and Montreal isotherm. Study populations in Montreal and Alma existed as monophyletic stands of native *P. australis*. Within the Quebec City isotherm, the only native *P. australis* populations which could be located existed as mixed stands of native and non-native *P. australis*, and ultimately all native sites from the Quebec City isotherm were located within these mixed stands. As well, the mixed stands consisted of a minority of native *P. australis*, which for all appearances seemed to be in the process of being eliminated from the stand through competition with the non-native *P. australis*. It is impossible to say for certain if competition with non-native *P. australis* explains the poor performance of Quebec City sites relative to sites from the Montreal and Alma isotherms. However if this were in fact the case, different conclusions could be drawn from this study. For instance, height in native *P. australis* is greatest in Montreal and decreases in Alma at a similar rate to non-native *P. australis*, which could suggest there is actually a relationship in native *P. australis* between height and seasonal GDDs. Alternatively, differences seen in native *P. australis* populations between isotherms could be attributed to different native haplotypes. While non-native *P. australis* populations in North America are believed to consist mainly of a single haplotype, native *P. australis* populations have been shown to have at least 11 different haplotypes (Kettenring et al. 2012). Unfortunately native haplotypes have so far only been identified through genetic studies, and as of yet no morphological characters are known to distinguish between haplotypes in the field. As well, it is uncertain if different haplotypes within the same lineage would result in different genotypes. Likely the

differences seen between the non-native and native lineages is a result of the different selective pressures experienced by these two groups in their native habitats, and not intrinsically linked to different haplotypes. Native *P. australis* has also been shown to be less tolerant of high soil salinity than non-native *P. australis* (Vasquez et al. 2005). Despite sites being placed to minimize abiotic differences, it is possible that salinity differences between sites could affect the results of native *P. australis* while showing no measureable effect on non-native *P. australis*. Nevertheless, what is clear is that the relationship between seasonal GDDs and the production of visually viable seeds in native *P. australis* is very different from non-native *P. australis*. The greatest rates of seed production in native *P. australis*, and in fact in the entire study, are from the most northern native populations in Alma. This suggests that unlike non-native *P. australis*, fewer seasonal GDDs do not restrict seed production in native *P. australis* within the study area.

No North American studies have examined clinal variation in either height or seed production with non-native or native *P. australis*. However as with development, several European studies have examined the clinal variation in height and seed production in Eurasian *P. australis* (Haslam 1969b; Spence 1964; Björk 1967; Nikolajevsky 1971; Kühl and Kohl 1992; McKee and Richards 1996; Clevering et al. 2001; Bastlova et al. 2004; Bastlova et al. 2006; Haslam 2010). Like development, height has been found to relate to current local temperature and to be fixed. Halsam (1969b) noted in her study that daily temperature had a direct positive effect on height in Eurasian *P. australis*, although plants from the colder climate of England saw a greater positive effect from increasing daily temperature than did plants from the warmer Malta region. As well, Bastlova et al. (2004) noted in their study that when plants from a latitudinal gradient of Sweden to Spain were planted in a common garden in the Czech Republic, increasing height was negatively correlated with latitude of origin. They also noted that several other vegetative characters, including the total number of nodes and the number of live leaves, could be accurately used to distinguish plants along

the geographical gradient (Bastlova et al. 2004). Not only do warmer days and nights increase overall growth rate, but warmer weather at the beginning of the growing season leads to an earlier emergence of buds which subsequently are more likely to grow taller during the growing season (Haslam 1969b; Spence 1964; Kühl and Kohl 1992; McKee and Richards 1996; Clevering et al. 2001; Bastlova et al. 2006; Haslam 2010). There also seems to be a correlation between height and sexual reproductive success, with taller plants being more likely to flower and producing larger seed (Haslam 1972; McKee and Richards 1996; Haslam 2010). However this study found that there was no direct relationship between plant height and seed production, and as of yet no other study has directly investigated the effect of biomass on seed production rates.

Seed production in both Eurasian populations of *P. australis* and North American non-native populations of *P. australis* has traditionally been seen as erratic and not associated with a temperature gradient, and no published studies have as yet examined seed production in native *P. australis* (Hauber et al. 1991; Gervais et al. 1993; McKee and Richards 1996; Koppitz 1999; Pellegrin and Hauber 1999; Keller 2000; Small and Catling 2001; Dianat et al. 2007; Brisson et al. 2008). However a study in Russia did note increasing seed fertility in Eurasian *P. australis* populations with decreasing latitude, and a study in Sweden noted depressed seed set in populations which had experienced cold summer temperatures (Björk 1967; Nikolajevsky 1971). As well, McKee and Richards (1996) noted that increasing local temperatures were associated with increasing seed weight with plants in Britain, and that increased seed weight could be positively correlated with increasing seed production. However they were unable to find a direct relationship between temperatures and seed production (McKee and Richards 1996). Several studies have attempted to explain extreme differences in seed sets between populations, particularly in northern areas. Before non-native *P. australis* was found produce seed in the field in North America, it was previously assumed that the clonal nature and possible self-incompatibility of non-native *P. australis* created monoclonal populations

incapable of pollination and proper seed production (Curran 1969; Gustafsson and Simak 1963; Satyamurty and Seshavatharam 1984; Clevering and Lissner 1999; Small and Catling 2001; Ishii and Kadono 2002; Mal and Narine 2004; Kettenring et al. 2011). Ishii and Kadono (2002) confirmed that populations of Eurasian *P. australis* in Japan were pollen limited, as additional pollen supplemented by researchers resulted in increased seed set. Several studies examining pollination and self-incompatibility in non-native *P. australis* have also noted that pollen is produced and germinates readily, suggesting that although seed production may be pollen limited, infertile or poor pollen production is not the issue (McKee and Richards 1996; Ishii and Kadono 2002; Meyerson et al. 2010; Kettenring et al. 2011). As well, several studies have also shown non-native *P. australis* to be self-fertile, although self-pollinated plants have been found to produce fewer seeds than cross-pollinated plants (Lambert and Casagrande 2007; Kettenring et al. 2010; Kettenring et al. 2011). These studies have demonstrated that neither infertile pollen nor self-incompatibility is consistently limiting seed production. Poor seed set, particularly in northern populations, may be explained by the physical changes which occur during flowering in non-native *P. australis*. This study observed that flowering in non-native *P. australis* followed distinct stages. Stigmas emerged first and remained healthy and full for several days. As stigmas started to brown and die, stamens would begin to emerge from the florets. The full emergence of stigmas from florets coincided with the death of the stamens. As well, typically all florets within a single inflorescence would undergo these stages at the same time. The separation of the stamen and stigma emergence from the floret would suggest that the flower prioritizes one stage of flowering at a time, first pollen production and then ovule fertilization. If this is the case, likely it is difficult for a non-native *P. australis* inflorescence to pollinate itself if in most cases the optimal stage of pollen production precedes the emergence of its stamens. This may not be a problem when stands consist of many plants undergoing flowering at different stages over an extended period of time, as was observed in southern populations of *P. australis*. However plants in northern populations were observed to undergo flowering very quickly and all plants in the

stand seemed to develop at very similar times and rates. It is possible that this synchronization exists because these smaller northern populations are represented by a single clone, and that low genetic diversity within the stand is currently the most important factor limiting seed production in northern populations. Kettenring et al. (2011) noted in their study that both self-pollination and low genetic diversity within stands depressed seed production. However the brief window for pollination which this rapid development allows also suggests that even if plants were completely self-compatible, there may not be enough overlap between the production of stamens and stigmas to allow for proper fertilization in the northern-most populations of non-native *P. australis*.

5.3. Germination

The germination rate of visually viable seeds of both native and non-native *P. australis* was not affected by seasonal GDDs. Germination rates in non-native *P. australis* ranged between 11.33%-23.00% and showed no pattern in relation to seasonal GDDs. Germination rates from Alma could not be accurately measured because of a lack of visually viable seeds. However seeds collected from this area during the 2011 preliminary study were able to germinate in vitro. Therefore, despite very poor seed production overall, it should not be assumed that non-native *P. australis* are incapable of producing germination-capable seeds at this latitude. Germination rates for native *P. australis* were also similar across isotherms, ranging from 12.00%-20.67%. Despite similar germination rates across isotherms, when germination rates are taken into consideration with the overall rates of seed production, a pattern based on seasonal GDDs does emerge in non-native *P. australis*. As seasonal GDDs increase, the production of germination-capable seeds increases until the Catskill and Secaucus region. It would appear that the Catskill region produces more germination-capable seed than the Secaucus region, but this may be an artefact of Hurricane Sandy's effect on plants within the Secaucus isotherm. Perhaps the most viable seeds are also among the first to mature, and these may have been selectively dispersed by the extreme

winds of Hurricane Sandy. Within native *P. australis*, excluding Quebec City where low seed production numbers skew results, the production of germination-capable seed actually increases as seasonal GDDs decrease. This would suggest that native *P. australis* actually has a competitive advantage in sexual reproduction over non-native *P. australis*. However during this study, native inflorescences were observed to be considerably smaller than non-native inflorescences in the field. It is possible that native *P. australis* may be producing fewer florets relative to non-native *P. australis*, which may eliminate any competitive advantage rendered by a higher per-floret production of germination-ready seeds. Ultimately this needs to be examined in more detail with a greater number of populations.

Clinal variation in seed germination remains very poorly studied in both native and non-native *P. australis*. McKee and Richard's (1996) study focuses on Eurasian *P. australis* and remains one of the few. However they found no overall pattern for seed germination and latitude of origin from plants across England. Gervais et al. (1993) in examining populations of North American *P. australis* (possibly including native populations) across a latitudinal gradient in Quebec did not note any differences in germination between plants from Alma to Dundee (Quebec). However they did note that overall germination was extremely low, about 1% of the total florets produced per plant (Gervais et al. 1993). This study found a production rate of germination-capable seed (comparable to what Gervais et al. examined in their 1993 study) in non-native *P. australis* of 1.6% and 0.39% in Montreal and Quebec City respectively, which averages to 1% across the Province of Quebec. Native *P. australis* was considerably higher at 5.42%, 0.06%, and 8.40% from Montreal to Alma respectively. Although it is not possible to determine if the populations in Gervais et al.'s (1993) study were native or non-native, it is interesting to note that the germination rate in non-native *P. australis* appears to have remained stable between 1993 and 2012. Kettenring and Whigham (2009) also noted in their study on non-native *P. australis* within the Chesapeake Bay area that seed viability was found to be less

than 1% in most patches, although a few patches demonstrated viability levels of 5-21%. This low rate of viable seed production indicates that likely non-native *P. australis* populations would need to reach a certain population density in order to produce enough propagules to become invasive.

5.4. Pathogen Occurrence

Species within the *Claviceps* genera are known to have a parasitic relationship with the plants they infect (Alderman et al.1999; Agrios 2005). Fungal *Claviceps* spores mimic germinating pollen in order to infect ovaries within the host plant, and then destroy the ovary in order to develop a sclerotium (Alderman et al.1999; Agrios 2005). The life history of the *Claviceps* genera and the location of sclerotia within the florets (where viable seeds would otherwise be located), suggests this fungi has a parasitic relationship with both native and non-native *P. australis* conspecifics. For native *P. australis*, seasonal GDDs were found to have no effect on *Claviceps* sclerotia occurrence, while a relationship was found to exist between *Claviceps* sclerotia occurrence and seasonal GDDs in non-native *P. australis*. Within non-native *P. australis*, levels of *Claviceps* sclerotia occurrence were the least in the coldest isotherm Alma (at 3.84%) and greatest in the warmest isotherm Secaucus (at 13.21%), but maintained similar values in the remaining three isotherms. Temperature has been shown to have an effect on sclerotia germination in *C. purpurea*, which is believed to be the species which commonly infects *P. australis* (Mitchell and Cooke 1968; Gupta et al. 1983). However generally it has been found that sclerotia germination declines with warmer temperatures, which would conflict with the results obtained in this study (Mitchell and Cooke 1968; Gupta et al. 1983). Likely environmental factors other than seasonal GDDs, such as rainfall and relative humidity, have the greatest effect on Claviceps occurrence (Gupta et al. 1983). With native *P. australis*, *Claviceps* occurrence shows even less relation to seasonal GDDs. No *Claviceps* fungus was found in native Alma *P. australis*, while the highest at 2.71% was found within Quebec City plants. Interestingly, native *P. australis* has

a much lower *Claviceps* occurrence than non-native *P. australis* within isotherms. With seed contamination during germination from non-native *P. australis*, rates ranged from 18.66% - 34.33% and showed no relation to seasonal GDD. Within native *P. australis*, seasonal GDD seemed to have an effect on seed contamination during germination. As seasonal GDD decreased, levels of seed contamination during germination increased. This would seem counterintuitive, as presumably a greater number of seasonal GDD would contribute to healthier plants and subsequently lower levels of seed contamination. However only three isotherms are included in the temperature gradient used to examine native plants, and the relationship seen here may be the result of a low number of isotherms sampled as opposed to representing a true relationship. As well, no pattern was found between native and non-native *P. australis* in seed contamination.

Fungi and parasitism in *P. australis* is very poorly studied in both Europe and North America, and as of yet no study has attempted to quantify fungal parasites or relate their occurrence to environmental conditions in *P.australis* (Haslam 2010). However the relative similar abundance of *Claviceps* sclerotia and contamination in germinating seeds across isotherms, as well as the overall low levels of occurrence, suggest that fungal parasites are endemic at low levels in *P. australis* regardless of isotherm. Unfortunately it is not possible in this study to determine how many viable seeds may have been lost because of fungal parasitism, and therefore the effect of fungal parasitism on sexual reproductive success. Even if *Claviceps* infects a floret it is not clear if that floret would have otherwise been fertilized, as this study observed numerous empty florets when examining seed dispersal units. Regardless of the occurrence of *Claviceps* sclerotia and contamination in germinated seeds, viable seeds are still produced, which indicates that these fungal parasites do not prevent sexual reproduction completely. However it is possible that greater levels of seed production would have been observed without the contamination of fungal pathogens. It is interesting to note that levels of *Claviceps* occurrence are higher in non-native *P. australis* than native *P. australis*. This runs counter to the enemy-release

hypothesis of invasion, which would suggest that non-native species are able to become invasive in part because their introduction to a new geographic location allows them to grow unhindered by natural enemies from their native range and therefore gives them a competitive advantage over native species (especially congeners and conspecifics) (Keane and Crawley 2002). However likely, the interactions between local pathogens and invasiveness implied by the enemy-release hypothesis consist of multiple interactions from many species on many different levels within the life cycle of the invasive species, and the effect of one particular pathogenic species (such as *Claviceps*) is not a good test of the enemy-release hypothesis as a whole.

5.5. Responses to Climate Change

Although it is clear that seasonal GDDs affect native and non-native *P. australis* in very different ways along the gradient that we examined, the question remains as to how increasing local temperatures from climate change will affect populations of native and non-native *P. australis*. This study has demonstrated that reproduction and growth in non-native *P. australis* is currently limited at the northern edges of its range, and seasonal GDDs play at least a role in this limitation. However this does not necessarily indicate that this limitation will be eased with increased temperatures. Within its native Europe, Eurasian *P. australis* has a history of fixed climatic adaptation (Clevering et al 1999; Lessmann et al. 2001; Bastlova et al. 2004; Bastlova et al. 2006; Hansen et al. 2007; Haslam 2010). However Eurasian populations have been established within their respective ecoclines for hundreds of thousands of years. Non-native *P. australis* has been established in the Montreal region since the 1960s, the Quebec City region since the 1970s, and the Alma region since the 1980s (Lelong et al. 2007; Catling and Mitrow 2011). As well, there is considerable documentation suggesting that within Quebec, non-native *P. australis* has moved linearly northward from founder populations in the south, as opposed to multiple separate introductions from Europe along the Quebec temperature gradient (Lavoie 2007;

Lelong et al. 2007; Lelong et al. 2009; Catling and Mitrow 2011). It is clear from this study that non-native populations are adapting to colder temperatures as they move rapidly northward. However what is unclear is if these adaptations will prove to be fixed (as in Eurasian populations) or the result of phenotypic plasticity, especially given the relatively short length of time non-native *P. australis* has had to undergo these adaptations within North America. If adaptations in non-native *P. australis* are mutable, then likely populations currently restricted by shorter growing seasons with fewer seasonal GDDs will respond positively to increased temperature from climate change. As well, responses to increased temperature from climate change are likely to be the greatest in areas with the fewest seasonal GDDs, suggesting populations in northern areas may be more plastic compared to those in southern areas (Deutsch et al. 2008). Haslam was also able to demonstrate in her study (1969a) on plant height that non-native *P. australis* populations adapted to northern climates showed a much greater positive response to increased temperature than did populations adapted to southern climates. This study has shown that non-native *P. australis* is currently producing germination-capable seed in most isotherms within the study area. Although overall germination-capable seed production is very low per plant, when populations commonly reach densities of 200 reproductive individuals per m^2, very low seed germination rates can still translate into incredible propagule outputs (Mal and Narine 2004). This study observed that these dense populations of non-native *P. australis* currently exist throughout much of the study area, and this may explain the current invasive nature of non-native *P. australis* within these isotherms. However non-native *P. australis* populations within the northern limit of the invaded range in Alma were found to be extremely limited in seed production. As well, it was observed in the field that within the Alma isotherm there were very few non-native *P. australis* populations relative to the other isotherms. It is currently not known how non-native *P. australis* commonly invades new habitats (Mal and Narine 2004). Previously it was believed that the transport of rhizome fragments through road work was a likely vector of invasion (Mal and Narine 2004). However recent studies on seed

dispersal and genetic diversity within non-native *P. australis* stands suggests that seed may in fact be the most important invasion vector in non-native *P. australis*. For instance, studies have noted that the wind-dispersed nature, shading intolerance, and high nutrient requirements of non-native *P. australis* seedlings make it very unlikely that seeds would germinate in an established non-native stand (Mal and Narine 2004). More likely, wind dispersed seeds mainly function to establish new populations (Lambertiti et al. 2008; McCormick et al. 2010a). One study from France has shown that new populations typically have a high genetic diversity compared to very old established stands, suggesting that while new stands may be established with seeds representing several genotypes, over time weaker genotypes are eventually eliminated within the stand through local competition and clonal growth (Alvarez et al. 2005). Lambertini et al. (2008) noted that although they could not directly correlate stand age to size, the only monoclonal stand they observed was an extremely large one in an undisturbed wetland, while all other stands which were polyclonal were also younger and characterized by disturbance. If seeds are truly the most important invasion vector for non-native *P. australis*, then the low seed production rate of plants within Alma would explain the relatively rare occurrence of non-native *P. australis* populations within the Alma isotherm despite the abundance of favourable habitat (Catling and Mitrow 2011). However only approximately 230 km south of Alma, populations of non-native *P. australis* within Quebec City are producing seed successfully and populations are widely distributed throughout the isotherm. If temperatures within the Alma region increase with climate change, likely non-native populations of *P. australis* within Alma would experience an increase in seed production to the point where Alma populations would undergo a population release. As a result, the invasion front for non-native *P. australis* would move northward, as the Alma isotherm became a climate more favourable to seed production in non-native *P. australis* (Catling and Mitrow 2011).

This study also found that colder temperatures and fewer GDDs are not currently limiting sexual reproduction in native *P. australis*, at least along the

partial isotherm gradient that we considered. That gradient may have been too short to capture the true northern response for native populations at these latitudes. As well, it is possible that factors other than temperature are most important in determining the production of viable seed and successful establishment within the native type. That being said, increases in temperatures from climate change could still have a significant impact on native *P. australis*. Many studies have noted significant decreases in native *P. australis* abundance and increases in non-native abundance in the field (Saltonstall 2002; League et al. 2006; Packett and Chambers 2006; Meadows and Saltonstall 2007; Meyerson et al. 2009; Mozdzer and Zieman 2010; Catling and Mitrow 2011, Plut et al. 2011). While direct competition between native and non-native has not often been studied in the field, those studies which have examined their relationship have generally found native *P. australis* to likely be a weaker competitor. Non-native *P. australis* produces greater aboveground biomass, grows taller, emerges earlier, and has greater genetic diversity than the native *P. australis* (League et al. 2006; Packett and Chambers 2006; Mozdzer and Zieman 2010; Plut et al. 2011). Likely native *P. australis* may not be able to compete directly with non-native *P. australis* over the long term and increases in temperature (especially in northern areas) will only increase both vegetative productivity and seed production in non-native *P. australis* relative to native *P. australis*. Therefore, even if temperature does not directly affect native *P. australis*, climate change may still have a negative impact on this native species.

6. Conclusions, contributions to knowledge and future research

This study represents a significant contribution to the fields of invasion ecology, climate change, and our understanding of the complex species, *P. australis*. Several biological processes were investigated in North America for the first time to our knowledge, and this study represents the only known scientific conclusions drawn on these processes. This is especially true of native *P. australis*. This project represents not only what is likely the first investigation of

clinal variation in native *P. australis*, but also what is believed to be one of the first to provide evidence of seed production and germination. In non-native *P. australis*, more studies have been done but the work covered in this study remains considerably novel. For instance, this study represents the first documentation of clinal variation in North America. As well, this study represents one of only a few studies relating seed production to local temperature in non-native *P. australis* worldwide, and is the only study within North America that makes a distinction between native and non-native populations within the study area. This work is also the first to conclusively demonstrate that there is no direct relationship between plant height and seed production in either native or non-native *P. australis*. This research is also the first known to quantify *Claviceps* sclerotia occurrence in either species of *P. australis*, and one of the few to quantify levels of seed contamination. Many important comparisons were also made between native and non-native populations of *P. australis* which are vital to understanding their behaviour in the field, and ultimately how we may conserve one while controlling the other. For instance, the knowledge that the production of germination-capable seeds was greater in native *P. australis* than non-native *P. australis* contradicts the common belief that non-native *P. australis* outcompetes native *P. australis* in all respects.

The information generated from this study has provided insights into the behaviour of native and non-native *P. australis* in relation to local temperatures, and provides insights into how these two conspecifics and the dynamics between them will respond to increased temperature from climate change. However work remains to be done to fully understand the relationship between climate and both vegetative and sexual reproduction in *P. australis*. A common garden experiment with plants from northern and southern isotherms should be conducted to determine if temperature adaptations are fixed in either non-native and native *P. australis*. This will provide a clearer example of how plants will respond to increased temperature. As well, plants from the northern range limit of native *P. australis* should be examined for their relationship to local temperature, as

temperature adaptations in native *P. australis* may become more evident in climates where seasonal GDDs are known to be limiting. A more detailed developmental study (particularly of native populations) would also greatly benefit our understanding of the ability of both species to interbreed in the field. Finally, more work should be done to investigate both the effect of *Claviceps* on sexual reproduction in native and non-native *P. australis*, and the potential of *P. australis* to act as a source of fungal infection in crop plants as many *Claviceps* species are not species-specific and are known to infect crops.

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8. Appendix

All raw data for both the 2012 main study and 2011 preliminary study can be obtained from the database of the Ecolab. Room R3-041 Raymond Building, Macdonald Campus of McGill University, 21 111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada. H9X 3V9.

Table S.1 Results of statistical analyses

Year	Conspecific	Independent Variable	Dependant Variable	Distribution	p-value	Chi-Sq
2011	Non-native	Seasonal GDDs	Plant Height	Normal	**0.0013**	10.327
2011	Non-native	Seasonal GDDs	Visually Viable Seed	Normal	**0.0349**	4.4488
2011	Non-native	Seasonal GDDs	Claviceps Sclerotia	Poisson	**0.0032**	8.6944
2011	Non-native	Plant Height	Visually Viable Seed	Normal	0.2685	1.2243
2011	Non-native	Pantyhose	Visually Viable Seed	Normal	0.2767	1.1831
2011	Non-native	Pantyhose	Claviceps Sclerotia	Normal	0.1143	2.4943
2011	Non-native	Pantyhose	Fungal Seed Contamination	Poisson	0.9544	0.0033
2011	Non-native	Pantyhose	Germination Rate	Poisson	0.3547	0.8567
2011	Non-native	Stratification	Fungal Seed Contamination	Poisson	**<0.0001**	47.309
2011	Non-native	Stratification	Germination Rate	Poisson	0.5079	0.4385
2012	Non-native	Seasonal GDDs	GDD Inflorescence Emergence	Normal	**<0.0001**	22.167
2012	Non-native	Seasonal GDDs	GDD Flowering	Normal	**<0.0001**	67.917
2012	Non-native	Seasonal GDDs	Plant Height	Normal	**0.0002**	14.43
2012	Non-native	Seasonal GDDs	Visually Viable Seed	Normal	**0.0044**	8.0973
2012	Non-native	Seasonal GDDs	Claviceps Sclerotia	Poisson	**0.0316**	4.6194
2012	Non-native	Seasonal GDDs	Fungal Seed Contamination	Normal	0.0588	3.572
2012	Non-native	Seasonal GDDs	Germination Rate	Normal	0.0973	2.7485
2012	Non-native	Plant Height	Visually Viable Seed	Normal	0.1125	2.5194
2012	Native	Seasonal GDDs	Plant Height	Normal	0.2038	1.6148
2012	Native	Seasonal GDDs	Visually Viable Seed	Normal	0.5593	0.341
2012	Native	Seasonal GDDs	Claviceps Sclerotia	Poisson	0.2008	1.6364
2012	Native	Seasonal GDDs	Fungal Seed Contamination	Normal	**0.0018**	9.7402
2012	Native	Seasonal GDDs	Germination Rate	Normal	0.0496	3.8564
2012	Native	Plant Height	Visually Viable Seed	Normal	0.4286	0.6265

Table S.2 Coordinates and elevation of study sites across all isotherms

Isotherm	Conspecific	Site	Coordinates		Elevation
			Latitude	Longitude	
Alma	Non-Native	1	48º30’21.83"N	71º39'37.71"W	120 m
		2	48º29'42.35"N	71º39'57.99"W	128 m
		3	48º32'07.77"N	72º15'20.18"W	145 m
		4	48º37'05.78"N	72º21'51.22"W	106 m
	Native	1	47°53’41.57”N	72°26’16.90”W	398 m
		2	48°23’12.23”N	71°39’55.77”W	142 m
		3	48°23’08.65”N	71°39’49.94”W	140 m
Quebec City	Non-Native	1	46º29'30.03"N	71º41'17.06"W	805 m
		2	46º42'21.71"N	71º17'58.32"W	67 m
		3	46º40'14.70"N	71º22'04.52"W	88 m
		4	46º50'39.50"N	71º16'21.07"W	27 m
	Native	1	46º50'39.50"N	71º16'21.07"W	27 m
		2	46º43’57.68"N	72º45'28.65"W	126 m
		3	46º41’07.06"N	72º42'06.85"W	144 m
Montreal	Non-Native	1	45º23'41.56"N	74º03'25.27"W	27 m
		2	45º25'27.80"N	74º06'19.72"W	31 m
		3	45º22'23.20"N	74º01'58.20"W	30 m
		4	45º23'07.33"N	73º59'28.56"W	26 m
	Native	1	45º02’29.83"N	74º27'45.19"W	47 m
		2	45º24’22.87"N	73º53'43.93"W	21 m
		3	45º23’52.18"N	73º53'35.79"W	21 m
Catskill	Non-Native	1	42º32’05.82"N	73º47'08.10"W	54 m
		2	42º25’42.09"N	73º48'26.61"W	55 m
		3	42º23’40.85"N	73º49'45.83"W	54 m
		4	42º23’53.74"N	73º49'30.92"W	51 m
Secaucus	Non-Native	1	40º47’58.28"N	74º03'56.24"W	2 m
		2	40º47’53.07"N	74º03'54.06"W	2 m
		3	40º47’51.22"N	74º03'57.06"W	2 m
		4	40º47’58.07"N	74º03'49.98"W	2 m

Table S.3 Coordinates and elevations of weather stations used to retrieve temperature data for GDD calculations

Isotherm	Weather Station Name	KORD-Station ID	Climate ID	Coordinates		Elevation
				Latitude	Longitude	
Alma	Roberval A	NA	7066685	48°31'00.00" N	72°16'00.00" W	178.60 m
	Mistook	NA	7065012	48°35'54.00" N	71°42'57.00" W	112.50 m
	St. Prime	NA	7067658	48°37’00.00” N	72°25’00.00“ W	121.90m
Quebec City	Ste-Foy (U. Laval)	NA	701Q004	46°46'49.00" N	71°17'15.00" W	91.40 m
	Beauport	NA	7010565	46°50'13.00" N	71°11'50.00" W	10.00 m
	Quebec/Jean Lesage INTL A	NA	7016294	46°48'00.00" N	71°23'00.00" W	74.40 m
Montreal	Ste-Anne-de-Bellevue 1	NA	702FHL8	45°25'38.00" N	73°55'45.00" W	39.00 m
	St-Anicet 1	NA	702FQLF	45°07'15.00" N	74°17'22.00" W	49.10 m
	Ste-Clothilde	NA	7027039	45°10'02.00" N	73°40'44.00" W	53.00 m
Catskill	Albany	KNYALBAN11	NA	42º40’29.56” N	73º44’23.10” W	15.00 m
	Catskill	KNYCATSK5	NA	42º14’23.71” N	73º55’17.85” W	35.00 m
	Freehold	KNYFREEH2	NA	42º21’38.34” N	74º02’53.00” W	179.00 m
	Teterboro	KNJHASBR2	NA	40º51’21.68” N	74º04’13.30” W	45.00 m
Secaucus	Harmon Cove	KNJSECAU5	NA	40º47’52.21” N	74º04’04.81” W	7.00 m
	North Arlington	KNJNORTH7	NA	40º47’27.40” N	74º07’55.96” W	8.00 m

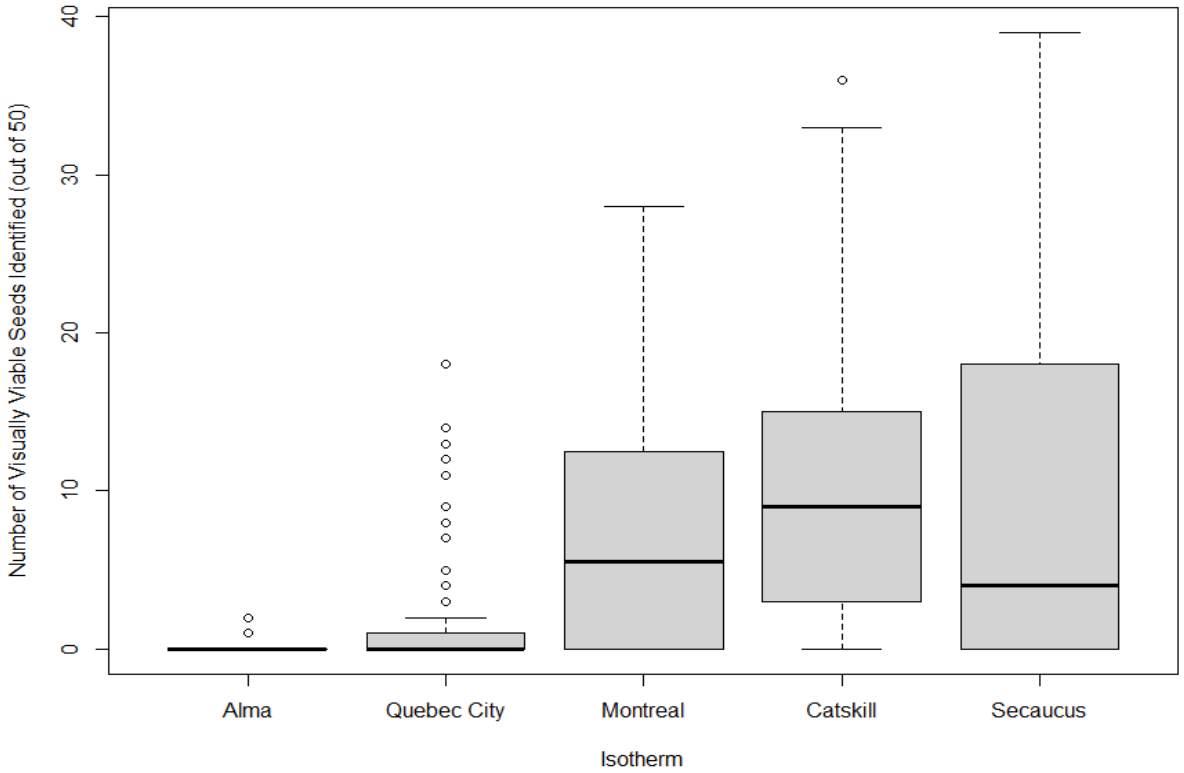

Fig. S.1 The number of visually viable seeds out of 50 intact seed casing in non-native *P. australis* populations across isotherms as determined by the classical unimbibed seed crush test

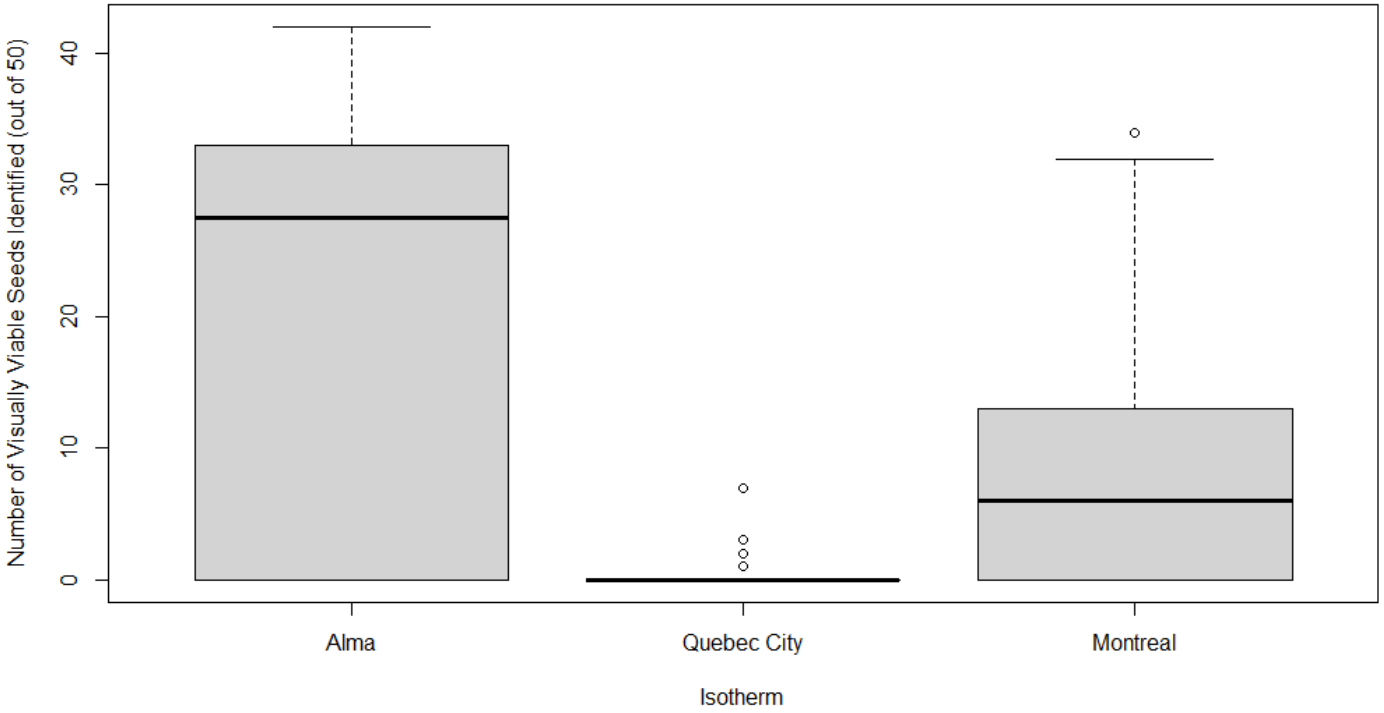

Fig. S.2 The number of visually viable seeds out of 50 intact seed casing in native *P. australis* populations across isotherms as determined by the classical unimbibed seed crush test

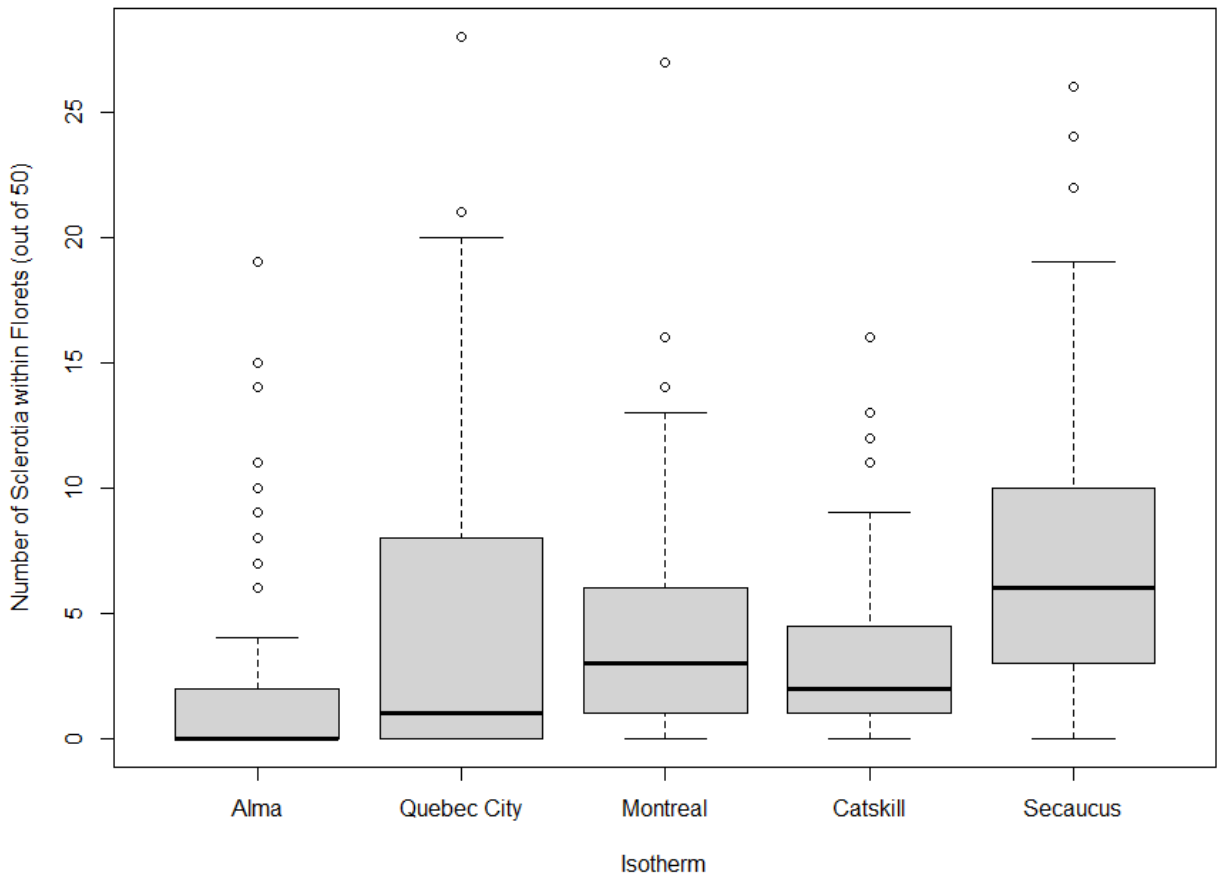

Fig. S.3 The number of *Claviceps* sclerotia out of 50 intact seed casing in non-native *P. australis* populations across isotherms

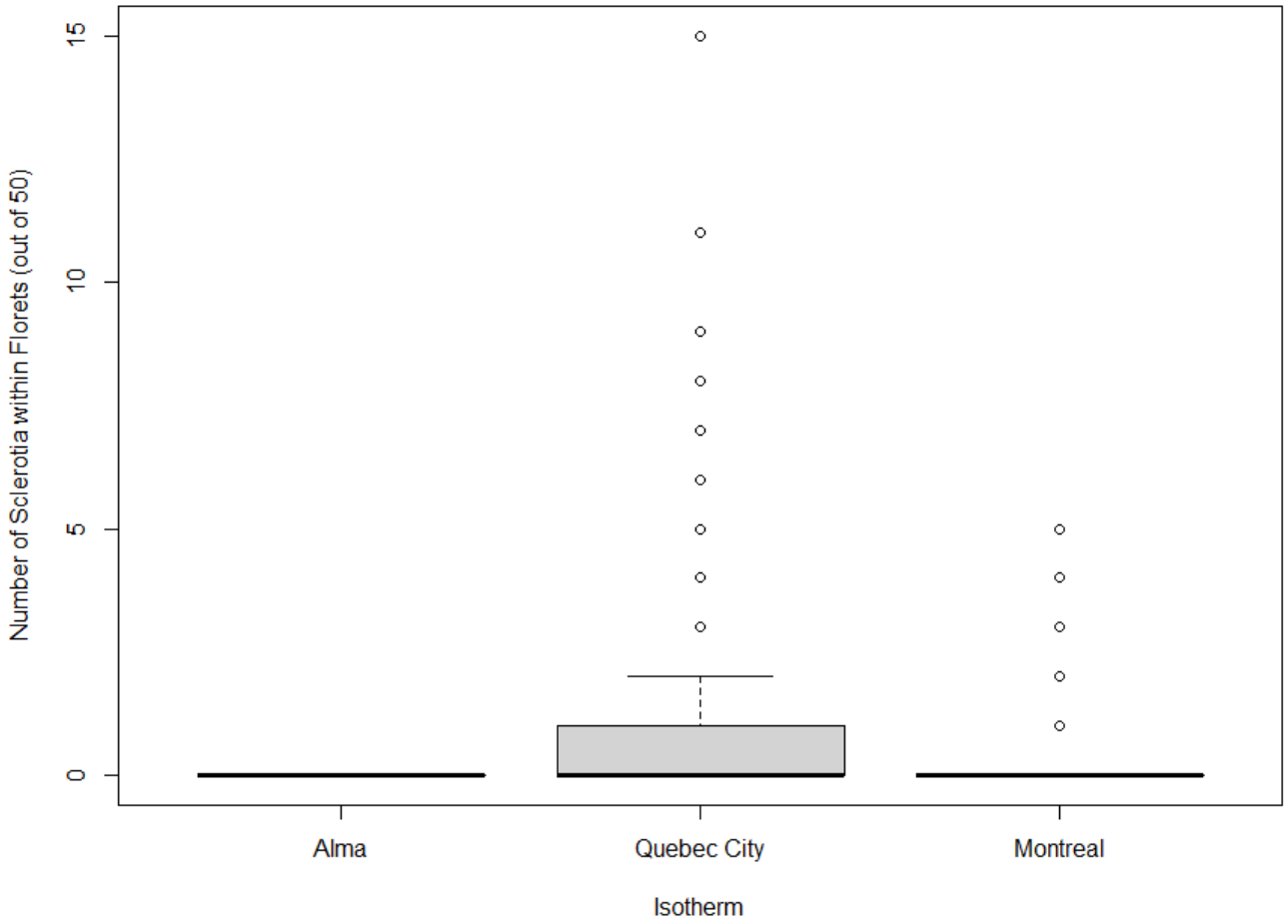

Fig. S.4 The number of *Claviceps* sclerotia out of 50 intact seed casing in native *P. australis* populations across isotherms

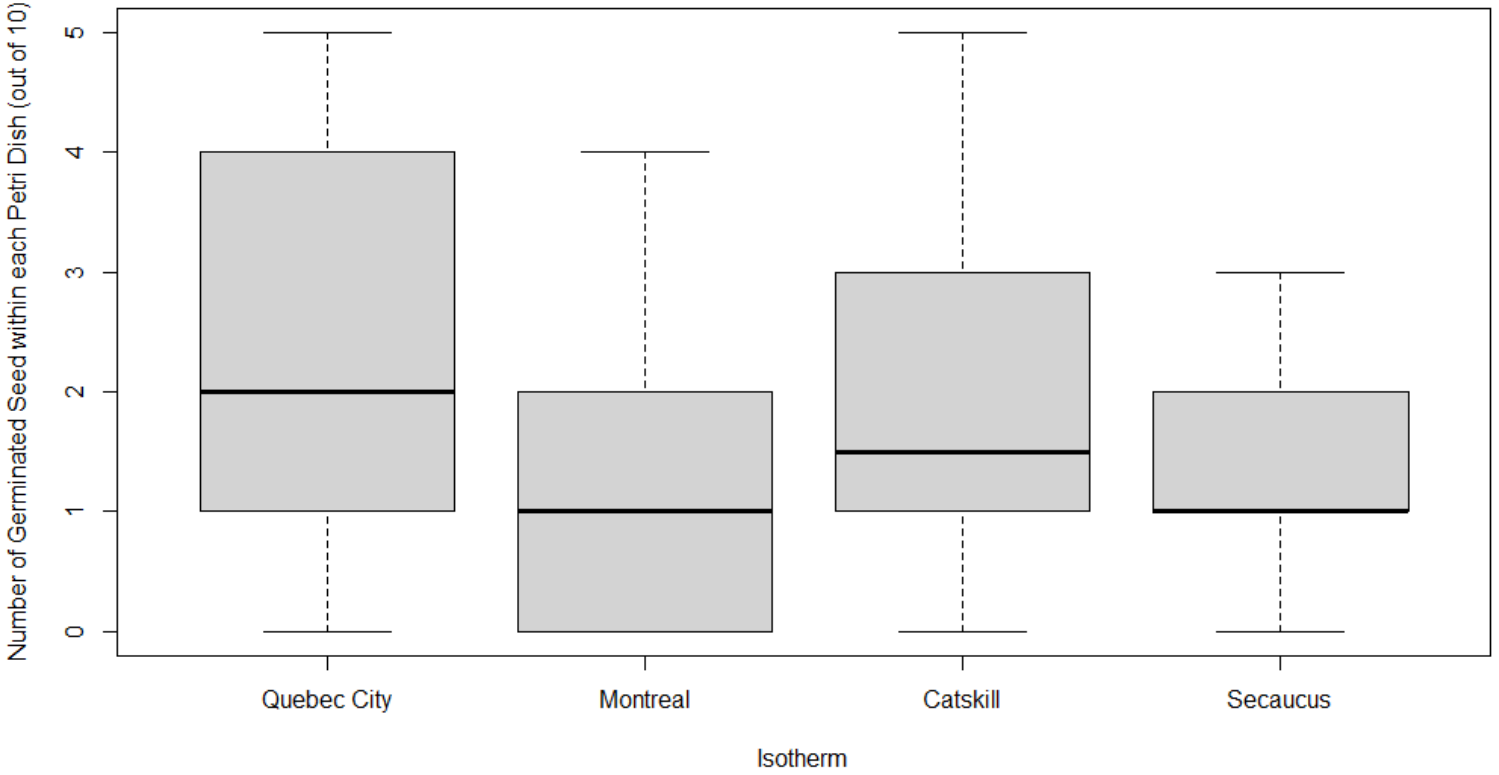

Fig. S.5 The number of germinated seeds out of 10 within each Petri dish in non-native *P. australis* populations across isotherms

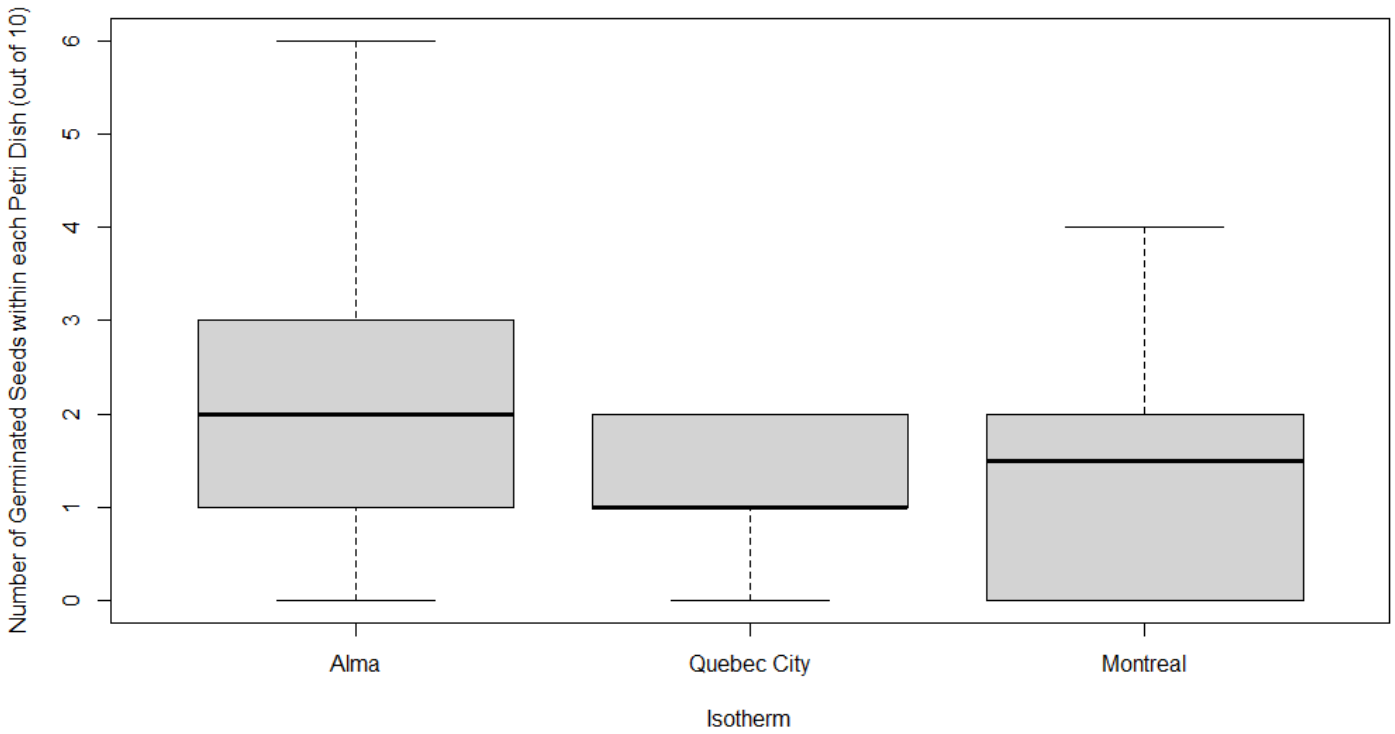

Fig. S.6 The number of germinated seeds out of 10 within each Petri dish in native *P. australis* populations across isotherms

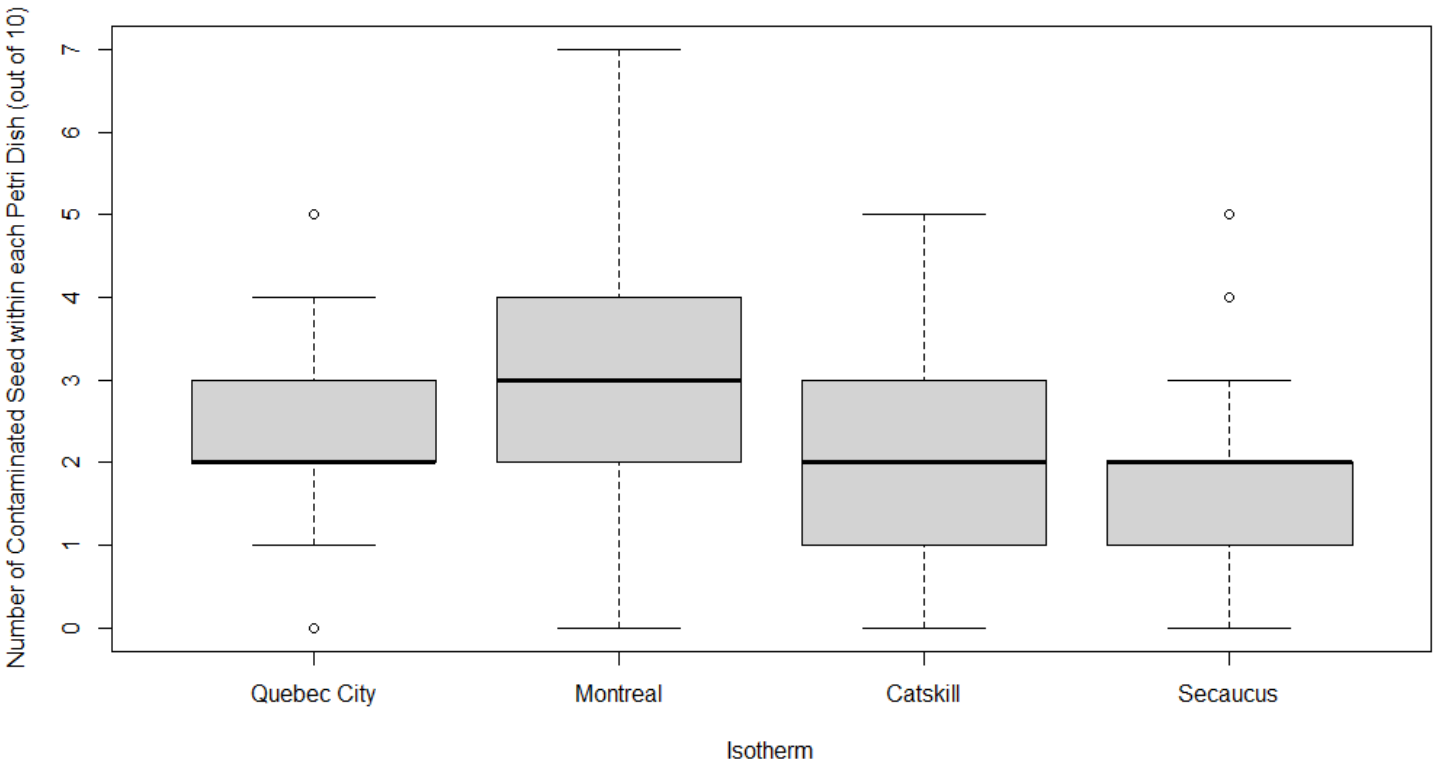

Fig. S.7 The number of contaminated seeds out of 10 within each Petri dish in non-native *P. australis* populations across isotherms

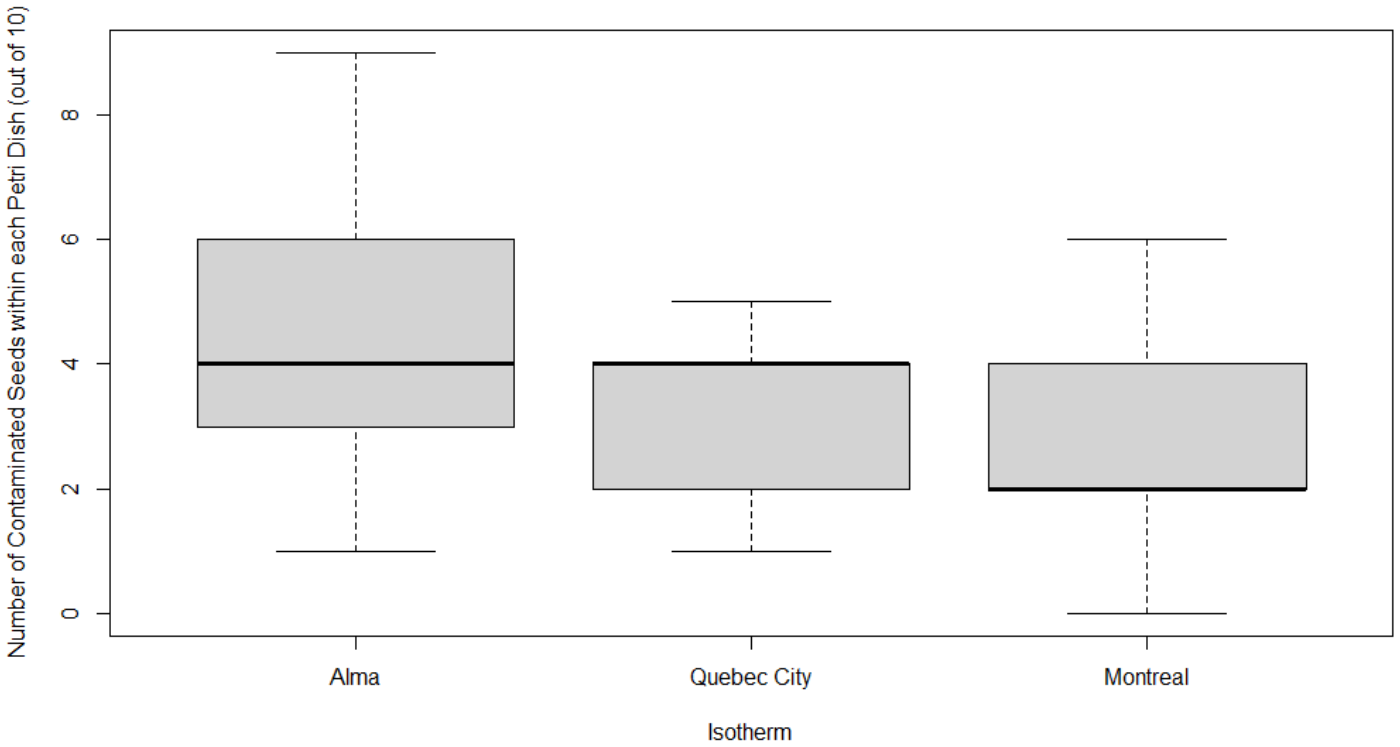

Fig. S.8 The number of contaminated seeds out of 10 within each Petri dish in native *P. australis* populations across isotherms