Will climate change influence disease susceptibility? A study of natural polyploids with manipulated flowering time exposed to contrasting water-availability conditions

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Introduction: Integration of Biological Principles

As with all subdiciplines in science, a variety of skills and diverse background knowledge is used to approach problems. However, sometimes the integration of macro principles, such as ecosystem dynamics and evolution, with finer-scale ideas such as immunobiology and cell-signaling does not get incorporated. It is at the junction of these two study scales that many scientific problems remain unsolved.

To investigate where relationships between broad-scale and fine-detail research lie requires the consideration of many subgenera of scientific research. Here, I ligate the large, overarching problem of climate change and the immense world of ecology with the refined bridge of genetics. Climate change -which has become of particular interest and global concern in the last decade- will influence the outcomes of many biological interactions. The study of ecology describes these interactions and the relationships of organisms in response to their biotic and abiotic pressures. The discipline of genetics outlines the details and constraints within organisms to actually respond to these pressures.

I use these principles to investigate the response of plants- one of the most basal, important groups to an ecological system- to climate change. Specifically, I target the response of plants to disease and changing disease dynamics, and how their genetic constructs may or may not allow them to survive these changes. The understanding of this is essential if we are to predict the outcomes of anthropogenic climate change. These fitness outcomes will ripple all the way up through the food chain and across the ecosystem. The implications of these studies are extremely varied, from the survivorship

of some biodiversity, to the survivorship of some key species, to the survivorship of life as we understand it.

Chapter 1

Determining the best method to measure the degree of infection of *Solidago altissima* by *Erysiphe cichoracearum*

Although powdery mildew, a common fungal pathogen of plants, has been measured in studies of many crop species; however, the methods employed are often highly variable and depend largely on the leaf morphology of the host plant species. It has less often been measured on native plant species, such as *Solidago altissima* (late goldenrod). Due to the variety of species of powdery mildews and host plants in the literature and the constraints on measurement time during field work, we sought to determine the most accurate and rapid field measurements of the powdery mildew species *Erysiphe cichoracearum* on our host plant species, *Solidago altissima*. We tracked the occurrence and extent of powdery mildew infection on *S. altissima* during peak season using three quantitative measurements (percent height of infection, percent of infected clones, percent of infected leaves) and three qualitative measurements (visual low/med/high, index score of percent of total plant covered, and an index adapted from the literature).We found that the mildew infects from the base of the plant upwards, and the quantitative measurement of the percent of total plant height infected captures this movement. Along with this quantitative measurement, qualitative visual measurements of low/medium/high designators and a scale that encompasses total percent of the plant covered with colonies were rapid and simple ways to determine extent of pathogen colonization.

Introduction

Powdery mildew has been long studied as a crop fungal pathogen (Moseman et al. 1965, Magyarosy et al. 1976, Donald et al. 2002, Caffara et al. 2012). It has been known to infect grains, vegetables, fruits, and ornamentals (Spanu and Panstruga 2012), and many researchers are using measurements of the intensity of infection in studies with the aim of finding resistance genes (e.g. mlo5 and Mla1; Lyngkjær et al. 1995, Prats et al. 2006). Because of their obligately biotrophic nature, it is not only hard to cultivate and facilitate the pathogenicity of these mildews, but visual enumeration is also difficult (Beaver and Cienfuegos 1990, Spanu and Panstuga 2012).

Manuals that have been written for legume infections (Ellwood et al. 2007) suggest comparing two of the most highly infected leaves to distinguish between highly susceptible and less susceptible genotypes. However, this fails to take into account the overall effect of multiple patches of infection on plant health and fitness. Others have suggested methods for detached leaf staining with LPTB (see Warkentin et al. 1995). However, this approach is also limited in inference about total plant infection because it only quantifies the mycelial bodies on one leaf per plant and is too time consuming to do on all leaves. In particular, for studies that include thousands of plants, this is not a feasible method. Similarly, SEM techniques, such as those used by Prats et al. (2006), provide fine-scale information about cellular changes that occur with powdery mildew infection, but are not suitable for large-scale investigations.

An alternative approach is to use visual methods to obtain an overall index of the degree of infection. For example, Schmid (1994) used a numerical scale to relate percent cover of mildew over the vegetative mass of the plant in this same powdery

mildew/goldenrod system. However, this is approach may be subject to observational biases because it is a qualitative score determined by the observer. The goal of this study was to use a diverse set of qualitative and quantitative measurements to find a technique that is accurate and rapid.

Materials and Methods

In the summer of 2011, data was gathered on an existing experiment on *Solidago altissima* to obtain information on the relationship between water availability, flowering time, polyploidy, and infection by powdery mildew. This experiment examined powdery mildew incidence on diploid and tetraploid plants that had experimentally manipulated flowering phenology and were exposed to two different watering treatments in a common garden.

Etterson (*unpublished data*) has been conducting artificial selection on flowering time in drought and well-watered conditions on diploid and tetraploid *Solidago altissima* for three generations (24 artificial selection lines = 2 ploidy levels x 2 watering treatments x 2 replicates x 3 flowering times: early, control, late). Plants from the second generation of artificial selection were maintained in pots at the UMD Research and Field Studies Center and exposed to two watering treatments: ambient-dry and supplemental watering.

The incidence of powdery mildew infections was measured on plants in 360 pots (15 genotypes / 2 ploidy levels x 2 watering treatments x 2 replicates x 3 flowering times) using several approaches (three quantitative and three qualitative) from late Aug. to early Sept. The three quantitative measurements were percentage infection of: total plant height, leaves, and clonal stems per pot. The three qualitative approaches included a subjective score of degree of infection (zero/low/medium/high), a qualitative assessment of percentage infection, as previously conducted by Schmid (1994) (0- no infection, 1- 1-20%, 2- 20-40%, 3- 40-60%, 4- 60-80%, and 5- 80-100%), and a literature index (adapted from Lyngkjær et al.1995; 0= no colonies, 1= a few colonies, 2= multiple

colonies on one leaf, 3= multiple colonies on many leaves). All of the qualitative measurements were done by one researcher for consistency.

Results

Quantitative Measurements

Using the metric of percent height of infection, we found that three factors- watering treatment, the combination of ploidy and watering treatments, and the combination of ploidy, watering treatment and flowering time- were all significant influences on mildew cover (Table 1.1). Percent of leaves infected was influenced by artificial flowering time selection line, the combination of ploidy and watering treatment, the interaction of ploidy, watering treatment and flowering time selection line, and also marginally by genotype (Table 1.1). Percent of clones infected was significantly influenced by watering treatment alone and the combination of ploidy and watering treatment. The percent of clones infected was also marginally influenced by the combination of ploidy, watering treatment and flowering time (Table 1.1).

Qualitative Measurements

Measurements of the visual index (low/med/high) indicated that ploidy; watering treatment; combinations of ploidy and watering treatment; combinations of ploidy and flowering time, and the combination of ploidy, watering treatment, and flowering time all influenced the visual intensity of mildew cover (Table 1.1). Measurements of percent cover scale (0-5) indicated that watering treatment; the combination of ploidy and watering treatment; and the combination of ploidy, watering treatment and flowering time were all significant influences on overall mildew cover, and genotype was a marginally significant factor (Table 1.1). The literature index adapted from Lyngkjær et al. (1995) indicated that the combination of ploidy, watering treatment and flowering time was a

significant influence on mildew cover, as well as the marginally significant combination of ploidy and watering treatment (Table 1.1).

Discussion

The best metrics for measuring future mildew on *Solidago altissima* were found to be percent height of infection, a percent cover scale (as adapted by Schmid 1994) and a visual low/medium/high scale. These three metrics had the greatest overlap in significant data found and involved a reasonable amount of effort in the field (Table 1.1). The percent height of infection follows the progression of the infection up the length of the plant, the percent cover scale allows for an overall coverage assessment, and the visual low/medium/high scale is a rapid field measurement.

When measuring a plant with morphological differences between cytotypes, such as *S. altissima* (Etterson, unpublished data), it is important to measure the morphotypes in a way that is not skewed towards one type or the other. For instance, in our goldenrod system, diploids tend to be shorter and have more numerous, smaller foliage. Tetraploids, on the other hand, are taller and have fewer, larger leaves. The rationale for excluding percent of leaves covered with mildew is to avoid this bias towards heavy infection counts on tetraploids. Even if more of their total leaves are exhibiting infection, the area of infection on these leaves may be minimal. The index percentage of cover (0-5) adapted from Schmid (1994) takes this into account and eliminated the bias of leafiness by looking at total area infected.

In the same way, percentage of height of infection is a relative measurement that removes the influence of inherent differences in plant height. Even though tetraploids are taller than diploids, because we are utilizing a percentage of the total height, the measurement is relative to each individual clone. Percent of clones infected is only useful when assessing a pot with more than multiple clones, and many experiments undertaken

hereafter only included one clone per pot. The literature index taken from Lyngkjær et al. (1995) was not useful for this system because there are numerous leaves on each plant, and measuring individual colonies is not amenable to rapid fieldwork. This measurement was also non-significant compared to the other methods when analyzing real data (Table 1). The most rapid of all of these techniques was a visual zero/low/medium/high assessment. Although this is highly subjective, I completed this assessment rapidly on 1400 plants by myself. If using only one observer, the inter-observer differences are eliminated within an experiment.

Overall, an index scale of infection (0-5), percent height of infection, and a visual (0/low/med/high) index are the most rapid and reliable methods to measure powdery mildew in this system. Using a quantitative method to support two qualitative methods strengthens the overall assessment, and the previously published use of the index scale makes it a more valid option.

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Table 1.1 ANOVA test statistics (*F*, *P*) of six measurements of *Erysiphe cichoracearum* (powdery mildew) infection on diploid and tetraploid *Solidago altissima* subjected to artificial selection on flowering time and reared in ambient dry and well-watered pots. Powdery mildew cover is measured using quantitative (percent height of infection, percent of leaves infected, percent of clones infected) and qualitative (visual infection index, percentage of infection index, literature index) methods.

Factors	**Quantitative measurements**			**Qualitative Measurements**		
	% Height of Infection	**% of Leaves Infected**	**% of Clones Infected**	**Visual Index (Low/Med/ High)**	**Index Percentage**	**Literature Index**
Ploidy[1,4]	1.12, 0.29	1.47, 0.23	0.32, 0.57	14.30, **0.003**	0.75, 0.39	2.34, 0.13
Watering Treatment[1,4]	9.16, **0.003**	1.82. 0.18	11.66, **0.0008**	21.63,**<0.0001**	7.63, **0.006**	2.42, 0.12
Flowering Time Selection Lines[2,4]	0.36, 0.70	3.73, **0.03**	1.65, 0.20	7.21, 0.30	0.30, 0.74	0.04, 0.96
Ploidy x Watering Treatment[1,4]	4.20, **0.04**	5.18, **0.02**	8.12, **0.005**	15.15, **0.002**	4.06, **0.05**	2.99, 0.09†
Ploidy x Flowering Time Selection Lines[2,4]	1.49, 0.23	1.31, 0.27	1.34, 0.26	14.08, **0.03**	0.46, 0.63	0.91, 0.40
Watering Treatment x Flowering Time Selection Lines[2,4]	0.38, 0.68	0.07, 0.93	0.61, 0.54	6.72, 0.35	0.83, 0.44	0.40, 0.67
Genotype[1,3]	1.21, 0.11	1.22, 0.09†	1.20, 0.12	N/A	1.26, 0.06†	1.22, 0.10
Ploidy x Watering Treatment x Flowering Time Selection Lines[2,4]	3.23, **0.04**	11.44, **<0.0001**	2.75, 0.07†	15.47, **0.02**	8.26, **0.0004**	5.77, **0.004**

[1]df numerator =1 [2] df numerator = 2 [3] df numerator= 162 [4] df denominator ≈ 354

Chapter 2

Infection by powdery mildew (*Erysiphe cichoracearum)* differs in sites with contrasting water availability and among plants with manipulated flowering time in a polyploid goldenrod (*Solidago altissima*)

Climate change is already influencing the prevalence and distribution of plant disease, posing a potential threat to native species. For plant populations that include mixed ploidy levels, diploids and polyploids may differ in their response to changing abiotic and biotic factors because of divergence in morphology, phenology, or plasticity in these traits. To examine how these factors influence the incidence of powdery mildew, we use diploid and tetraploid *Solidago altissima* lineages that had been artificially selected to expand the breadth of flowering time and planted them into two sites with contrasting water availability. In general, powdery mildew infection was more widespread in the wetter site. We also found that tetraploids bore lighter powdery mildew loads than their diploid counterparts. Within each ploidy level, the extent of disease damage was higher among earlier flowering genotypes suggesting that advanced flowering phenology may result in increased duration of exposure to pathogens. This work indicates that the genetic composition of plants may influence their disease susceptibility in a changing climate, and phenological changes influence plant fitness in response to disease.

Key Words: Polyploidy, climate change, flowering phenology, powdery mildew, water availability, disease dynamics

Introduction

Pathogenicity in crops and native plant species will be difficult to predict as climate rapidly changes especially given predictions of enhanced variability and extreme weather events (Coakley 1999, Chakraborty et al. 1998, Chakraborty 2000, Garrett et al. 2006). Future disease dynamics have been most carefully modeled for crop species and predict that many pathogens will, in general, expand their ranges into increasingly northern latitudes (Rosenzweig et al. 2001), and some even predict overall range-shifts from current areas of high virulence (Lafferty 2009). Some pathogens will be negatively affected by an overall reduction in precipitation, especially those that utilize water to infect their hosts (Bald 1996). In contrast, other pathogens may spread via extreme weather events, leaving inundated areas fraught with disease (Garrett et al. 2006, Rosenzweig et al. 2001). Pathogen loads may increase because of mild winters that enhance the survival of fungal pathogens (Pfender and Vollmer 1999). Rapid changes in soil moisture, for example in extreme rain events, may reduce the effectiveness of plant resistance genes as has been shown in the Mlo genes of barley when drought stress is suddenly removed from the host plant (Newton and Young 1996). While overall climate is expected to become less predictable and more prone to extreme weather events, overall drying trends may increase physiological stress on plants making them more vulnerable to colonization by fungal pathogens (Chakraborty et al. 1998, Chakraborty 2000).

Climate change is also advancing plant phenology (Fitter and Fitter 2002, Primack et al. 2004, Menzel et al. 2006, Cleland et al. 2007, Amano et al. 2010) which may alter the timing of exposure to disease-causing organisms (Biere and Honders 1996, Elzinga et al. 2007). Earlier flowering may enhance fitness in a warmer climate because of pollinator

preference to early flowering individuals and increased effective mating opportunities (Ashman et al. 1996, Elzinga et al. 2007) but, at the same time, it may expose plants to pathogens for a longer period of time after they have expended energy in reproduction, leaving them more susceptible to colonization (Reekie and Bazzaz 1987, Biere and Honders 1996). It is important to bear in mind that pathogens will also respond to these same pressures (Anderson et al. 2004, Garrett et al. 2006) with unpredictable outcomes.

Another important factor that will influence plant disease dynamics across the landscape is polyploidy. As many as 80% of angiosperm species are a product of a polyploid event (Masterson 1994, Comai 2005). Some species contain a ploidy series, most typically with diploids and polyploids separated geographically (Rothera and Davy 1986, Johnson et al. 2003) but with some species harboring mixed populations that contain different ploidy frequencies (Husband and Schemske 1998, Petit et al. 1999). In general, a larger genome size may confer adaptive advantages, such as flexibility in up- or down-regulation of genes and gene products in response to the environment (Flagel and Wendel 2009, 2010), co-option of genes for novel functions (Leitch and Bennett 1997, Comai 2005), and enhanced opportunities for adaptive plasticity to evolve (Comai 2005). These genomic differences may influence adaptation to changes in water availability (e.g. drought tolerance, Li et al. 1996) and seasonality (e.g. flowering time, Levin 1983, Pires et al. 2004). Disease resistance may also be influenced by polyploidy, either in the immediate term when diverse progenitor genomes combine (Valkoun et al. 1985) or in the longer term as resistance genes diversify post-polyploidization (Innes et al. 2008).

While many have speculated on the effects of each of these factors on plant fitness, few have simulated artificial climate change conditions to study the natural response of pathogens and their plant hosts. The goal of this experiment was to enhance our understanding of the interaction of climate change, plant phenology, polyploidy, and disease dynamics by planting lineages of a native polyploid species with manipulated flowering times into sites with contrasting water availability. Our hypotheses were threefold. First, we anticipated that drought-stressed plants would be weakened and become a target of pathogen infection. Secondly, we hypothesized that early flowering plants in both environments would acquire a greater disease load by the end of the growing season. Third, we expected that polyploid plants may be able to contend with all of the aforementioned stressors better than their diploid counterparts.

Materials and Methods

Study Site

This research took place in the state of Minnesota which is characterized by strong gradients in temperature and precipitation (Tester 1989, Tester 1995, Danz et al. 2011). These climate factors strongly influence vegetation and contribute to the position of the three major biomes that meet in the state: boreal forest, temperate deciduous forest, and prairie (Tester 1989, Danz et al. 2011). It is at these ecotone boundaries, largely governed by moisture availability and temperature, that the impact of climate shifts will be most apparent (Danz et al. 2011). We took advantage of the climate gradients in MN in the design of this experiment that primarily used two sites: 1) a cooler, mesic site in eastern MN at the UMD Research and Field Studies Center (St. Louis Co. 46.47 º N, 92.6 º W), and 2) a warmer, drier site in western MN at the University of Minnesota Itasca Field Biological Station (Clearwater Co., 47.3º N, 95.2º W). These two sites differ in annual precipitation, and the eastern Duluth site (St. Louis Co., hereafter SLC) receives an average annual precipitation of 78.74 cm whereas the western Itasca site (Clearwater Co., hereafter CC) is drier and experiences 68.68 cm/ year (National Climatic Data Center 2011). The use of these two sites allowed for natural environmental treatments in common garden experiments, as seen in previous work by Etterson (2004).

Study System

Solidago altissima, or late goldenrod, is an herbaceous dicot perennial in the family Asteraceae (Beaudry and Chabot 1959). This species has a broad distribution across North America and is native to MN. *S. altissima* encompasses multiple ploidal levels, including diploid (2n, 2x=18), tetraploid (2n, 4x=36), and hexaploid (2n, 6x=54)

cytotypes in MN and elsewhere (Beaudry and Chabot 1959, Halverson et al. 2008); populations with mixed ploidy levels are common. *S. altissima* is self-incompatible and reproduces sexually by seeds and asexually by clonal rhizomes (Halverson et al. 2008).

Powdery mildews of the *Erysiphaceæ* family are a group of Ascomycetes that include powdery mildew, downy mildew, and blight (Reed 1913). *Erysiphe cichoracearum,* or powdery mildew, is an obligate parasite that infects host species by burrowing haustoria. These haustoria delve into the epidermal tissue of the host plant and parasitize them (Schulze-Lefert and Vogel 2000). Once the fungi develop, they produce asexual conidia that bud off of the main fungal body (Schulze-Lefert and Vogel 2000). The mycelial body that covers the leaf tissues is known to negatively affect photosynthesis in colonized plants (Magyarosy et al. 1976). Ascomycetes can also reproduce sexually, forming ascospores that are borne in ascocarp fruiting bodies within cleistothecia (Reed 1913). This clestothecia is the resting, over-wintering structure that persists to the next growing season, whereby spores are expelled and travel via wind to the next host (Reed 1913). Only one previous study has examined the effects of powdery mildew on *S. altissima s*pecifically (Schmid 1994) and showed that small-scale genetic diversity (i.e. genotypic diversity of clonal stands), positively influenced overall population fitness by reducing the incidence of disease.

Data Collection and Analysis

In the summer of 2011, data was gathered on an existing experiment on *Solidago altissima* to obtain information on the relationship between water availability, flowering time, polyploidy, and infection by powdery mildew. The starting material included diploid and tetraploid lineages that had been subjected to artificial selection for earlier

and delayed flowering for two generations (8 artificial selection lines = 2 ploidy levels x 2 replicates x 2 flowering times: early and late). Plants from the second generation of artificial selection were maintained in pots at the UMD Research and Field Studies Center.

Seedlings were cloned from the existing artificial selection second generation plants, transported in small cardboard pots, and planted directly into the soil in a randomized block design at each site. Disease incidence and reproductive state (as a metric of flowering time) was assessed on these field plots on consecutive days in August (August 16-17, 2011). Reproductive state was assessed using a numeral scale (0=no flowers, 1=green buds, 2=yellow buds, 3=open flowers, 4=post-flowering, 5=seeds dehiscing). Two quantitative and one qualitative estimates of the degree of infection were used, including percent total height and the percent leaves infected, as well presence of infection (y/n). The results were analyzed using a mixed model analysis of variance (JMP Pro SAS Institute, Cary, North Carolina 2012). In the analyses, block was a random effect nested within location. Reproductive state was nested within ploidy because of marked differences in flowering phenology between ploidy levels (diploids flower 9-15 days earlier than tetraploids, Etterson, *unpublished data*). Box-Cox best transformations were made for percent height of infection and percent of leaves infected (percent height^{1}-1/1; percent leaves$^{0.2}$-1/0.01890197237832)

Results

On average, the sites differed with respect to the prevalence of disease caused by powdery mildew. *Solidago altissima* plants grown in the wetter SLC site had higher infection levels of mildew than their counterparts in the drier CC site. Although percentage of leaves infected was non-significant (Figure 2.1A); overall, plants in the wetter SLC site had 30% higher progression of infection from the base of the plant ($p<0.0001$, Figure 2.1B). There was also greater numbers of plants overall that were infected by mildew at the SLC site ($p=0.002$, Table 2.1).

Ploidy significantly affected the infection rates of *S. altissima* with *Erysiphe cichoracearum.* Diploid plants had about 8 percent more leaves covered with mildew than tetraploid plants ($p=0.002$, Table 1, Figure 2.2A) and overall greater incidence of mildew infection than tetraploids ($p=0.001$, Table 2.1). No significant effects from ploidy were found for height of infection (Figure 2.2B). Within ploidy levels, flowering time also had an effect on mildew cover ($p=0.02$, Table 2.1), with late flowering tetraploid plants having more leaves infected than early flowering plants ($\beta_{tetraploid}=-2.37$, $p=0.03$) and early flowering diploids having greater numbers of infected leaves than late flowering diploids ($\beta_{diploid}=1.80$, $p=0.09$). There was a significant effect of flowering time within the ploidy levels ($p=0.009$, Table 2.1), with lower heights of infection in later-flowering diploids ($\beta_{diploid}=3.09$, $p=0.005$) but no significant effect in tetraploids (($\beta_{tetraploid}=1.39$, $p=0.20$).

No significant results were found for the interaction between ploidy and location in percent of leaves infected (Figure 2.3A). This interaction was only marginally significant for percent height of infection, and tetraploids were less infected in the drier

CC location than diploids (p=0.06, Table 2.1, Figure 2.3B). Both cytotypes bore higher mildew loads in the wet SLC location.

The combination of location and within-ploidy flowering time significantly influenced the presence of infection (p=0.01, Table 2.1), with tetraploids in the SLC location showing a significant increase in mildew incidence over the flowering season ($\beta_{tetraploid/SLC}$=-0.53, p=0.01; $\beta_{diploid/SLC}$=0.15, p=0.29). The height transversed by the mildew was also significantly affected, with tetraploids in SLC experiencing more infection early in the growing season than those in the CC location ($\beta_{tetraploid/SLC}$=4.08, p=0.0002; $\beta_{diploid/SLC=}$1.27, p=0.25). Block had no significant effects on any mildew measurements.

Discussion

The wetter Duluth location in St. Louis Co. (SLC). had greater incidence of mildew than the drier Itasca location in Clearwater Creek Co. (CC). Powdery mildew utilizes moisture to travel along the leaf tissue and infect the plant (Knogge 1996, Schulze-Lefert and Vogel 2000), so the importance of water appears to outweigh the benefits of colonizing a drought-stressed host. Because plants utilize water as an electron donor during photosynthesis, the availability of water also affects their ability to produce photosynthetic assimilates, which is the material that the pathogen is extracting for its own nutritive benefit (Williams and Aryes 1981, Blankenship and Hartman 1998).This finding has implications for areas of the world, such as high latitude regions, were moisture is expected to increase within the upcoming years (Garrett et al. 2006). Also, changes in storm models and occurrence may alter pathogenicity and pathogen movement, and increases in precipitation due to these storms could have potent effects on crop and native species (Garrett et al. 2006, Anderson et al. 2004).

We found that polyploidy influenced the degree to which *Solidago altissima* became infected with powdery mildew. Diploids had a greater percentage of total mildew and had a greater extent of the disease cover up the length of the plant. This was found to be the case regardless of the water availability of the location. Other studies have found that polyploid cytotypes may contain more secondary compounds, such as alkaloids, which may discourage colonization of pathogens and predation by insects (Beest et al. 2012). It is known that the genetic makeup of *S. altissima* can affect the ability of powdery mildew to colonize its tissues; Schmid (1994) found that greater genotypic

variability of *S. altissima* stands decreased the incidence of powdery mildew found on the plants; however, Schmid's study did not deal with differences in cytotype.

Interestingly, flowering patterns were different between cytotypes. Tetraploids experienced more incidents of mildew colonization later in the season, but the extent of the mildew damage was less as the season progressed. Conversely, diploids experienced less incidence of mildew infection early in the season, and the area of mildew cover (percent height of infection and percent of leaves infected) increased throughout the season. Although our initial hypothesis predicted that early flowering plants would become more infected, it appears that the two cytotypes have different strategies for escaping peak mildew season, by either flowering before or after it takes place. However, the strong effect of flowering time is consistent with our initial hypothesis. As flowering time becomes progressively advanced in the growing season, this may cause a loss of fitness, especially in these locations that experience more moisture due to climate change. This may lead to a fitness peak somewhere between flowering early enough in the season to outcompete neighbors for mating opportunities, yet flowering late enough to avoid extensive pathogen colonization. Other studies, such as those by Aragón et al. (2008), have found fitness peaks to be highly influenced by changes in plants' abiotic environment. These changes include drought-which can affect flowering phenology, and small changes in timing of flowering can have large effects on reproduction. This becomes increasingly complex in species such as *S. altissima* that have both asexual and sexual methods of propagation.

Schmid (1994) has suggested that that disease may select upon the propagation method of these plants with dual reproductive modes. Sexual propagation may allow for

an escape from diseases such as powdery mildew. Not only that, but researchers also found that sexual reproduction and seed dispersal may play a key role in invasive properties of a plant (Meyer and Schmid 1999). *S. altissima* is a notable invasive in Europe and Asia, and is found to have similar distributions of phenotypic variation across climatic gradients in Japan as is found in its native North America range (Etterson et al. 2008). In some cases, the increases in pathogenicity can curb the spread and noxiousness of certain invasive weeds, such as in the case of *Bidens pilosa* in South Korea, whose fitness is suffering as a consequence of powdery mildew infection (Cho et al. 2013). However, in most cases these infections are detrimental to the delicate balance of an ecosystem (Gilman et al. 2001).

Climate change is already altering disease dynamics in plants in several ways. Drying in certain regions of the globe may limit the spread of pathogens, while more intense rainfall in other areas may increase virulence in some cases. Temperature increases that affect phenological events in plants, such as emergence and flowering time, may shift the timing of these events. These changes may affect how pathogens interact with their hosts and could offer a potential means of escape from disease. Genetic differences in host plants may also play a role in how severe the effect of these factors is on the fitness of the plants

Future work to be undertaken includes the analysis of the effects of all of these factors- flowering time, water availability, and cytotype- in a controlled environment to eliminate the effects of local factors such as other plant predators, extreme weather events, and the differences in wind available for spores to disperse upon. Additionally, tracking the maternal effect of mildew infection would shed light on the affect that

mildew had across generations of these plants, both within their asexual and sexual propagules. No previous work has been done to look at the interaction of factors that contribute to pathogenicity during climate change in such detail, and this is what must be done to truly understand the multifaceted nature of ecological change.

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Tables

Table 2.1 ANOVA test statistics (*F*, *P*) of three measurements of *Erysiphe cichoracearum* infection on diploid and tetraploid *Solidago altissima* that were artificially selected for early and late-flowering and reared in two locations in Minnesota with contrasting water availability.

	Three Measurements of *Erysiphe cichoracearum* Infection		
Factors	**Presence/ Absence**	**% Infected Height**	**% Infected Leaves**
Location[1,3]	**9.64, 0.002**	**58.92, < 0.0001**	0.66, 0.42
Block[Location][2,3]	1,79, 0.41	0.87, 0.42	1.82, 0.16
Ploidy[1,3]	**10.50, 0.001**	1.45, 0.23	**9.29, 0.003**
Reproductive stage[Ploidy][2,3]	**68.66, <0.0001**	4.79, **0.009**	**3.83, 0.02**
Ploidy x Location[1,3]	0.88, 0.35	3.68, 0.06†	1.10, 0.16
Reproductive Stage [Ploidy] x Location[2,3]	**8.71, 0.01**	**7.61, 0.0006**	0.31, 0.73

[1] *df* numerator =1 [2] *df* numerator = 2 [3] *df* denominator = 299

Figure Legends

Figure 2.1 Differences between study site in average percent of A) total leaves and B) height of *Erysiphe cichoracearum* infection on *Solidago altissima* plants at two locations with contrasting water availability: the drier, western Clearwater Co. site (CC) and the wetter, eastern St. Louis Co. site (SLC). *Solidago altissima* plants were artificially selected for early- and late-flowering and are of both diploid and tetraploid cytotypes. Dots indicate least square means and bars represent one standard error. On the y-axis, one through thirty are omitted for clarity.

Figure 2.2 Differences between diploid and tetraploid cytotypes of *Solidago altissima* in average percent of A) total leaves and B) height of *Erysiphe cichoracearum* infection on plants reared in at two locations with contrasting water availability and artificially selected for early- and late-flowering. Dots indicate least square means and bars represent one standard error. On the y-axis, A) one through thirty and B) one through fifty are omitted for clarity

Figure 2.3 Differences between the interaction of ploidy and study site in average percent of A) total leaves and B) height of *Erysiphe cichoracearum* infection on diploid and tetraploid *Solidago altissima* plants reared in at two locations with contrasting water availability: the drier, western Clearwater Co. site (CC) and the wetter, eastern St. Louis Co. site (SLC) and artificially selected for early- and late-flowering. Dots indicate least square means and bars represent one standard error. On the y-axis, one through thirty are omitted for clarity.

Figure 2.1

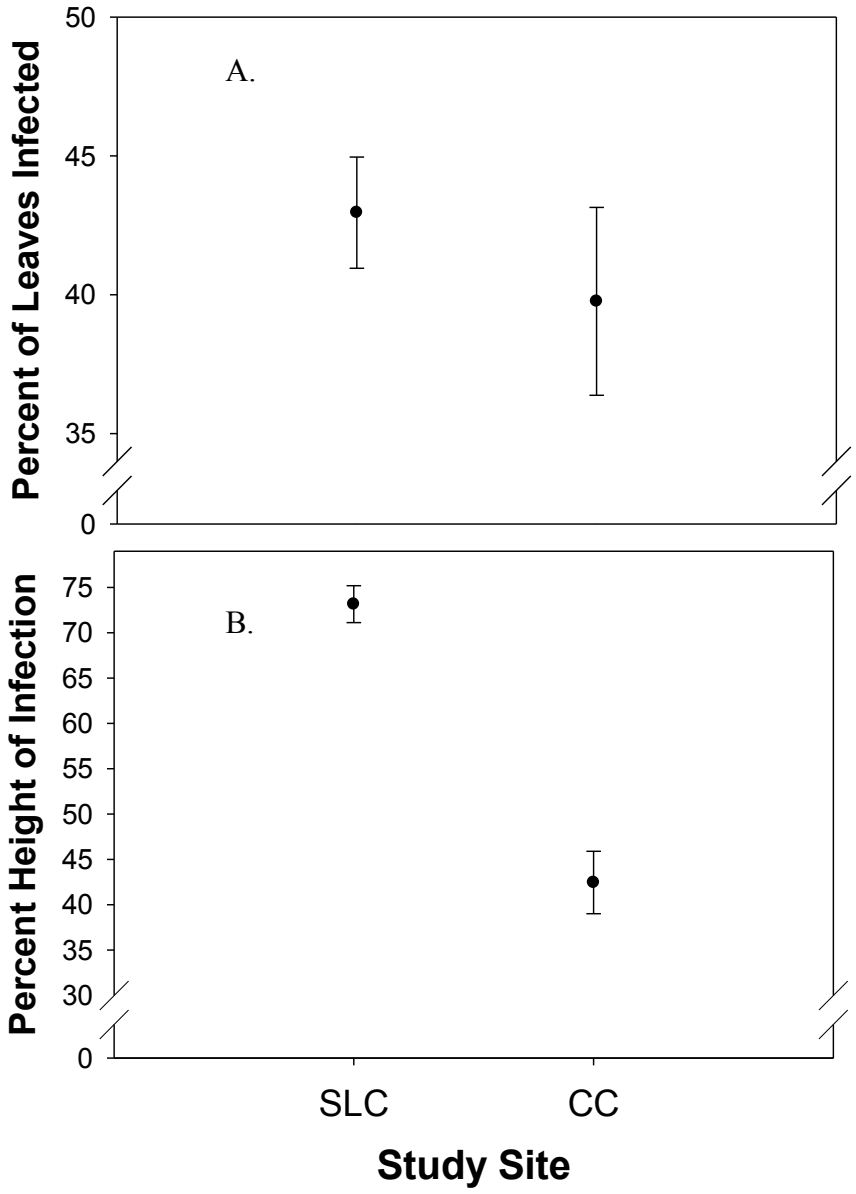

A.
Percent of Leaves Infected
50
45
40
35
0
B.
Percent Height of Infection
75
70
65
60
55
50
45
40
35
30
0
SLC
CC
Study Site

Figure 2.2

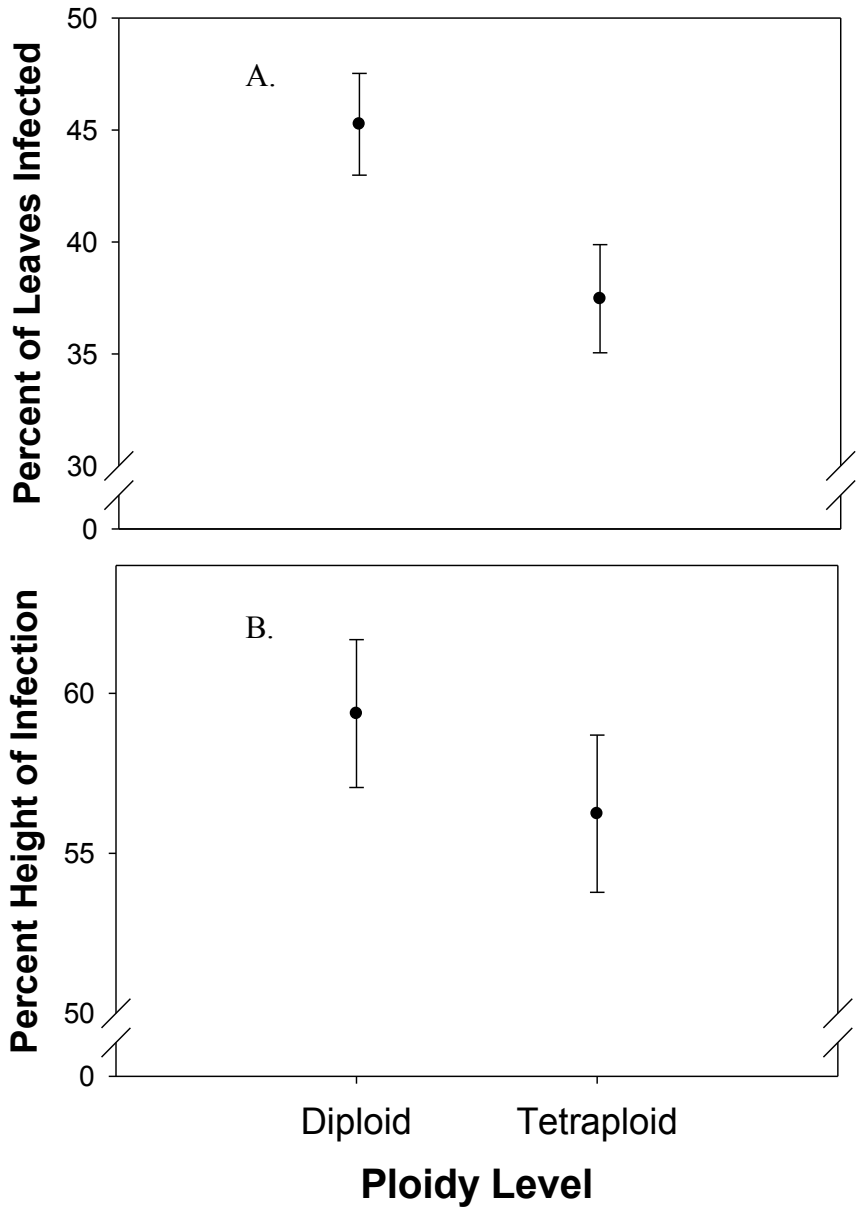
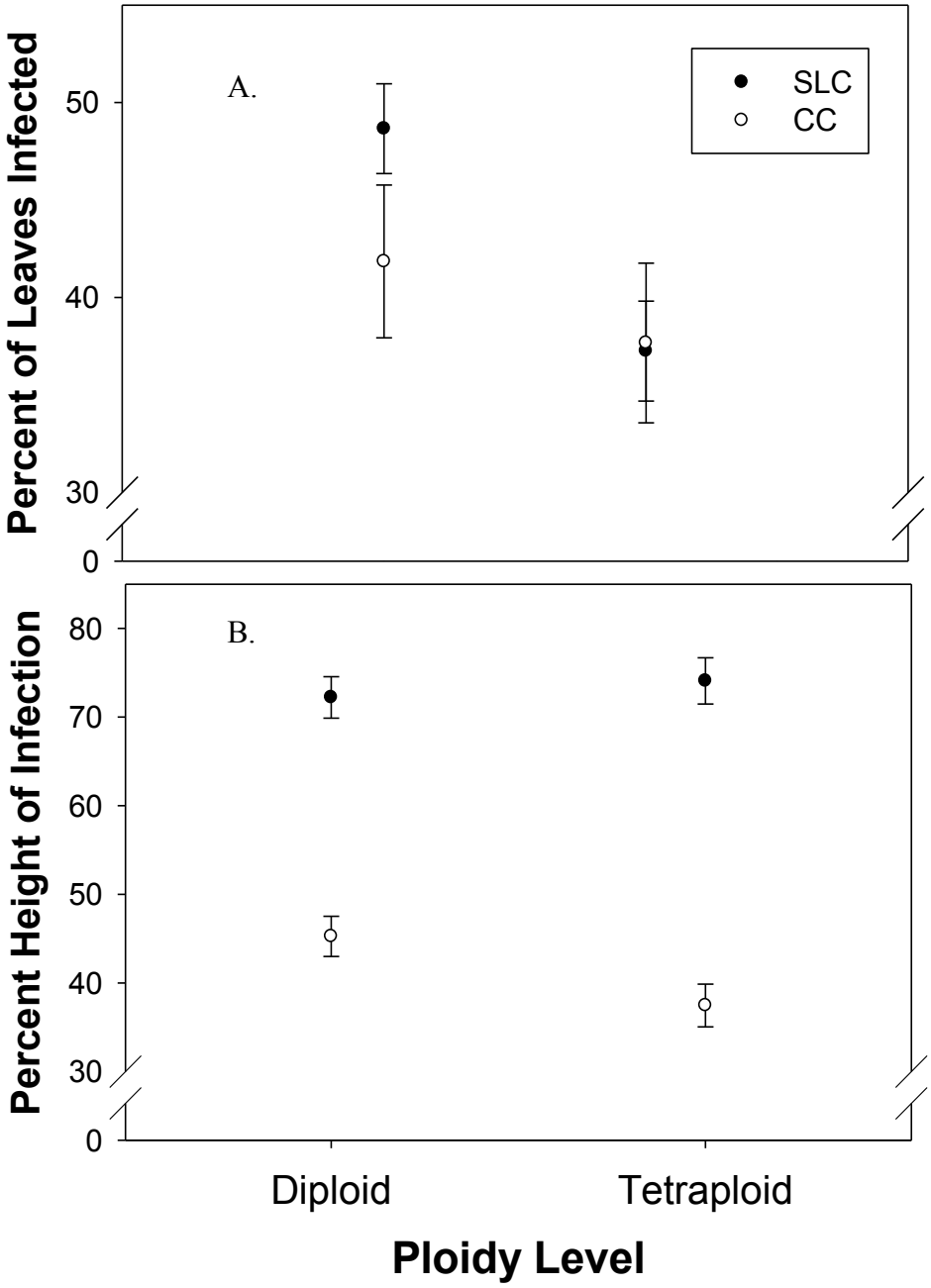

A.
Percent of Leaves Infected
50
45
40
35
30
0
B.
Percent Height of Infection
60
55
50
0
Diploid
Tetraploid
Ploidy Level

Figure 2.3


A.
SLC
CC
Percent of Leaves Infected
50
40
30
0
B.
Percent Height of Infection
80
70
60
50
40
30
0
Diploid
Tetraploid
Ploidy Level

Chapter 3

Maternal effects of *Erysiphe cichoracearum* infection, manipulated flowering time and water availability on polyploid *Solidago altissima* (late goldenrod)

The biotic and abiotic environment that a maternal plant experiences can affect fitness of its offspring. These environmental factors can include drought stress, pressures for earlier flowering phenology, and pathogen infection, which are all strongly influenced by changes in the global abiotic climate. We investigated the effects of maternal water availability, maternal flowering time, and maternal leaf health on both diploid and tetraploid *Solidago altissima* seedlings. We took seed from diploid and tetraploid mother plants with different amounts of powdery mildew, dry and wet watering treatments, and differing flowering times and germinated and reared seedlings in a common greenhouse environment. We measured germination, seedling height, stem diameter and leaf number. Mothers that experienced moderate levels of *Erysiphe cichoracearum* (powdery mildew) infection produced more robust offspring than completely healthy or heavily infected maternal plants, and this relationship changed with maternal water availability. Maternal flowering time also influenced overall germination success. The amount of genetic material had no bearing on the fitness of the seedlings. These findings suggest that *E. cichoracearum* may pose selection pressures on progeny fitness and these pressures may vary in response to a changing climate.

Key Words: Maternal effects, polyploidy, climate change, pathogen, flowering phenology, *S. altissima, E. cichoracearum*

Introduction

Fitness costs of disease are not only apparent in the health of the generation afflicted by the disease, but can also affect the reproductive efforts of the plant system. Maternal effects are conditions that the mother plant experiences that may affect the fitness of its progeny (Roach and Wulff 1987, Lacey et al. 2003). Maternal effects can manifest themselves via seed set output and seed quality, as well as by inherited adaptive plasticity to environmental pressures (Galloway 2005). The effects of maternal disease can be manifested by changes in growth and development of offspring (Carriére et al. 2001, Lacey et al. 2003), the inheritance of disease resistance or tolerance (Machlanahan 1978, Simms and Triplett 1994) or via inheritance of the disease across generations (Jirtle and Skinner 2007, Anway and Skinner 2008, Skinner et al. 2010). Transgenerational inheritance of disease lines can be transmitted by epimutations in the germ tissue (Skinner et al. 2010). Because environment has such a strong effect on the epigenetics of tissues, as well as on disease frequency, this set of far reaching consequences has become of increasing interest, as we investigate the effects of a changing global environment across generations (Räsänen and Kruuk 2007).

As temperature and moisture availability vary, so too will fungal disease prevalence (Chakraborty et al. 1998, Chakraborty et al. 2000, Anderson et al. 2004, Garrett et al. 2006). Fungi are typically moisture-limited, so overall drier conditions, such as those anticipated for Minnesota by Galatowitsch et al. (2009) may lead to reduced habitat for parasitic fungi (Chakraborty et al. 2000, Garrett et al. 2006). However, drought-weakened host plants may be susceptible to colonization, so predictions of

disease prevalence are difficult to interpret (Chakraborty et al. 2000, Anderson et al. 2004).

Other plant functions may be affected by climate change. In addition to potential drought stress and different disease interactions, phenological changes may also occur that can affect disease exposure (Alexander et al. 1996, Garrett et al. 2006). Scientists have overwhelming evidence that plants have been flowering earlier and earlier in response to a warming climate for the past 250 years (Fitter and Fitter 2002, Balasubramanian et al. 2006, Amano et al. 2010, Brunet and Larson-Rabin 2012). This extended exposure to environmental stressors, such as disease and drought, may reduce the fitness of the early-flowering plants, regardless of the potential benefits of early flowering, such as increased pollen dispersal and escape from herbivory (Lacey and Pace 1983, Walck et al. 2001, Elzinga et al. 2007). The maternal effects of flowering phenology have been investigated by other researchers and have been found to influence offspring germination, growth and reproduction (Lacey and Pace 1983).

Interactions between parasites and plant fitness are complicated and often difficult to interpret (Chakraborty et al. 1998, Anderson et al. 2004). Organisms that prey upon plants are known to affect several plant characteristics and processes, including resource allocation, leaf area, reproductive strategies, production of secondary compounds, trichome density, seed characteristics, and in extreme cases, hypersensitive response (Agrawal 1998, Agrawal 2000, Lam et al. 2001). Amidst much speculation, one largely unknown constituent of plant health is the effect of polyploidy on plant defenses and susceptibility to disease (Shoen et al. 1992, Wright et al. 1998 Innes et al. 2008). Polyploid plants are characterized by having multiple chromosomal copies, and this is

thought to allow for up-regulation of genes and gene products (Comai 2005, Chen and Ni 2006). This genetic malleability may enhance the ability of polyploid plants to be successful in their environments.

While many researchers have examined the effects of flowering phenology, climate change, ploidy, and maternal effects, none have investigated the complex interactions of all four in tandem with fungal pathogen infection (Schmid 1994, Chakraborty et al. 2000, Lacey et al. 2003, Garrett et al. 2006). To more completely understand the changes that will occur within our ecosystems, a broad-scale investigation of all factors is necessary. Artificial selection studies are ideal when examining changes that may occur in a phenotype (such as flowering time) over successive generations. We utilized artificial selection on differing cytotypes, as well as controlled environmental treatments and natural field mildew colonization to subject mothers to environmental stressors, then grew seedlings from these mothers in a controlled environment to parse out the maternal environmental effects.

We hypothesized that detrimental effects from maternal disease would be found in progeny; with diseased plants producing seeds less likely to germinate and seedlings with reduced growth. We anticipated that because fungal pathogens would colonize early-flowering, drought-stressed plants to a greater degree than their late-flowering, well-watered counterparts, these plants would also have less fit offspring. Finally, we investigate the possibility that polyploid plants may have fewer detrimental maternal effects than diploid plants due to their greater genetic potential.

Materials and Methods

Study Organisms

Solidago altissima, or late goldenrod, is an herbaceous dicot perennial in the family Asteraceae (Halverson et al. 2008). This species has a broad distribution across North America and is native to MN. This species is also found in Europe and Asia as an exotic species and is a particularly strong invader of areas such as Japan (Etterson et al. 2008). *S. altissima* encompasses multiple ploidal levels, including diploid (2n, 2x=18), tetraploid (2n, 4x=36), and hexaploid (2n, 6x=54) cytotypes in MN and elsewhere (Beaudry and Chabot 1959; Halverson et al. 2008); populations with mixed ploidy levels are common. *S. altissima* is self-incompatible, pollen limited, and reproduces both sexually by seeds and asexually by clonal rhizomes (Halverson et al. 2008). *S. altissima* is pollen limited and relies on insect pollination (Gross and Werner 1983, Santandreu and Lloret 1999). Other research has been done by Schmid and Dolt (1994) examining the parental effects of environment and genotype in *S. altissima,* but their investigation was targeting the effects of soil nutrients on offspring, rather than the maternal effects of disease.

Erysiphe cichoracearum is an ascomycete and a common obligate leaf parasite. It infects host plants via haustoria, which enter the epidermal leaf tissue and utilize the plant's photosynthetic assimilates (Schulze-Lefert and Vogel 2000). Once the fungi develop, they produce asexual conidia that bud off of the main fungal body (Schulze-Lefert and Vogel 2000). Ascomycetes can also reproduce sexually, forming ascospores that are borne in ascocarp fruiting bodies within cleistothecia (Reed 1913). This clestothecia is the resting, over-wintering structure that persists to the next growing season, whereby spores will be expelled and wind dispersed to the next host (Reed 1913).

Studies by Schmid (1994) researched the effects of powdery mildew on *S. altissima* specifically and found that genotypic variation affected host plant susceptibility. Schmid's (1994) experiment was conducted in the exotic range of *S. altissima* in Switzerland, whereas ours takes place in the native MN, United States range.

Study Site and Conditions

S. altissima seeds were taken from parental plants grown at the University of Minnesota Duluth Research and Field Studies Center on Jean Duluth Road in Duluth, Minnesota. These parental plants were raised from seed in 2010, with parental plants of known genotypes, flowering times and ploidy levels. Artificial selection crosses for early, control, and late flowering times were made and seed from these plants reflect those matings. This was the second generation of artificial selection for flowering time. Early flowering plants in this generation flowered an average of 6 days sooner than late flowering plants (Figure 3.1A).

After senescence and overwintering in 2010, these plants were rhizomatously propagated in the spring of 2011. Plants were grown in 6x6x12 inch pots, 1440 pots were placed in 12 blocks, each containing two populations of 60 plants each in a single ploidy level, either a diploid or tetraploid. Also, half of the pots were subjected to ambient dry conditions, while the other half of the pots were well watered (Figure 3.1B). The difference between watering treatments was monitored via TH2O Soil Moisture Meter (Dynamax, Houston, TX) measurements throughout the season. (1440 plants comprised of 2 ploidy levels (diploid, tetraploid) x 3 artificial flowering treatments (early, control, late) x 2 watering treatments (ambient dry and well-watered) x 2 replicates x 60

plants/line). A few plants from late-flowering lines were missing as they did not produce flowers or seed in the previous season.

In the fall of 2011, seed from these stock maternal plants was collected for use in the greenhouse study of maternal effects. It was stored for use in the summer of 2012 at approximately 7 degrees C. In June of 2012, these seeds were planted at the University of Minnesota Duluth Experimental Greenhouse at standard greenhouse conditions. Control flowering seed was not used to allow for greater replication of the early and late flowering lines. Four seeds of each combination of maternal infection state, ploidy, artificial selection flowering time line, and watering treatment (Figure 3.2) were planted into each 6.3 cm x 6.3 cm x 15 cm cardboard container filled with ProMix soil. These containers were placed in crates (approx. 27 containers/crate). Three crates were then placed inside one of six containment shelters. These shelters were constructed out of CPVC pipe and nylon ripstop fabric. This fabric allowed for some gas exchange and sun penetrance without compromising the cleanliness of the shelter.

Three shelters held plants to be infected and three shelters contained plants that were left to grow untainted. The placement of the shelters in the greenhouse was randomized. *S. altissima* plants were monitored for date of germination over the months of July and August. Germination counts and plant survival (out of 4 seeds planted) were taken, and containers were weeded down to one plant/pot. At this time, substitutions of the same ploidy/parental treatment/parental flowering time/parental infection were made to pots with failed germination or dead plants.

Data Collection and Analysis

At peak season, plants in three of the six shelters were subjected to a facilitated infection of *E. cichoracearum.* This was done by collecting infected field *S. altissima,* running a stiff-bristled paintbrush over the visible mildew, and running a finger across the top of the brush bristles as the brush was held a few inches above the healthy plants. Powdery mildew was found on plants in these shelters a few weeks later. *S. altissima* plants were measured a few weeks later for basic growth, in addition to germination counts taken previously. Measurements included height, leaf number, and stem diameter. Data was analyzed using a mixed-model ANOVA using JMP SAS software (JMP Pro 10, 2013). Date of first flowering was nested within ploidy level as it is strongly influenced by cytotype (diploids flower 9-15 days earlier than tetraploids; Etterson, *unpublished data*). Data was also transformed using the Box-Cox best transformation (height=height$^{0.4}$-1/0.15392821463125, stem diameter= stem diameter$^{0.2}$-1/0.13461193196111, leaf number=leaf number$^{0.2}$-1/0.033760621356).

Results

Germination

Maternal water availability was marginally influential on germination rates (p=0.08, Table 3.1). Powdery mildew in the maternal generation was also marginally significant (p=0.07, Table 3.1, Figure 3.3A), and mother plants with moderate levels of infection had the greatest germination success. Within each ploidy level, germination was influenced by the flowering time in the maternal plant (p=0.0499, Table 3.1), with maternal tetraploid plants with an earlier date of first flower having less germination success than those that flowered later in the season ($\beta_{tetraploid}$= 0.44, p=0.03; $\beta_{diploid}$= -0.15, p=0.14). The shelter in which the seeds were grown also influenced the germination rate of the seedlings (p=0.009, Table 3.1).

Powdery Mildew Facilitation

While a few plants became infected with mildew, not enough replicates were present to complete a full analysis. The method of infection was sufficient, but growth conditions perhaps were too arid for powdery mildew penetration and growth on the leaf tissue.

Late Season Seedlings

Powdery mildew in the maternal generation significantly affected seedling leaf number (p=0.01, Table 3.1, Figure 3.3B). Seedlings from mothers with low or moderate levels of infection averaged more leaves, or were bushier, than those from mothers without mildew and with high mildew (Figure 3.3 B). The interaction of maternal mildew cover with maternal water availability also marginally significantly influenced seedling leaf number (p=0.02, Table 3.1, Figure 3.4A). When examining the interaction of

maternal water availability and mildew cover, mothers that were well-watered and had low levels of mildew produced the leafiest seedlings (Figure 3.4A). Mothers grown in ambient dry conditions that were moderately infected yielded bushier seedlings than the mothers with other levels of mildew cover.

Powdery mildew in the maternal generation also affected seedling height ($p=0.03$, Table 3.1, Figure 3.3C) and seedlings from mother plants that experienced low and moderate levels of infection grew the tallest (Figure 3.3C). Ploidy was also a determining factor of seedling growth ($p=0.02$, Table 3.1). Tetraploid seedlings were found to be 24% taller than diploid seedlings, although in combination with maternal infection levels, ploidy was non-significant. Height was also influenced by the combinations of maternal watering availability and mildew cover ($p=0.04$, Table 3.1, Figure 3.4B). Within well-watered mothers, having low mildew cover yielded the tallest offspring. Mothers that experienced ambient dry conditions and moderate infection also produced taller offspring than those that experienced zero, low, or high powdery colonization (Figure 3.4B).

Stem diameter was found to be marginally significant for ploidy ($p=0.08$, Table 3.1). This was not found to be significant in any other univariate tests or in combination with any other factor.

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Discussion

We found that maternal health has effects on offspring fitness, but these effects were different depending on maternal water availability. Germination was marginally greater in offspring from mothers that experienced high water availability. Water availability has been found to be a large influence on plant fitness in other studies, and decreased water availability can be evidenced in decreased seed weight (Roach and Wulff 1987). In contrast to our experiment, studies by Luzuriaga et al. (2006) found that increased water availability reduced germination rates in their system. Additionally, studies by Dudley (1996) found that high water availability can influence leaf morphology, and phenotypic changes can influence energy input into vegetative biomass versus reproductive biomass. However, overall plant health is bolstered by adequate water supply (Farooq et al. 2009), and intuitively, these effects should be transmitted to the offspring (Roach and Wulff 1987, Galen 2000). Floral attractive traits, such as nectar volume, have also been found to be reduced by drought stress (Carroll et al. 2001). For a pollen limited species, such as *S. altissima,* (Walck et al. 2001, Santandreu, and Lloret. 1999) this could have strong influences on progeny fitness.

The effect of water availability continued throughout the growth of the seedlings. Powdery mildew on maternal plants, in combination with water availability, had an effect on both seedling height and leaf number. Maternal plants with limited water availability and moderate (medium) levels of infection produced the tallest offspring. Well-watered plants, on the other hand, produced the tallest seedlings under low levels of infection. In addition to the role of sexual propagation as a way of escape from current biotic stressors,

this mode of escape may also be useful to combat abiotic stressors, such as drought (Barnabás et al. 2008).

Blocking effects, or shelter locations, were significant for germination, but because we substituted extra germinants into pots that did not germinate, we eliminated these effects from further growth measurements. Growth measurements (height, leaf number) later in the season indicate that moderately infected mothers produced seedlings with greater growth. This finding of greater seedling robustness in progeny from moderately infected mothers rather than from mothers that were disease-free is surprising. This may indicate a selection for sexual reproduction in systems with dual reproductive modes. Other researchers have hinted at the different pressures for sexual vs. asexual reproduction maintained by plant pathogens, specifically regarding the Red Queen Hypothesis of continued defense adaptation against invading pathogens (Hamilton 1980, Clay and Kover 1996, Kelley 1996, Otto and Nuismer 2004, Busch et al. 2007). In the case of *S. altissima,* moderate infection may encourage energy output into sexual propagules (seeds), rather than asexual propagules (rhizomatous clones). Schmid (1994) not only reached similar conclusions about the role of pathogens influencing sexual propagation, but also suggested that pathogens may encourage lateral clonal growth away from infected stands. Because our clones were enclosed in pots, perhaps the only means of escape was via seeds.

Tetraploid individuals produced larger seedlings than their diploid counterparts; however, ploidy itself affects the size of seedlings. Diploid and tetraploid cytotypes of *S. altissima* are morphologically different regardless of maternal plant health, and this is evident in previous studies in other systems (Hardy et al. 2000), as well as from

experimentation in this system (Etterson, *unpublished data*). Diploids also flower earlier in the season inherently (Etterson, *unpublished data*) but because we nested flowering time within ploidy level, we accounted for this phenological difference and only saw significance within that factor for germination. Other studies cite flowering time as a major contributor to overall seed production and seedling fitness (Lacey et al. 1983, Lacey et al. 2003). This inherent difference in flowering phenology between the ploidy levels may be a key contributor to fitness differences in a changing environment.

Future studies investigating the third generation of plants could provide insight into the transgenerational effects of powdery mildew, and this would detail how far these effects can be detected throughout the generations. Also, while our attempt at examining the maternal effects of infection and the strengthening of defense in progeny was unsuccessful, other studies have aimed to uncover the inheritance and changes in gene expression leading to local adaptation in progeny following a stressor in the maternal environment (Galloway 2005). Our study provides insight into the complexity of plant-pathogen interactions, especially in systems that have both sexual and asexual modes of reproduction, during abiotic stress or change. These types of studies are imperative if we are to understand the complex and dynamic nature of the multigenerational effects of disease and climate change.

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TH2O Soil Moisture Meter, Dynamax, Houston, TX

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Tables

Table 3.1 ANOVA test statistics (*F*-statistics, and *p*-values) of offspring traits measured in the greenhouse from seed collections on maternal S*olidago altissima* plants with different levels of *Erysiphe cichoracearum* infection, ploidy, water availability, and flowering time.

Factors	**Percent Germination** [4]	**Height** [5]	**Stem Diameter**[6]	**Leaf Number**[5]
Maternal Infection Index [3]	2.35, 0.07†	3.06, **0.03**	0.45, 0.72	3.66, **0.01**
Ploidy [1]	0.00, 0.95	5.42, **0.02**	3.10, 0.08†	0.28, 0.59
Maternal Watering Treatment [1]	3.15, 0.07†	0.047, 0.49	0.01, 0.93	0.01, 0.94
Date of Flowering[Ploidy][2]	3.02, **0.0499**	1.23, 0.29	1.22, 0.30	1.12, 0.33
Ploidy x Infection [3]	0.75, 0.52	0.16, 0.92	0.30, 0.83	0.45, 0.72
Watering x Infection [3]	2.08, 0.10	2.87, **0.04**	0.56, 0.64	3.20, **0.02**
Shelter[1]	6.86, **0.009**	0.73, 0.40	0.29, 0.59	0.08, 0.77

[1]*df* numerator =1 [2] *df* numerator = 2 [3]*df* numerator=3 [4]*df* denominator = 478 [5]*df* denominator =270 [6]*df* denominator =222

Figure Captions

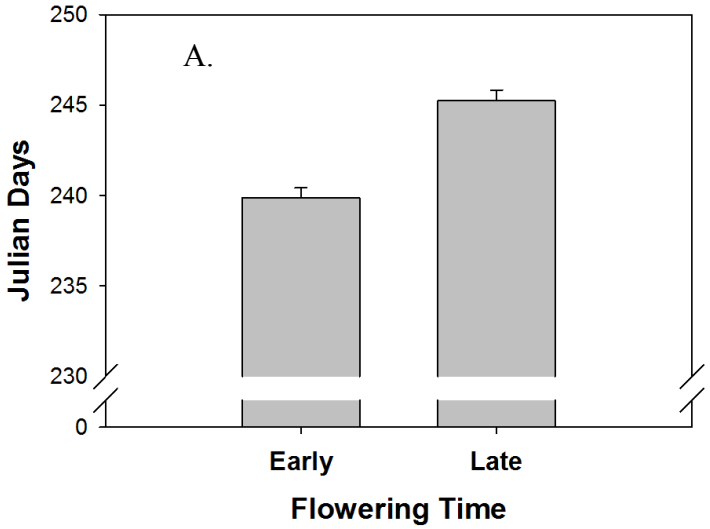
Figure 3.1 Least square means and one standard error of the maternal A) artificial selection line flowering time (1 through 230 omitted for clarity on y-axis) and B) soil water saturation in well-watered and ambient dry treatment pots of *Solidago altissima* in the second generation of artificial selection on early- and late-flowering.

Figure 3.2 Maternal lines of *Solidago altissima* planted in each of 6 shelters within a greenhouse consisting of 16 combinations of ploidy, watering treatment, flowering time, and infection state. Diploid lines were omitted for clarity.

Figure 3.3 Least square means and one standard error of the average A) germination, B) leaf number and C) height (cm) of *Solidago altissima* seedlings grown in a common greenhouse environment from maternal plants with zero, low, medium and high levels of *Erysiphe cichoracearum* infection.

Figure 3.4 Least square means and one standard error of the average A) leaf number and B) height in *Solidago altissima* seedlings grown in a common greenhouse environment from maternal plants subjected to dry and well-watered treatments and infected by zero, low, medium, and high levels of *Erysiphe cichoracearum.*

Figure 3.1


A.
250
245
240
235
230
0
Julian Days
Early
Late
Flowering Time

.

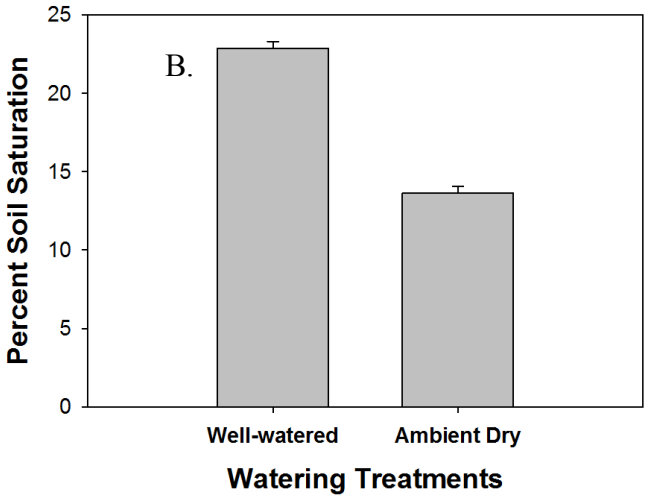

B.
25
20
15
10
5
0
Percent Soil Saturation
Well-watered
Ambient Dry
Watering Treatments

Figure 3.2

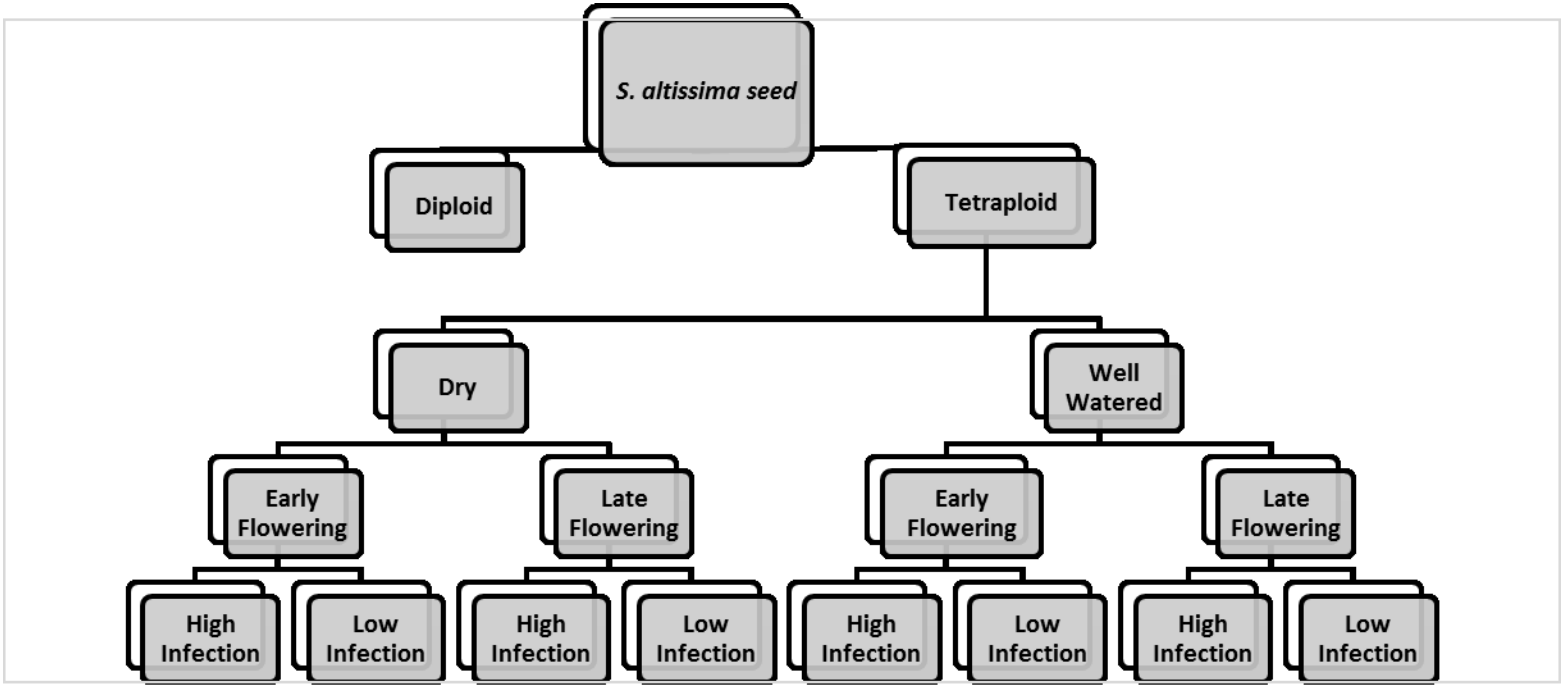

S. altissima seed
Diploid
Tetraploid
Dry
Well Watered
Early Flowering
Late Flowering
Early Flowering
Late Flowering
High Infection
Low Infection
High Infection
Low Infection
High Infection
Low Infection
High Infection
Low Infection

Figure 3.3

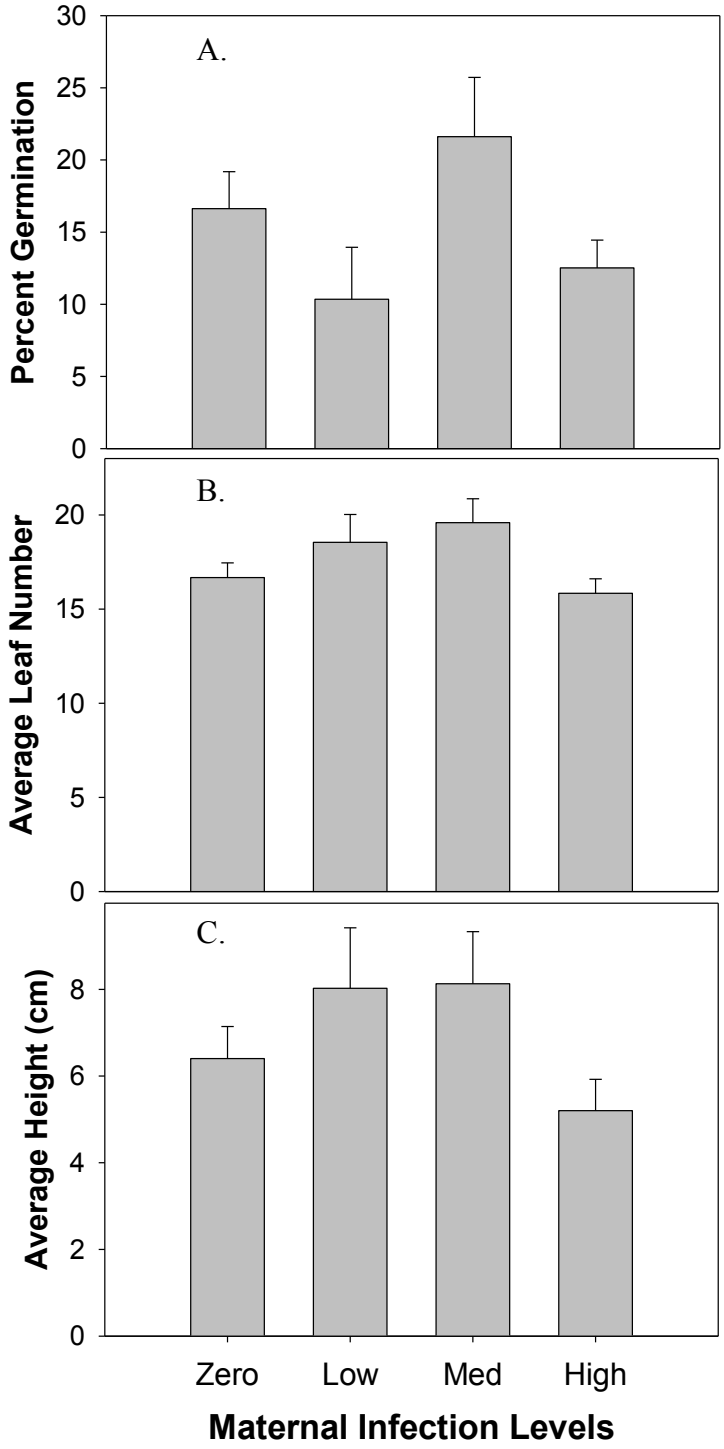
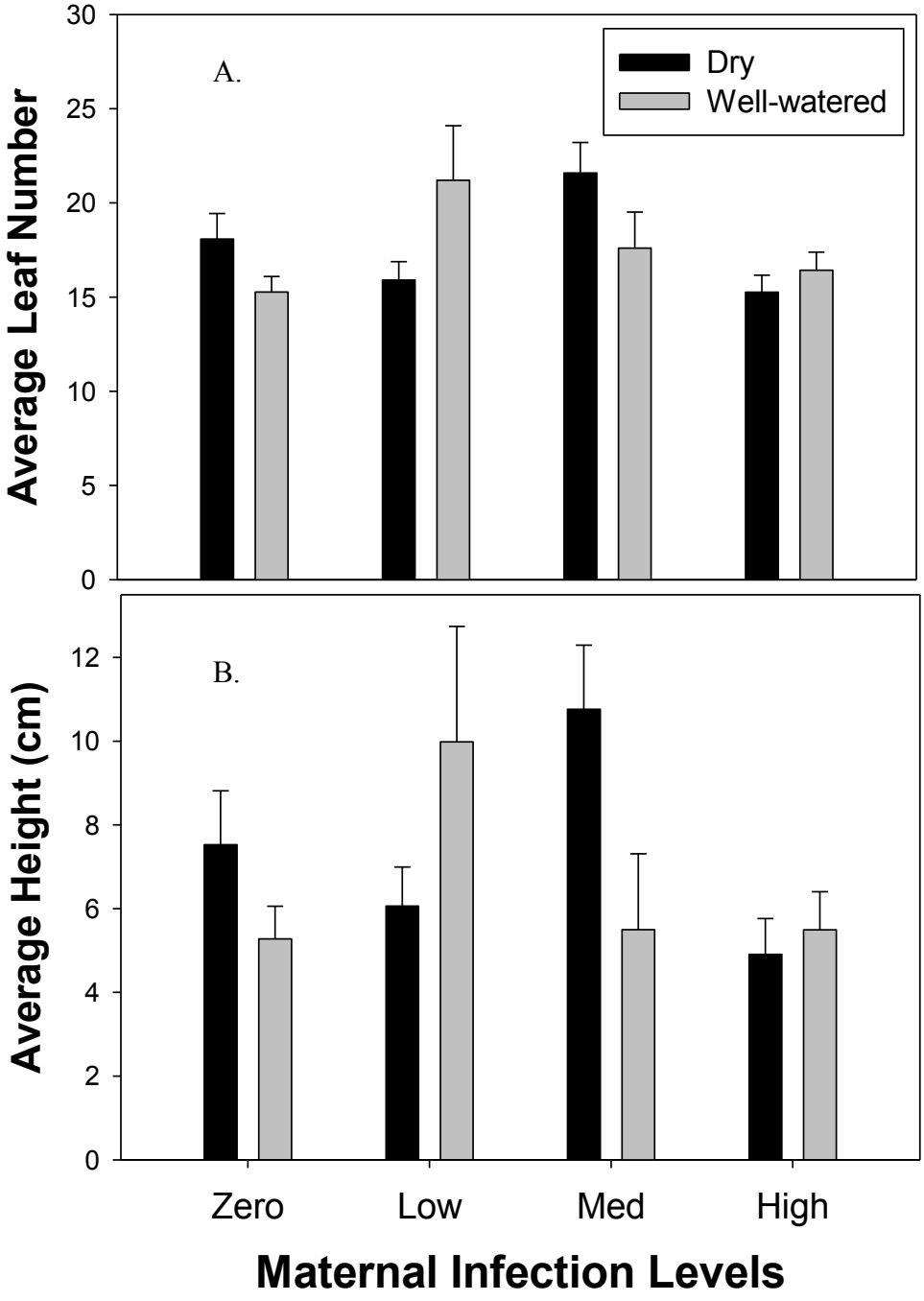

A.
Percent Germination
30
25
20
15
10
5
0
B.
Average Leaf Number
20
15
10
5
0
C.
Average Height (cm)
8
6
4
2
0
Zero
Low
Med
High
Maternal Infection Levels

Figure 3.4


A.
B.
Dry
Well-watered
Average Leaf Number
Average Height (cm)
0
5
10
15
20
25
30
0
2
4
6
8
10
12
Zero
Low
Med
High
Maternal Infection Levels

Chapter 4

Effects of flowering time, water availability, and *Erysiphe cichoracearum* (powdery mildew) infection on diploid and tetraploid *Solidago altissima* (late goldenrod)

Climate change is predicted to have many effects on plants, including changes in flowering phenology, moisture availability, and disease susceptibility. These effects may even be detected in following generations and affect offspring fitness. Polyploid plants, having multiple chromosomal copies, have often been looked to as a possible source of genetic flexibility in response to a changing environment. We tested the health of diploid and tetraploid *Solidago altissima* artificially selected for flowering time and subjected to drought treatments against attempted *Erysiphe cichoracearum* (powdery mildew) infection. These plants were grown from parental plants also experiencing well-watered and drought-stressed treatments. During peak season, we measured the height of infection, visual infection score (low/medium/high infection) and overall percent mildew cover. We found surprising evidence that diploid plants were inherently healthier than their tetraploid counterparts, and this may be due to morphological leaf traits such as trichome density. Powdery mildew was more prevalent in well-watered plants, and the water-availability in parental plants had consequences for the health of the next generation. Early-flowering plants were exposed to the pathogen for greater periods of time and accumulated greater mildew cover over the season. These findings are significant in light of the earlier growing seasons and drier climates we are expected to experience in Minnesota, and this has implications for fungicide applications in crops and preservation of native species.

One Sentence Summary: Diploid *Solidago altissima* remains healthier in response to attempted *Erysiphe cichoracearum* infection than tetraploid *S. altissima* during climate-change simulation conditions.

Introduction

Climate has been used as a predictor of plant health for years (*1*). However, it is largely unknown what the effect of anthropogenically-derived climate change will be on plant- disease interactions (*2).* According to the traditional disease triangle, disease occurs at the intersection of susceptible host, pathogen virulence, and amenable environment (*1, 3*). Changes that influence these dynamics could be detrimental to plant fitness, and this has implications for crop health and survival of native plant species (*1, 4, 5*). To further complicate predictions of disease prevalence, phenological changes, such as earlier flowering time, may expose plants to their environment and pathogens for an increasingly greater period of time each season (*6*).

Earlier flowering time has been observed to be closely tied with increases in temperature, such as those taking place in the last century (*6, 7, 8, 9*). While early flowering time can be advantageous for several reasons, such as increased fecundity and herbivory escape (*10*) flowering is an energetically costly process. Once plants reproduce, there may be little resources left over to defend against disease infection (*11*). This may have important consequences for the offspring of these plants, as well. The maternal effects of drought and disease may be evidenced in the fitness of the successive generations (*12, 13*).

Polyploidy, or housing multiple chromosomal copies, is common in angiosperm species (*14, 15*). Although highly debated in the literature, polyploidy may confer potential adaptive advantages, such as novel gene functions (*16, 17*) and up- and down-regulation regulation of gene products (*18, 19*). Morphological traits can also be different between the cytotypes, including such traits that may aide or hinder disease progression,

i.e. stomatal size, stomatal density, and trichome cover (*14, 20*). These morphological traits are a part of a greater group of ecophysiological traits that are likely contributors to plant overall fitness, such as certain leaf attributes, biomass allocation, and growth (*21*). These traits will likely be selected on based on environmental pressures, such as drought and disease *(22*). Stomata and trichomes are known to protect plants from desiccation, especially in dry, windy environments (*23*).

The aim of this study was to investigate how the interaction of climate change, flowering phenology, maternal influences, polyploidy and disease interact to form a response by the host plant. We tested this idea by planting seeds from parents with artificially selected flowering times and with contrasting water availability into a common garden and subjecting the current generation to well-watered and drought stressed treatments. Even though many scientists have studied individual aspects of this problem, none have combined all of these intertwined factors into one experiment. Our hypotheses were four-fold. First, we hypothesize that diploid and tetraploid cytotypes will respond differently to environmental pressures, with tetraploids responding to environmental pressures more rapidly and therefore accumulating less disease over the growing season. Second, drought-stressed and early flowering plants are hypothesized to be more susceptible to disease, due to both enhanced levels of stress on the plant and a greater amount of time to accumulate disease. Thirdly, plants that have a maternal environment that is subject to differing water availability may evidence these effects within their progeny. Finally, disease levels will be affected by morphological traits that hinder disease such as increased trichome density and decreased stomatal density.

Results

Parental effects of water availability on generation 4 of artificial selection (AS-4)

Watering Treatment

Solidago altissima plants in well-watered conditions experienced greater frequency of infection ($p<0.0001$, Table 4.1) and maintained greater levels of powdery mildew infection than plants in drought-stressed conditions. Well-watered plants had approximately 57 percent height of infection as compared to 46 percent for plants in dry treatments ($p<0.0001$, Table 4.1). Drought- stressed plants also had a percentage cover-based infection score of approximately 2.1, whereas well-watered plants scored approximately 2.8 ($p<0.0001$, Table 4.1).

Previous Parental Watering Treatment

Parental water availability had no influence on the frequency or height of powdery mildew infection in offspring. However, parental water availability affected overall mildew cover, and well-watered parents produced more susceptible offspring. Offspring of drought-stressed parents had an average infection score of approximately 2.3 versus a score of 2.5 for offspring of well-watered parents ($p=0.04$, Table 4.1).

Flowering Time

Flowering time within each ploidy level had a strong effect on the visual presence of powdery mildew on the host plant ($p<0.0001$, Table 4.1), with diploids experiencing more frequent colonizations later in the season ($\beta_{diploid}= 0.02$, $p=0.008$, $\beta_{tetraploid}= -0.004$, $p=0.70$). Flowering time also influenced the height of powdery mildew infection in a similar response ($\beta_{diploid}= -0.42$, $p<0.0001$; $\beta_{tetraploid}= -0.20$, $p=0.002$). This

influenced the overall infection score ($p<0.0001$, Table 4.1), decreasing the colonization as the flowering season progressed ($\beta_{diploid}= -0.19$, $p<0.0001$; $\beta_{tetraploid}= -0.009$, $p=0.01$).

Ploidy

Ploidy level affected powdery mildew cover, and tetraploid *S. altissima* maintained greater levels of infection by powdery mildew than diploid cytotypes. For the quantitative measure of percent height of infection, this was apparent by an average of 6% greater heights of infection on tetraploid plants ($p=0.0006$, Table 4.1). For the qualitative scale of percentage mildew cover, tetraploids ranked approximately a 2.6 score out of 5, versus a score of approximately 2.3 for diploids ($p=0.003$, Table 4.1).

Block

Position within the experiment, specifically which shelter block a plant was growing in, affected the mildew cover of the plant. Plants from shelters 7 and 8 had the least amount of mildew for both percent index ($p<0.0001$, Table 4.1) and percent height of mildew cover ($p<0.0001$, Table 4.1).

Higher Order Interactions

When examining the combined effect of ploidy and water availability, the height of mildew cover was greater in well-watered tetraploids, with infection reaching up to 62 percent of the extent of the plant ($p=0.003$, Table 4.1, Figure 4.1 A). Water availability had little effect on diploid cytotypes, with well-watered plants having slightly higher average infection scores: 2.4 for wet vs. 2.2 for dry. However, watering treatment had a large effect on tetraploid plants, with well-watered individuals scoring an average of 1.3 greater than dry plants ($p<0.0001$, Table 4.1, Figure 4.1B).

High water availability in the parental generation compounded with high water availability for the current (2012) progeny led to an increase in overall mildew cover in the offspring. These increases were as much as 0.5 to 1 on the infection scale (p=0.02, Table 4.1, Figure 4.2); however, these effects were not found for the extent of mildew growth up the plant.

Over the flowering period, plants that were drought stressed experienced more mildew colonization up the length of the plant (p=0.0053, Table 4.1) and this was apparent in both diploids ($\beta_{diploid}$=0.13, p=0.02) and tetraploids ($\beta_{tetraploid}$=0.13, p=0.04). This also occurred within the measurement of overall infection score ($\beta_{diploid}$=0.01, p=0.002; $\beta_{tetraploid}$=0.009, p=0.02).

Analysis with Morphological Characteristics from Generation Three of Artificial Selection (AS-3)

Trichome and stomatal density alone had no significant influence over powdery mildew susceptibility on *S. altissima.* However, in combination with within-ploidy flowering time, presence of mildew is influenced by trichome density (p=0.04, Table 4.2). Over the flowering season for tetraploids, leaf hairiness decreased mildew cover ($\beta_{tetraploidflowering*avgtrichomes}$= -0.0007, p=0.002) and mildew cover increased for diploids over the flowering season ($\beta_{diploidflowering*avgtrichomes}$=0.005, p=0.003). Over the flowering season, trichome density also moderately affected extent of mildew cover up the plant (p=0.07, Table 4.2) and overall plant area covered with infection (p=0.05, Table 4.2).

Analyses on the comparison of leaf area in tetraploids versus diploids in relation to trichome densities reveals that there are fewer trichomes on tetraploids and this is

significantly correlated with the size of tetraploid leaves ($p<0.0001$). Nesting leaf area with ploidy also revealed a similar trend ($p=0.0009$) No data was collected for stomatal densities and leaf size due to the destructive nature of stomatal peels.

Discussion

We found that powdery mildew relies more heavily on a conducive, wet environment for successful colonization of leaf tissue, rather than upon the weakness of its host plant. This is an interesting deviation from the hypothesized outcomes. In addition, water availability in the parental generation influenced the total mildew cover, with well-watered parents producing more susceptible offspring. This finding may be important when predicting overall disease prevalence during climate change. It is expected to become warmer and drier in Minnesota, and the pressure of this climate may reduce the virulence of powdery mildew (*24*). However, if extreme weather events bring sudden precipitation, this will have negative consequences for native plant species, especially if they have constitutive defenses that may be lost over time rather than induced defenses (*25, 26, 27*).

Powdery mildew also relies on wind to disseminate its spores (*28*), so our highly significant shelter blocking effect was due to the placement of the plants in relation to wind direction. This has implications for position within wild clonal stands of *Solidago altissima.* While our plants were held within pots, in wild stands, plants on the lagging edge of the disease may be able to escape via lateral clonal growth, as suggested by Schmid (*29*).

Contrary to initial predictions, we found that tetraploid *Solidago altissima* was more susceptible to mildew colonization than diploid plants. This may be due to inherent leaf morphology characteristics. Tetraploids have a larger leaf area than diploid *S. altissima* leaves and also have less dense trichomes as a result. Trichome densities were only significant in tandem with changes over the flowering period. These are both

interesting findings, as early flowering times are one of the predicted conditions associated with climate change (*30*). More work could be done in the future to examine whether or not trichome cover is an effect of mildew colonization or a cause of prevention and whether or not *S. altissima* trichomes are glandular. Other studies have found that trichomes, especially ones that contain secondary compounds, are great fungal deterrents (*31*). As of yet, no information on the presence or composition of secondary compounds in *S. altissima* has been published.

The mode of polyploidization may have an effect on the level of disease resistance conferred. Other studies, such as by Oswald and Nusimer (*32*) examined newly-formed autotetraploids and found them to be more resistant than their diploid counterparts. However, wide-scale gene loss and gene-silencing have been observed in allopolyploid plants (*33*) and this may decrease any of these initial positive effects. In addition, researchers have found increased parasitic species richness on polyploid animals, such as freshwater fish (*34*). At this time, it is uncertain what the mode of polyploidization is for *S. altissima.*

Additionally, the presence of overall more mildew on early flowering *S. altissima* supports our original hypothesis, and may provide a challenge towards plant health as climate selection pressures for early flowering increase (*6,8*), especially since the energetic cost of flowering is high (*35*). Because the effect of flowering time was so strong, and was even an intense influence of mildew colonization in combination with water availability, this will merit further investigation and future studies.

The overall understanding of the complex interactions between ploidy, flowering time, drought-stress, and maternal effects are essential when considering the larger

picture of plant health and its relationship with climate change. The next important step will be to determine the evolutionary potential of diploid *S. altissima* in response to disease and climate change (*36*) by examining the heritability of these resistances or susceptibilities over time. This will allow us to better understand how to conserve native species and develop crops that can cope with both drought-stress and disease pressures (*37*). The delicate balance of ecosystem health can be quickly undermined by changes in disease virulence (*38*). Under the umbrella of plant conservation, we can hope to protect other species within the ecosystem.

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Tables 4.1-4.2

Table 4.1 ANOVA test statistics (*F*, *P*) of three measurements of *Erysiphe cichoracearum* infection on diploid and tetraploid *Solidago altissima* that were artificially selected for early, control and late-flowering and reared in two watering treatments. These plants are in the fourth generation of artificial selection and came from parent plants that were exposed to well-watered and drought-stressed treatments.

	Three Measurements of *Erysiphe cichoracearum*		
Factors	**Percent Height of Infection**	**Visual Infection Scale (0/L/M/H)**	**Percentage of Infection Scale**
Ploidy[1,6]	11.91, **0.0006**	2.40, 0.49	8.76, **0.003**
Watering Treatment[1,6]	39.35, **<0.0001**	37.32, **<0.0001**	57.46, **<0.0001**
Date of Flowering [Ploidy][2,6]	29.84, **<0.0001**	36.91, **<0.0001**	17.31, **<0.0001**
Previous Watering Treatment[1,6]	1.10, 0.29	4.10, 0.25	4.16, **0.04**
Ploidy x Watering Treatment[1,6]	8.74, **0.003**	16.01, **0.001**	27.57, **<0.0001**
Ploidy x Previous Watering Treatment[1,6]	0.11, 0.74	3.92, 0.27	1.71, 0.19
Watering Treatment x Date of Flowering[Ploidy][2,6]	4.82, **0.008**	11.50, 0.07†	7.90, **0.0004**
Watering Treatment x Previous Watering Treatment[1,6]	1.99, 0.16	2.04, 0.56	5.96, **0.01**
Previous Watering Treatment x Date of Flowering[Ploidy][2,6]	0.15, 0.86	4.48, 0.61	0.17, 0.84
Shelter[5,6]	13.25, **<0.0001**	150.05, **<0.0001**	13.35, **<0.0001**

[1]*df* numerator=1, [2]*df* numerator=2, [3]*df* numerator=7, [4]*df* denominator≈980

Table 4.2 ANOVA test statistics (*F*, *P*) of three measurements of *Erysiphe cichoracearum* infection on diploid and tetraploid *Solidago altissima* that were artificially selected for early, control and late-flowering and reared in two watering treatments. These plants are from the third generation of artificial selection and leaf morphology characteristics (stomatal density and trichome density) were included in the analysis.

	Three Measurements of *Erysiphe cichoracearum*		
Factors	**Percent Height of Infection**	**Visual Infection Scale (0/L/M/H)**	**Percentage of Infection Scale**
Ploidy[1,3]	14.67,**0.0001**	3.45, 0.33	7.15, **0.008**
Watering Treatment[1,3]	5.49, **0.02**	19.36, **0.0002**	0.67, 0.41
Date of Flowering[Ploidy][2,3]	0.18, 0.84	17.46, **0.008**	3.80, **0.02**
Average Trichomes[1,3]	0.81, 0.37	5.14, 0.16	0.05, 0.82
Average Stomata[1,3]	0.02, 0.88	6.25, 0.10	0.75, 0.39
Watering Treatment x Date of Flowering [Ploidy][2,3]	5.91, **0.003**	28.82, **<0.0001**	7.77, **0.0005**
Average Trichomes x Date of Flowering [Ploidy][2,3]	2.71, 0.07†	13.16, **0.04**	2.99, 0.05†
Average Stomata x Date of Flowering [Ploidy][2,3]	0.82, 0.44	2.83, 0.83	0.80, 0.45
Watering Treatment x Ploidy[1,3]	2.27, 0.10	1.76, 0.62	3.56, 0.06†

[1] *df* numerator =1 [2] *df* numerator = 2 [3] *df* denominator ≈ 627

Figure Captions

Figure 4.1 Least square means and one standard error of the average A) percent height of infection and B) infection score (0= no infection, 1= 1-20% infected, 2= 21-40% infected, 3= 41-60% infected, 4= 61-80% infected, 5= 81-100% infected) of *Erysiphe cichoracearum* on diploid and tetraploid *Solidago altissima* plants subjected to drought-stressed and well-watered treatments.

Figure 4.2 Least square means and one standard error of the average infection score 0= no infection, 1= 1-20% infected, 2= 21-40% infected, 3= 41-60% infected, 4= 61-80% infected, 5= 81-100% infected) of *Erysiphe cichoracearum* on drought-stressed and well-watered *Solidago altissima* plants whose parental generation was subjected to drought-stressed and well- watered treatments .

Figure 4.1

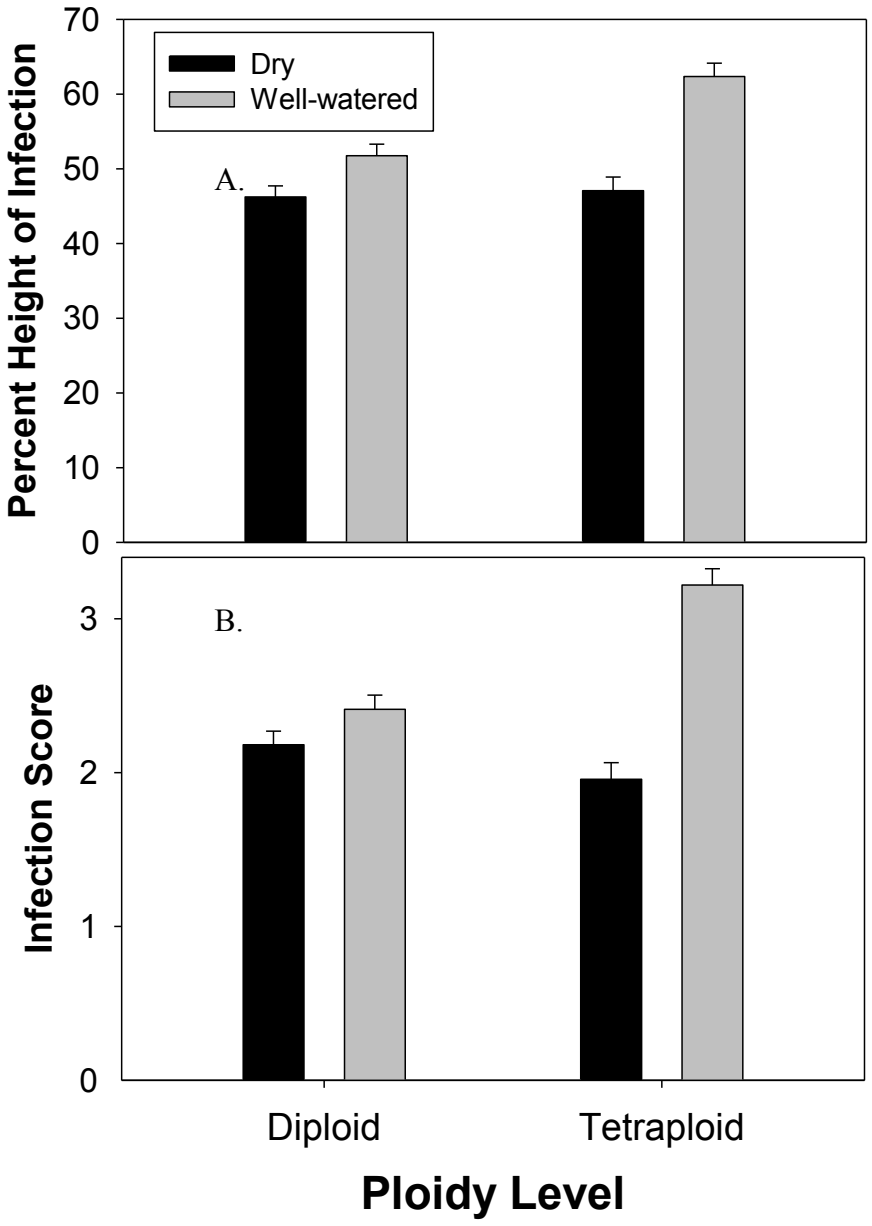
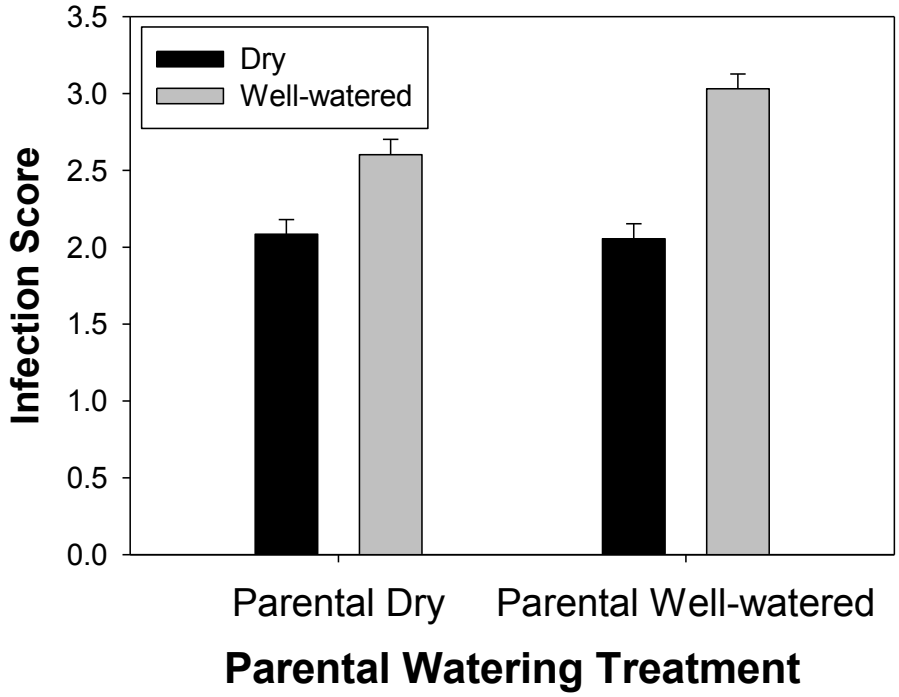

Dry
Well-watered
A.
B.
Percent Height of Infection
Infection Score
70
60
50
40
30
20
10
0
3
2
1
0
Diploid
Tetraploid
Ploidy Level

Figure 4.2


3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Dry
Well-watered
Infection Score
Parental Dry
Parental Well-watered
Parental Watering Treatment

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Supplementary Materials

Materials and Methods

Figures 4.1S-4.2S

Materials and Methods

Study Organisms

Solidago altissima, or late goldenrod, is an herbaceous dicot perennial in the family Asteraceae that is native to Minnesota, with a broad distribution across North America (*39*). *S. altissima* is genetically polymorphic, includes diploid (2n, 2x=18), tetraploid (2n, 4x=36), and hexaploid (2n, 6x=54) cytotypes (*39, 40*), and populations with mixed ploidy levels are common. *S. altissima* is self-incompatible and reproduces sexually by seeds and asexually by clonal rhizomes.

Erysiphe cichoracearum, or powdery mildew, is an Ascomycete obligate parasite that covers host leaves with mycelial fungal bodies (*28*). These then produce haustoria that delve into the epidermal leaf tissue of the host plant and parasitize them, utilizing plant photoassimilates to meet their own energetic needs (*41*). Once the fungi develop, they produce asexual conidia that bud off of the main fungal body (*41*). The leaf surface of host plants, specifically leaf hair morphology, can affect the conidiophore development of powdery mildews (*41*). Powdery mildew can also reproduce sexually, via ascospores that are contained within cleistothecia over winter months (*28*). These clestothecia persist to the next growing season, whereby spores will be released on the wind to colonize a new host (*28*). Only one previous study has examined the effects of powdery mildew on *S. altissima s*pecifically, and researchers found that increase in small-scale genetic diversity has a positive effect on overall fitness and plant health (*29*).

Study Site and Conditions

Plants were grown at the University of Minnesota in Duluth MN at the UMD Research and Field Studies Center facilities (St. Louis Co. 46.47 º N, 92.6 º W) in the summer and fall of 2012. Two sets of plants were used for this study: one of which was in its second year of growth after three generations of artificial selection (AS-3) for early, control, and late flowering times and the other was in its first year of growth after four generations of artificial selection (AS-4) for flowering time (early, control, and late).

Diploid and tetraploid *S. altissima* plants from the third generation of artificial selection (AS-3) were selected as a subset of a larger, previous experiment (Etterson, *unpublished data*). Etterson has been conducting artificial selection on flowering time in drought and well-watered conditions on diploid and tetraploid *Solidago altissima* (24 artificial selection lines = 2 ploidy levels x 2 watering treatments x 2 replicates x 3 flowering times (early, control, late)). These plants were grown in 6x6x12 inch pots with ambient-dry and supplemental watering. 360 plants were used in the study subset (15 genotypes / 2 ploidy levels x 2 watering treatments x 2 replicates x 3 flowering times). These plants were stock plants and only used to measure stomatal and trichome characteristics.

Diploid and tetraploid *S. altissima* plants from the fourth generation of artificial selection (AS-4) were planted as seed in late April 2012. These were transplanted once they reached rosette stage into a completely randomized design underneath a rainout shelter which allowed for 98% light penetrance. Plants were then subjected to droughted or well-watered treatments. The two watering treatments were split evenly among plants whose parents were subjected to either dry or well-watered pots. This allowed for a parental effects investigation of previous water availability on offspring.

Watering treatments were implemented by an automated drip-irrigation system. Well-watered plants were watered for 2 hours, 3 times per week. Drought-stressed plants were only watered by hand when wilting (on August 2nd, October 8th), and were watered until soil was soaked and water ran through the pot. Plants were also fertilized with Osmocot (19-6-12) using 11 ml per pot during the first week of August.

Data Collection and Analysis

Plants from the third generation of artificial selection (AS-3) were monitored throughout the season for *E. cichoracearum* infection. Date of first infection was noted for each plant to ensure that infection date did not affect the overall infection levels later in the season. The first detectable incidence of infection took place on July 18th, 2012. Measurements taken at first infection included height of the whole plant and height of infection, a qualitative visual infection scale (0 = no mildew, Low = small circular patches of mildew, mildew pale in color, Medium= mildew patchy and usually doesn't reach as high up plant as in "high", still fairly white in color, High- mildew reaches high up plant, very thick on leaves and very white in color), and a qualitative infection percentage scale adapted from Schmid (*29*, 0= no infection, 1= 1-20% infected, 2= 21-40% infected, 3= 41-60% infected, 4= 61-80% infected, 5= 81-100% infected). Height of the plant and height of infection were used to calculate a percentage of the plant that was experiencing mildew colonization. This took inherent differences in plant height into account. Also, any additional infections on the plant, including rust, were noted and date of first flower (indicated by the emergence of a ray flower) was taken.

These measurements were repeated on August 14th 2012. Soil-water measurements were also taken via a soil-water moisture meter (*42*) to ensure the

effectiveness of the watering treatment on August 1st, 10th, 15th, and 29th in 2012. Additionally, leaf peels were taken on August 20th using a nail varnish lacquer and clear tape. These were affixed to slides and examined at a later date for stomata and trichome densities. Stomata were enumerated at 40x magnification on a light microscope. To examine the number of trichomes, the software ImageJ (*43*) was used in congruence with a light microscope. Leaf hairs were looked through the highest magnification at 112.5x and two images (adaxial and abaxial leaf views) of both clean and infected leaves were taken with a scale of 1mm. Due to the tendancy for powdery mildew to colonize mainly the upper leaf surface, individual and average counts were taken for adaxial and abaxial stomata and trichomes, rather than using a ratio as executed in previous experiments (*44*).

Plants from the fourth generation of artificial selection (AS-4) were measured on August 15, 2012 for the same qualitative and quantitative measurements as used on AS-3. This included height of the plant, height of infection, visual infection scale, and infection percentage scale. Any additional pathogen colonizations to the plant (rust, etc.) were noted. Soil-water measurements were taken on throughout the growing season to ensure differences between watering treatments (Figure 1S). Most of the mildew colonization occurred in September; qualitative and quantitative measurements were noted. In addition, date of first flower was recorded for all plants (Figure 2S). Data was analyzed using a mixed-model ANOVA in JMP SAS Software (*45*). Date of first flowering was nested within ploidy, as diploids inherently flower earlier than tetraploids (diploids flower 9-15 days earlier than tetraploids; Etterson, *unpublished data*). In AS-4, shelter effects (block effect) and random effects were included to account for wind dispersal of spores.

Figure Captions 4.1S-4.2S

Figure 4.1S Least square means and one standard error of the average percent water saturation of the soil in 6 x 6 x 12 inch pots containing *Solidago altissima* in both well-watered and drought stressed treatments the 4th generation of artificial selection on flowering time experiment. On the y-axis, 1 through 6 omitted for clarity.

Figure 4.2S Least square means and one standard error of the average flowering time difference of *Solidago altissima* in Julian date between early and control artificial selection flowering lines in the 4th generation. On the y-axis, 1 through 230 omitted for clarity.

Figure 4.1S

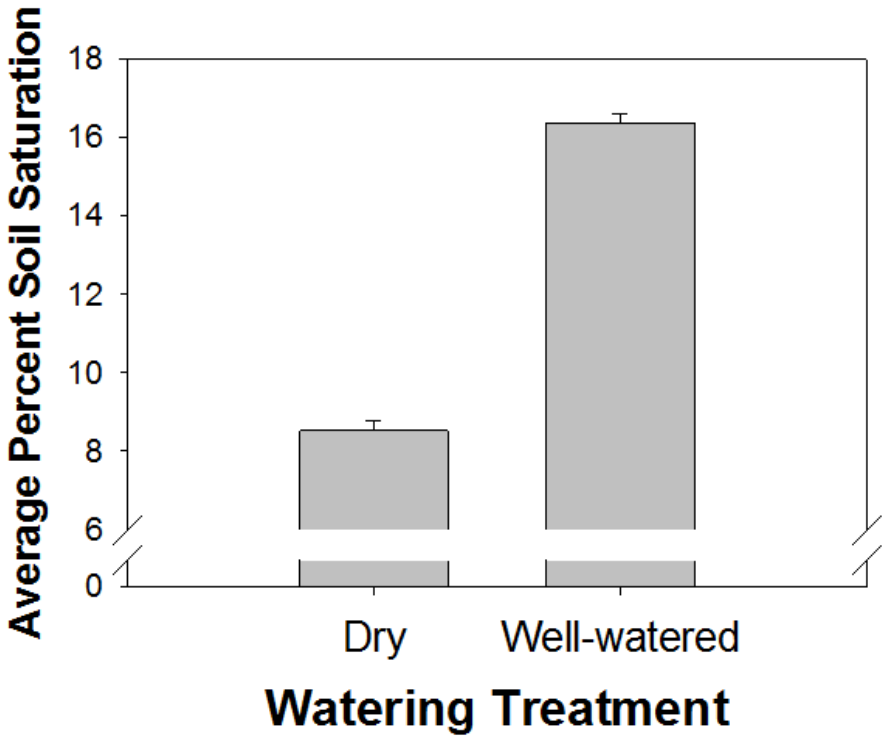
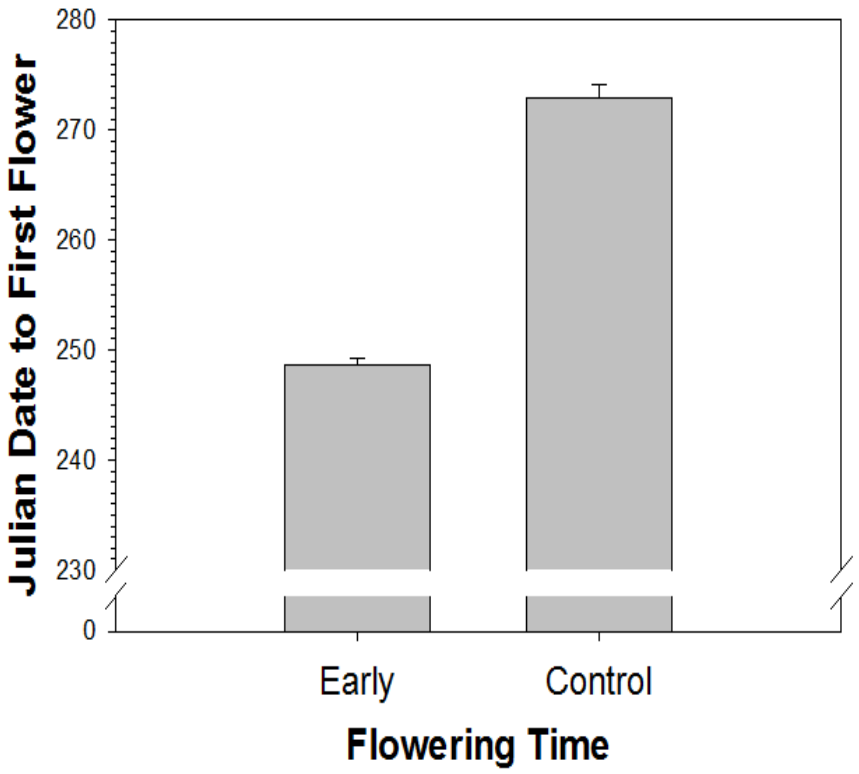

Average Percent Soil Saturation
18
16
14
12
10
8
6
0
Dry
Well-watered
Watering Treatment

Figure 4.2S


Julian Date to First Flower
280
270
260
250
240
230
0
Early
Control
Flowering Time

Comprehensive Bibliography

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