Wild Plant–Pathogen Interactions Across Gradients of Urbanization and Latitude

Quinn N. Fox

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Wild Plant–Pathogen Interactions Across Gradients of Urbanization and Latitude

by

Quinn N. Fox

A dissertation presented to
Washington University in St. Louis
in partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

May 2023
St. Louis, Missouri
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Quinn Fox

Washington University in St. Louis

May 2023
Dedicated to Mary Jane Groff

*What is a legacy?*

*It's planting seeds in a garden you never get to see.*

- Lin-Manuel Miranda
ABSTRACT OF DISSERTATION

Wild Plant–Pathogen Interactions Across Gradients of Urbanization and Latitude

by

Quinn Nicole Fox

Doctor of Philosophy in Biology and Biomedical Sciences
Ecology and Evolutionary Biology
Washington University in St. Louis, 2023
Professor Rachel Penczykowski, Chair

In light of large-scale urbanization and rising global temperatures, it is essential to understand how environmental and climatic changes impact the ecology and evolution of species interactions. This thesis investigates interactions between wild plants and their pathogens across gradients in land use and latitude, and specifically tests the effects of multiple scales of climate variation on such interactions. In this work, I focused on a system of common herbaceous plants in the genus *Plantago* and their fungal powdery mildew pathogens. I studied their interactions across an urbanization gradient transecting the metropolitan area of St. Louis, Missouri and along a latitudinal transect from southern Mississippi to northern Wisconsin. Both of these transects feature climate gradients, where temperature generally increases with the level of urbanization and with decreasing latitude. Moreover, microclimate conditions experienced by plants can vary dramatically within populations due to fine-scale heterogeneity in habitat features. As such, my thesis projects examine how plant–pathogen interactions change across three distinct spatial scales of climatic variation. However, climate conditions are not the only factors varying within and between these populations. Therefore, my projects also tease apart the roles of variation in host-pathogen compatibility, pathogen encounter rates, and other covariates (e.g., herbivory and mowing damage) in explaining patterns of disease. This research is
important for understanding the factors that drive current variation in disease pressure in wild plant populations, and for informing predictions of how continued land use and climate change may impact future plant–disease dynamics. My thesis research focused on three main questions: 1) How does the phenology of plant–pathogen and plant–herbivore interactions change with increasing intensity of urbanization? 2) What is the effect of within-site microclimate variation on plant–pathogen interactions across an urbanization gradient? And finally: 3) Are pathogens locally adapted to host populations or temperatures along a latitudinal gradient?

In Chapter One, I studied how the prevalence of disease and herbivory on two co-occurring Plantago species changed across an urbanization gradient over the course of two growing seasons. I found that Plantago in more urban populations experienced more early-season herbivory damage as well as earlier and larger powdery mildew epidemics. Results of field and laboratory experiments suggested that the observed differences in disease prevalence were not driven by variation in genetically based host susceptibility across the urbanization gradient but may reflect variation in pathogen encounter rates. Chapter Two focuses on the effect of microclimate variability on infection prevalence across the same urbanization gradient. In observational field surveys, I found that plants located in shaded microhabitats were more likely to be infected. This effect of shade varied through time but was consistent across levels of urbanization. I then performed a field experiment manipulating shade while controlling for host genetic background and pathogen inoculation status. Results of this experiment suggested a causal role of shade in promoting pathogen growth and transmission. In Chapter Three, I performed a laboratory experiment to test whether powdery mildew strains are locally adapted to host genotypes and temperature regimes along a latitudinal gradient. I found evidence for
powdery mildew local adaptation to temperature, but not to sympatric host genotypes. Finally, in Conclusions and Future Directions, I summarize and synthesize each chapter.
Introduction

After all, the word 'ecology' means the study of home
- Dr. Kathryn M. Flinn, “This is a place”, 2018

The Anthropocene is marked by human-driven changes in climate. Since the pre-industrial era, the Earth’s average yearly surface temperature has risen by approximately 1 °C (NOAA National Centers for Environmental information 2023). While 1 °C may seem inconsequential, this global increase has resulted in progressively more extreme regional temperatures and weather events (Chakraborty et al. 2000). At the same time, humans have transformed landscapes in ways that impact the climatic conditions experienced by organisms at smaller spatial scales, including through urban and suburban development. The replacement of vegetation with built surfaces and increased density of industrial and vehicular emissions in cities typically results in "urban heat islands" where temperatures are higher than in surrounding less-urban land (Zhou et al. 2017). Yet even within a given urban, suburban, or rural habitat, there can be substantial fine-scale variability in climatic conditions due to heterogeneity in natural and human-made features that cast shade, alter wind flow, or retain moisture. As global climate change continues, there is an urgent need to understand how climate variability at regional, local, and microclimate scales affects species interactions, including those between hosts and their pathogens.

The inherently temporal nature of anthropogenic climate change poses a challenge for studying its effects on the ecology and evolution of species interactions. Instead, existing spatial gradients in climate (e.g., gradients in temperature across latitudes or levels of urbanization) are often used as "natural laboratories" for studying species responses to climate change (De Frenne et al. 2013). Of course, latitudinal and urbanization transects span not only temperature variation,
but also reflect the legacy of complex environmental, ecological, and evolutionary processes that have played out through time. Therefore, studies that leverage spatial gradients to infer climate effects should ideally be complemented by experiments that allow for control over genetic background and other environmental effects (De Frenne et al. 2013). The integration of observational (field-based) and experimental (field- and/or lab-based) approaches is essential for generating robust understanding of how climate variation shapes interactions between species.

In this thesis, I use observational and experimental approaches to investigate variation in wild plant-pathogen interactions at three spatial scales: across a latitudinal gradient (~600 km extent), across an urbanization gradient (~50 km extent), and over fine-scale microclimate variation within habitats (few meters). While climate variation is highlighted here as a unifying theme across thesis chapters, these studies are also not only about climate. More generally, this thesis seeks to enhance our understanding of how variation in all three corners of the disease triangle—host, pathogen, and environment—contribute to patterns of disease at different spatial scales.

For a successful infection to occur, an infective pathogen needs to meet a susceptible host in suitable environmental conditions. This three-dimensional relationship between host, pathogen, and the environment is called the “disease triangle” (Stevens 1960). Spatial or temporal variation in any three corners of the disease triangle can change the probability of infection and severity of disease. Depending on regional climate, magnitude of temperature changes, and thermal sensitivity of host and pathogen physiological traits, temperature shifts have the potential to increase or decrease prevalence and severity of disease (Wang et al. 2009, Egerer et al. 2020, Desaint et al. 2021, van Dijk et al. 2022). For example, the thermal range of
the amphibian chytrid pathogen is smaller than that of its host, thus giving the host lower and upper thermal refugia from infection (Gsell et al. 2013). However, globally increasing temperatures may limit the host’s access to the lower thermal refuge, thereby increasing disease prevalence (Gsell et al. 2013). Furthermore, strains of the chytrid pathogen have been shown to vary in their thermal tolerances, such that a given climate could be prohibitive for growth of some pathogen genotypes but permissive for others (Voyles et al. 2017).

While now broadly recognized by disease ecologists, the disease triangle concept was first formalized in the field of plant pathology (Stevens 1960). In modern agricultural systems, several strategies are conventionally used to reduce the coincidence of susceptible hosts, infective pathogens, and disease-conducive environmental conditions. These strategies include deployment of resistant crop varieties, use of pesticides, and management of nutrient and moisture levels. Often, these strategies are applied uniformly across fields with the aim of achieving homogeneously low risk of disease. By contrast, in wild plant populations, host genotypes are typically more diverse and microclimatic conditions are more variable. Moreover, many wild plant species (e.g., weeds) are nearly continuously distributed across large geographic ranges spanning wide environmental variation and land use categories, while crop species tend to be grown in certain regions where they are economically fruitful. The disease triangle should therefore be much more spatially dynamic for wild plants than for crops. Yet diseases of wild plant populations have been subject to far less research than those in agricultural settings, due to their lower economic and societal value (Velásquez et al. 2018). Even less is known about how urbanization impacts the disease triangle and resulting patterns of infection in plant populations scattered across urbanized areas.
It has been recognized since the late 19th century that the dramatic environmental changes brought about by urbanization can alter the ecological and evolutionary processes of the species that live there (Kettlewell 1955). However, not all species receive equal attention. Many studies have looked at the impact of urbanization on animals, but plants have remained understudied despite their important role in human and environmental well-being (Johnson and Munshi-South 2017, Kondo et al. 2018, Rivkin et al. 2019). Additionally, while wildlife have been shown to face significantly greater risk of parasite infection in urban areas (Murray et al. 2019), only one study has tested the effects of urbanization on a tree disease (van Dijk et al. 2022). To date, no published studies have specifically investigated how diseases of wild herbaceous plants vary across levels of urbanization.

Disease outbreaks on both wild and cultivated plants have the potential to cause significant ecological, agricultural, and economic damage (Velásquez et al. 2018). Despite this, there remain many open research questions, especially for wild plant-pathogen systems. Here I ask: 1) Does the timing and prevalence of disease outbreaks vary across an urban–rural gradient? 2) Do within-habitat microclimate differences impact the prevalence of disease outbreaks across an urban–rural gradient? And lastly, 3) are pathogens adapted to temperatures across a latitudinal gradient? By addressing these questions, I hope to inform on the ecological and evolutionary dynamics at play in wild plant pathosystems, and to lay groundwork for understanding disease patterns in a changing world.
Dissertation Overview

Study system

All three chapters are focused on interactions between Plantago species and their fungal powdery mildew pathogens over spatial gradients. Chapters One and Two focus on a 60-km urbanization transect across the St. Louis, Missouri metropolitan area. Chapter Three focuses on broadscale temperature change along a North-South latitudinal transect from northern Wisconsin to southern Louisiana – a stretch of over 1,500 km. Across these gradients, I studied Plantago lanceolata and Plantago rugelii and two powdery mildew species that are specialists on Plantago host plants. Both P. lanceolata and P. rugelii are small herbaceous weeds that grow in open environments such as pastures and in disturbed habitats such as lawns, parks, and road verges (Penczykowski and Sieg 2021). Both species are found abundantly across the St. Louis metropolitan area. Plantago lanceolata is native to Eurasia, where it exhibits strong spatial genetic structure associated with geographic distance and precipitation seasonality (Smith et al. 2020). It was likely first introduced to the United States by early European settlers (Mack and Erneberg 2002). In its introduced ranges, P. lanceolata has greater within-population genetic diversity and exhibits weak spatial genetic structure, likely indicative of repeated long-distance introductions (Smith et al. 2020). Plantago rugelii is native and endemic to North America, but its population genetic structure has not been studied thus far.

The most conspicuous pathogens of these host plants are powdery mildews – obligate parasitic fungi that grow on the surface of leaves. Two specialized powdery mildew species infect Plantago hosts: Podosphaera plantaginis and Golovinomyces sordidus (Braun and Cook 2012). Powdery mildew spores are primarily passively transported via wind. While up to 90% of
spores land within 1-meter of the host plants, occasional long-distance transport is a critical component of pathogen metapopulation dynamics (Ovaskainen and Laine 2006, Tack and Laine 2014). After a spore has reached a susceptible leaf and germinates, hyphae on the leaf surface produce structures called haustoria which enter the epidermal tissue, allowing the mildew to syphon nutrients from the host. Chains of asexual spores, called conidia, then proliferate on the leaf surface, giving infected leaves a notable “powdery” appearance.

The *Plantago*–powdery mildew system is extremely tractable for studies across environmental gradients for many reasons (Penczykowski and Sieg 2021). Importantly for my thesis projects, both species of *Plantago* under consideration are highly abundant in the focal study regions, including in lawns and roadsides within rural, suburban, and urban settings. Yet while *Plantago* is classified as a “weed” by many, it is not targeted for removal or treated with herbicide in parks and nature reserves in the St. Louis region (Fox, *personal correspondence with park managers*). Another advantage of this study system is that the focal species of powdery mildew are specific to *Plantago*, meaning they pose no threat to agriculturally valuable species, and thus do not warrant eradication. Additionally, powdery mildew is a visually conspicuous infection, meaning it can be easily and confidently identified in the field.

*Chapter One: Phenology of plant foliar infection and herbivory change along an urbanization gradient*

In Chapter One, I used repeated observational surveys, a field experiment, and a lab experiment to investigate disease and herbivory dynamics across an urbanization gradient. By surveying infection prevalence in *Plantago* populations in 22 parks monthly from early summer
to autumn for two years, I showed that urbanization is associated with earlier and larger powdery mildew epidemics on *P. rugelii*, while *P. lanceolata* experienced less infection overall. Early-summer herbivory on both plant species was also accelerated in urban sites. When I placed greenhouse-grown sentinel plants of replicate genotypes across the urbanization gradient, prevalence of powdery mildew infection on *P. rugelii* sentinels was greater in suburban and urban than rural sites. Moreover, the almost complete lack of infection on *P. lanceolata* sentinels was consistent with low abundance of circulating spores infective to *P. lanceolata*. In a laboratory inoculation experiment, I then confirmed that *Plantago* genotypes from all three land use types (urban, suburban, and rural) were susceptible to local powdery mildew strains. Taken together, the experimental results suggest that the greater prevalence of infection observed in more urban populations was not driven by greater susceptibility of hosts in those sites. The results of this study prompted questions on between- and within-site differences in climatic conditions, which motivated Chapter Two of my dissertation.

*Chapter Two: Effects of microclimate on disease prevalence across an urbanization gradient*

In Chapter Two, I performed observational surveys and a field experiment to test the hypotheses that shade promotes mildew growth and that the effect of shade is stronger in more urban areas (compared to rural areas) due to higher urban temperatures. In the field surveys, I found that powdery mildew infection was more commonly found in shaded than sunny microhabitats. The effect of shade varied across months but was consistent across land use types. In the field experiment, I placed trays of inoculated *Plantago* into sun and shade treatments. The experimental results were consistent with a causal role of shade in increasing infection risk. The
strong role of microclimate in infection success motivated my investigation of pathogen thermal performance in Chapter Three.

Chapter Three: Local adaptation of a fungal pathogen to temperature over a latitudinal gradient

In Chapter Three of my dissertation, I investigated whether pathogens collected from along a latitudinal gradient were locally adapted to 1) their sympatric host genotypes and 2) temperature. I did this by testing mildew and host genotypes, collected from five locations across a latitudinal gradient, in sympatric and allopatric host-pathogen pairings. These pairings were then placed in each of seven temperature treatments, which aimed to capture the entire thermal range of powdery mildew. Every other day, I used a categorical scale to score the mildew growth on each host-pathogen pairing. If there was local adaptation to hosts, I expected to find significantly increased mildew growth on sympatric pairings. Using these data, I was also able to create thermal performance curves and calculate the optimum temperature for each mildew line. If there was local adaptation to temperature, I expected to see increased optimal temperatures for mildews collected from decreasing latitudes. I found that pathogen genotypes demonstrated local adaptation to temperature, but not to host genotypes.

Author contributions

The candidate, Quinn N. Fox (QNF), is a first author for all chapters – role of candidate in each work are as follows: Chapter One - QNF, Mahal J. Bugay (MJB), and Rachel M. Penczykowski (RMP) conceived of the study design. QNF, MJB, Eleanor Grant (EG), Olivia S. Shaw (OSS), and Keiko N. Farrah (KNF) performed field surveys. QNF, OSS, and KNF performed the sentinel experiment. QNF and KNF performed the infection assay experiment.
QNF and RMP performed statistical analyses. QNF wrote the first draft, and all authors contributed to subsequent drafts of the manuscript. Chapter Two - RMP conceived of road verge survey design. QNF and RMP conceived of urbanization survey and shade experiment study design. QNF, KNF, OSS, Armando Sánchez-Conde, and Michelle Pollowitz (MP) performed urbanization surveys. QNF and KNF performed the shade experiment. QNF, MP, and RMP performed statistical analyses. QNF, MP, and RMP wrote the first draft, and all authors contributed to subsequent drafts of the manuscript. Chapter Three – RMP and QNF conceived of study design. QNF performed experiment. QNF and RMP performed statistical analyses. QNF and RMP wrote the first draft, and all authors contributed to subsequent drafts of the manuscript.
Chapter One:
Phenology of plant foliar infection and herbivory change along an urbanization gradient
Abstract

Urbanization involves numerous environmental changes that may affect the timing of foliar damage by pathogens, herbivores, and human activities such as mowing. Yet such relationships have not been examined simultaneously in plant populations across levels of urbanization. To help fill this research gap, we conducted monthly surveys of 22 populations of Plantago lanceolata and P. rugelii in parks spanning an urbanization gradient. We collected data on the prevalence of powdery mildew infection, insect herbivory, and mowing damage. To control for plant genotype, we placed potted “sentinel” plants into field populations to directly measure infection and herbivory rates. Additionally, we performed an infection assay to experimentally test the susceptibility of plant genotypes from across the urbanization gradient. We found that mildew epidemics on P. rugelii started earlier and achieved greater prevalence in more urban sites. Correspondingly, P. rugelii sentinels only became infected in suburban and urban sites. There was less infection on P. lanceolata, including sentinels, suggesting low availability of pathogen genotypes able to infect this species. Early-summer herbivory on both plant species was accelerated in urban sites. Moreover, there was greater prevalence of mowing damage in urban and suburban sites. In the infection assay, we found that all P. rugelii maternal lines that we collected from across the urbanization gradient were susceptible to the same two strains of powdery mildew that we tested. This suggests that the greater prevalence of disease we observed in more urban field populations was not driven by greater susceptibility of urban than rural strains. Overall, our study documents differences in the prevalence of multiple types of foliar damage across an urbanization gradient and highlights that the magnitude of those differences across land use type vary between species, months, and sources of damage.
**Introduction**

Life on Earth is becoming increasingly urbanized. As of 2018, 55% of the world’s population resides in urban areas, and that metric is anticipated to grow to 68% by 2050 (United Nations, 2018). Urbanization involves dramatic changes to the environment which can alter ecological and evolutionary processes and impact the fitness of organisms, including increases in habitat fragmentation, frequency of disturbance, coverage by impervious surfaces, temperature, pollution, and introduction of invasive species (Johnson and Munshi-South 2017). A meta-analysis of more than 100 published studies revealed wildlife face significantly greater risk of parasite infection in urban areas (Murray et al. 2019). However, effects of urbanization on plant fitness are poorly understood (Johnson and Munshi-South 2017, Rivkin et al. 2019). Only one study has tested effects of urbanization on tree diseases (van Dijk et al. 2022), and none have tested effects of urbanization on diseases of wild herbs. No studies have simultaneously quantified the relationships between urbanization and the timing of damage from pathogens, herbivores, and human activities.

A well-known consequence of urbanization is the urban heat island effect (UHI), in which cities are warmer than surrounding environments due to retention of solar radiation by urban materials and release of heat from high densities of vehicles and other machinery (Zhou et al. 2017). Urban heating may increase or decrease prevalence of plant diseases, depending on regional climate, magnitude of UHI, and thermal sensitivity of host and pathogen physiological traits. Here, we focus on fungal pathogens because these are the most well studied in wild plant populations (Alexander 2010, Burdon and Laine 2019). Across life history processes, plant-associated fungi typically have temperature minima of 5-15 °C, optima of 18-28 °C, and maxima
of 26-38 °C (Chaloner et al. 2020). In regions where baseline climate is cooler than optimal for a pathogen, UHI may promote disease. Indeed, UHI is linked to increased severity of powdery mildew on English oak in Europe (van Dijk et al. 2022). However, in regions that are already warmer than optimal for a pathogen, additional heat in cities may inhibit pathogen growth. Effects of UHI on disease can further depend on microclimate heterogeneity (Penczykowski et al. 2018), temporal climatic variation (Egerer et al. 2020), and thermal sensitivity of plant defense responses (Wang et al. 2009, Desaint et al. 2021).

Fragmentation of urban green spaces can lead to greater isolation of plant populations compared to more continuous rural habitats (Dubois and Cheptou 2017). Alternatively, plant or pathogen species that grow or disperse along roadways may be more highly connected in cities with denser road networks. Fungal infection is more likely in populations of Plantago lanceolata (ribwort plantain) along roadsides, particularly at hubs of a road network, suggesting that vehicular traffic facilitates spore transport (Numminen and Laine 2020). Increasing road density and traffic volume from rural to urban environments could therefore drive increases in fungal infection prevalence.

Rates of insect herbivory can also be altered by abiotic (e.g., temperature, light, and sound) and biotic (e.g., biodiversity of plants, insects, and predators) features of cities (Miles et al. 2019). UHI may accelerate emergence of insect herbivores in spring (Bale et al. 2002). If host plant phenology is similarly accelerated, this should lead to higher rates of herbivory earlier in the growing season. Contrastingly, if UHI causes phenological asynchrony between insects and their host plants less herbivory is expected (Bale et al. 2002). From the few published studies linking urbanization to herbivory both positive (Cuevas-Reyes et al. 2013, Just et al. 2019,
Moreira et al. 2019) and negative (Meineke et al. 2019) relationships been documented (Miles et al. 2019). Other evidence for changes in herbivory pressure comes from declines in production of an antiherbivore chemical defense (hydrogen cyanide) in white clover with urbanization (Johnson et al. 2018, Santangelo et al. 2022).

The frequency and intensity of disturbance from human activities may also increase with urbanization. For herbs growing along roadways or lawns, human activities may damage or remove leaves and reproductive tissues directly or affect the probability of damage by pathogens or herbivores. On golf courses, increased human footsteps and lowered mower blade height increased severity of fungal infections on grass (Williams et al. 2001, Inguagiato et al. 2009, Roberts and Murphy 2014). Effects of walking and mowing on plant populations across levels of urbanization remain to be explored.

We examined the phenology of plant fungal infection, insect herbivory, and lawn mowing damage across a 56-km land use gradient in the St. Louis metropolitan area in Missouri, United States (Fig. 1.1, Table S1.1). We conducted monthly field surveys of *Plantago lanceolata* (ribwort plantain) and *P. rugelii* (blackseed plantain) populations co-occurring in 22 sites in summer–autumn 2019 and 2020. In a field experiment performed in 2020, we placed groups of healthy, pathogen-free “sentinel plants” of these species into a subset of sites to directly measure rates of infection and herbivory across the urbanization gradient. Furthermore, we conducted an infection assay to test whether there were significant differences in susceptibility to the focal fungal pathogen between plant lines collected from across the urbanization gradient, which may have contributed to the observed patterns in infection prevalence. We hypothesized that urbanization would be associated with: (1) earlier and larger powdery mildew epidemics, (2)
earlier and overall more prevalent insect herbivory, and (3) more mowing damage on leaves. We further hypothesized that rates of infection and herbivory on sentinels would positively correlate with prevalence of infection and herbivory in the field populations where they were placed. Lastly, we hypothesized that there would be no significant difference in susceptibility between plant lines collected from across the urbanization gradient.

**Materials and Methods**

*Study system*

This study focused on two co-occurring herbs, *Plantago lanceolata* (ribwort plantain) and *Plantago rugelii* (blackseed plantain). These short-lived perennials grow as rosettes in pastures or human-disturbed landscapes (e.g., lawns and roadsides), and are tractable model organisms for studies of plant–pathogen and plant–herbivore interactions across land use gradients (Penczykowski and Sieg 2021). *Plantago lanceolata* is native to Eurasia but cosmopolitan in its distribution, and *P. rugelii* is endemic to eastern North America (Penczykowski and Sieg 2021).

The most globally and locally common foliar fungal pathogens of *Plantago* are the specialist powdery mildews *Podosphaera plantaginis* (Castagne; U. Braun and S. Takamatsu) and *Golovinomyces sordidus* (L. Junell) V.P. Heluta (Braun and Cook 2012). Powdery mildews – obligate fungal pathogens in the order Erysiphales – grow on the surface of leaves and extract nutrients from their host's epidermal tissue (Bushnell 2002). Chains of asexual spores produced from mycelia on the leaf surface give infected leaves a white, dusty appearance. The spores are passively transmitted via wind, and more than 90% land within 2 m of the source plant (Tack et al., 2014). However, occasional long-distance spore transport allows for pathogen persistence at
the regional scale (Ovaskainen and Laine 2006). Powdery mildews overwinter via sexual resting structures that release spores when conditions are favorable in spring (Tack and Laine 2014).

Plantago are also host to numerous invertebrate herbivores (Penczykowski and Sieg 2021). This study focuses on leaf mines and chewing damage. Leaf mines on Plantago are exclusively created by larvae of the leaf miner fly Phytomyza plantaginis (Penczykowski and Sieg 2021). Chewing damage on leaf edges or between leaf veins could be due to a variety of arthropod taxa, particularly larval lepidopterans. In the region of our study, these include specialist common buckeye caterpillars (Junonia coenia) as well as generalist species (Penczykowski and Sieg 2021). Rather than focusing on the identity of the herbivore species, here we are concerned with the type of damage being inflicted on the focal plants.

**Focal populations**

We surveyed 22 sites (parks and nature reserves) with co-occurring P. lanceolata and P. rugelii populations extending southwest from downtown St. Louis City along the I-44 interstate highway corridor (Fig. 1.1a, Table S1.1). In a few large parks or nature areas, we surveyed two sites separated by at least 0.45 km. The study began with 19 sites in July 2019, two sites were added at Shaw Nature Reserve in August and September 2019, and a site was added at Forest Park in June 2020 (Table S1.1).

We classified eight sites within St. Louis City as “urban”, eight sites in St. Louis County east of Missouri Route 141 as "suburban", and six sites west of Missouri Route 141 as “rural”. These classifications were based on a principal component analysis (PCA) of environmental and spatial variables performed with the 'prcomp' function in R version 3.6.2 (R Core Team, 2019).
The PCA included average temperature from June through October (Temp), nighttime radiance (NightLight), percent impervious area (%Imp), percent tree cover (%Tree), and estimated intersection density of walkable roads (IntDen; walkable roads defined as having speed limits between 6-55 miles per hour) (Fig. 1.1b). The temperature data represented long-term (1970-2000) averages at these sites during the focal months of our study (WorldClim 2; Fick and Hijmans 2017). Nighttime radiance data came from the NASA Black Marble data product for December 2020 (NASA Worldview).

For most sites, percent impervious area, percent tree cover, and intersection density were obtained through the EnviroAtlas Community dataset for St. Louis (US EPA, 2021a,b,c). Some rural sites were located outside the boundaries of that urban-centered dataset. Thus, for rural sites #1, 2, and 3 (Table S1.1), we estimated percent impervious area and percent tree cover from the National Land Cover Database (MRLC, 2019). Specifically, we used the Summarize Categorical Raster tool in ArcGIS Pro to extract land use pixel count data from the NLCD layer for the census blocks containing the sites. We calculated percent impervious area as percent of pixels in low, medium, or high intensity development classes. Similarly, we calculated percent tree cover as the percent of pixels in deciduous forest, evergreen forest, mixed forest, shrub scrub, or woody wetlands classes. Intersection density data was not available through EnviroAtlas for rural sites #1, 2, 3, 5, or 6 (Table S1.1) thus we estimated intersection density using the streets layer from the ESRI Streetmap Premium dataset as a base (ESRI, 2020). After limiting to streets with speed limits between 6-55 mph, we manually digitized all intersections within a 750-m radius of the focal sites and used the Kernel Density tool to generate an intersection density
layer. From that layer, we extracted values of intersection density at the study sites, following methods in the EnviroAtlas metadata (US EPA, 2021c).

**Fig. 1.1.** (a) Google Earth satellite view of the focal urbanization gradient in St. Louis, Missouri, USA. Urban sites are plotted as purple diamonds, suburban as green squares, and rural as yellow circles. (b) Biplot of a principal component analysis of environmental variables at the study sites. Black arrows represent loadings of percent impervious area (%Imp), percent tree cover (%Tree), estimated intersection density of walkable roads (IntDen), average temperature from June–October (Temp), and nighttime radiance (NightLight). Sites are numbered from west to east (see Table S1.1 for additional site information).

**Field survey methods**

We performed monthly surveys in the focal populations from July to October 2019 (four surveys) and from June to October/November 2020 (five surveys). This time frame was chosen to represent the majority of the growing season of the plants, their fungal pathogens, and their insect herbivores. In each survey, we collected data for 50 individuals each of *P. lanceolata* and *P. rugelii*. On a meandering path through each population, we selected the nearest plant of either species that was at least 1 meter (m) from the previously surveyed individual of that same
species. The average sizes of the surveyed areas were 1004, 1714, and 2699 m$^2$ for rural, suburban, and urban sites, respectively (Fig. A.1; sizes estimated from polygons in Google Earth). Rural survey areas tended to be smaller because those sites were typically bounded by tall grassland or woodlands where $Plantago$ do not grow. By contrast, urban and suburban sites typically had larger total areas with $Plantago$ present, and our surveys covered more ground.

For each selected plant, we recorded the powdery mildew infection status for each plant as the presence or absence of conspicuous white mycelia and/or conidia on the leaf surface. In 2020, to assess whether the infection statuses of the selected plants were representative of nearby conspecifics, we additionally recorded the numbers of infected conspecifics within a 1.5-m radius of each focal individual, using the following categories: “0” = 0, “1” = 1-10, “2” = 11-50, “3” = 51-100, and “4” = 101 or more plants. In 2020, we recorded the presence or absence of two main types of herbivory damage (chewing and leaf mines on leaves) and mowing damage (straight cuts across leaves).

**Sentinel experiment**

In July 2020, healthy, greenhouse-grown “sentinel” plants were placed into 15 field survey sites to directly test rates of powdery mildew infection and herbivory across the urbanization gradient. These sites (five of each site type) were chosen from the full set of 22 sites based on presence of mildew on at least one of the $Plantago$ species in 2019. We selected sites based on that criterion to avoid biasing the outcome of the experiment towards rejection of the null hypothesis that infection prevalence would be the same across site types.
Sentinel plants were grown from *P. lanceolata* and *P. rugelii* seeds collected from wild, uninfected plants in St. Louis City and County during July–October 2019. To capture variation in plant genetic background, we planted seeds from six maternal lines of *P. lanceolata*, and five of *P. rugelii*. Each maternal line consisted of a single seed spike from a mother plant. Seeds were sown in BM6 All-Purpose soil (Berger) on 16 May 2020 in the Jeanette Goldfarb Plant Growth Facility at Washington University in St. Louis. All six *P. lanceolata* lines germinated, but only three of the *P. rugelii* lines successfully germinated. On 1 June, we moved the seedlings to a hoop house at Tyson Research Center (Eureka, Missouri) and transplanted each into a 4.5-in diameter pot. To ensure that sentinels remained uninfected before deployment into the field, we covered each with an autoclaved, spore-proof pollination bag (PBS International, model 3D.55), which we secured around the pot with a silicone band. Three times per week, we placed the sentinels in a shallow pool of water for 10 min, allowing water to soak up through the base of the pot.

Sentinels were deployed into field sites between 7-9 July 2020. Due to much higher germination success for *P. lanceolata* than *P. rugelii*, we were able to place 20-23 *P. lanceolata* into each of 15 sites (*n* = 322) but only 3-4 *P. rugelii* into each of 9 sites (*n* = 36). Within sites, sentinels were randomly divided between three seedling trays at the base of a single tree (i.e., all sentinels were at one shaded location per site). We removed the pollination bags and collected initial data on the number of leaves, length and width of largest leaf, and flower maturity stage. We confirmed that no sentinels were infected with powdery mildew, and recorded any existing damage on leaves (e.g., from greenhouse or hoop house insects encountered prior to enclosure with pollination bags). We also recorded the distance from the trays of sentinels to the nearest
infected Plantago in the surrounding wild population. We placed a temperature datalogger (HOBO MX2201) just below the soil surface in one pot per tray to monitor temperature differences between sites. The trays were filled with an inch of water at the time of deployment and once the following week. It did not rain during the week of the experiment, so this watering scheme achieved healthy moisture levels in the pots.

Between 14-16 July 2020, sentinels were retrieved from the field sites in the same order they were deployed. Before removal from the field, we recorded the number of leaves, length and width of largest leaf, and presence/absence of chewing and leaf mine herbivory for each leaf. Then we re-covered each sentinel with a pollination bag and moved them to a common location 5 km from the nearest field site to allow any powdery mildew infections to develop for another week. During that week, we watered plants twice (in shallow pools for 10 min), and at the end of the week we recorded infection status of each leaf.

**Infection assay**

Powdery mildew material used for the infection assay was cultured from wild Golovinomyces sordidus collected from across the St. Louis urbanization gradient in October and November 2021 and 2022. The urban line was collected from a road verge in St. Louis, the suburban line was from Buder South Park, and the rural line was from West Tyson Park, Chubb Trail. Infected leaves were collected from source plants in urban, suburban, and rural locales. To isolate a singular genotype, individual conidial chains from these leaves were isolated using a single paint brush hair, placed on a sterilized leaf, and allowed to grow into a source lesion. Each
mildew line was isolated at least once. Due to poor growth of the rural line, only urban and suburban powdery mildew lines were available for use.

Plant material used for the infection assay were grown from *P. rugelii* seeds collected from survey sites across the St. Louis urbanization gradient in October and November 2021 and 2022. Seeds were collected from mature plants >1 m apart. Following the sentinel experiment, we found that *P. rugelii* seeds require a cold stratification treatment to increase germination success (Goodson, unpublished). Therefore, on 1-September-2022 the seeds were placed between two layers of wet filter paper in Petri dishes in a 6-7 °C incubator for 3 months prior to being moved to a 20 °C incubator until germination. On, 16-December-2022 seedlings were sown in BM6 All-Purpose soil (Berger) on in the Jeanette Goldfarb Plant Growth Facility at Washington University in St. Louis. When the seeds reached seedling stage, individuals were transplanted into 4-inch cells containing BM6 All-Purpose soil and were watered every other day. We originally planted 105 seed lines, however due to poor germination success, only 68 total lines reached maturity (urban: n = 31, suburban: n = 23, rural: n = 13; Table A.2). On 10-January-2023 most plants had reached maturity and the infection assay began.

For each mildew line, we inoculated a series of Petri dishes containing four 2 cm leaf pieces – one piece from a unique plant line from each site type, as well as a control plant line, grown from seeds collected from >450 km outside St. Louis. After 12 days, binary growth success was recorded for each leaf piece. If mildew growth was not successful for a mildew × host pairing, we repeated the pairing up to three times.
**Statistical analyses**

All statistics were performed in R version 3.6.2 (R Core Team, 2019). Field survey data were analyzed separately for each species and year. In models testing effects of survey month (ordered factor), site type (urban, suburban, or rural), and their interaction on a given response variable, we included site (population) identity as a random effect. We performed post-hoc Tukey's tests of contrasts between site types within each month if there was at least a marginally significant (P < 0.10) site type x month interaction, or between site types averaged across months if there was a significant (P < 0.05) main effect of site type but no interaction (package ‘emmeans’; Lenth, 2022). We report all site type contrasts with P < 0.10, reserving the word "significant" for P < 0.05.

Prevalences of powdery mildew infection, leaf mines, and mowing were logit-transformed and analyzed in linear mixed effects models (package 'nlme'; Pinheiro et al., 2012) with first order autocorrelation structure. For prevalence values of zero or one, we added (to zeros) or subtracted (from ones) 0.0001 before logit-transforming. We detected no temporal autocorrelation in prevalence of chewing herbivory, so this was analyzed in a generalized linear mixed model (GLMM, package 'lme4'; Bates et al., 2015) with binomial error distribution and logit link function.

To test if the infection statuses of the surveyed plants were representative of nearby conspecifics, we used binomial generalized linear models (logit link). Specifically, we modelled binary infection status of surveyed plants in response to the categorical abundance of infected conspecifics in the surrounding 1.5 m radius, with site identity as a random effect.
In the sentinel experiment, we analyzed the proportion of leaves on each plant with powdery mildew, leaf mines, and chewing damage (i.e., "severity" of each damage type) using binomial GLMMs (logit link) with random effects of tray identity nested within site identity (because there were three trays of sentinels in each field site). In addition to testing effects of site type on infection and herbivory severity, we included covariates reflecting the initial size of sentinels. Specifically, these were scores on the first two principal components axes from a PCA of number of leaves and length and width of longest leaf at the start of the experiment. For *P. lanceolata*, the first principal component axis (PC1) explained 65.06% of variation in initial plant size data and the second (PC2) explained 34.72%; for *P. rugelii*, PC1 explained 75.36% and PC2 explained 21.92% of variation. For analysis of infection severity, we included distance to nearest infected wild plant as a covariate. For analyses of severity of leaf mines and chewing, we additionally included the initial severity (chewing) or presence/absence (mines) of these damage types as covariates, to account for any herbivory that occurred prior to placement in the field. For both infection and herbivory, we tested whether prevalence on sentinels was correlated with prevalence in the wild populations where they had been placed.

Due to susceptibility of all *P. rugelii* individuals to both powdery mildew lines, statistical analysis was not necessary.

**Results**

*Field site characteristics*

In the PCA of environmental variables at the study sites, the first two principal components (PC1 and PC2) together explained 89.85% of the total variation in the data (Fig.
1.1b). The pre-classified site types (rural, suburban, and urban) were primarily separated along PC1, which was associated with greater temperature, nighttime radiance, intersection density, and percent impervious area, and with lower percent tree cover (Fig. 1.1b). Variation within site types occurred primarily along PC2, which was associated with greater temperature, nighttime radiance, and percent tree cover, and with lower percent impervious area (Fig. 1.1b).

Field surveys: powdery mildew infection

There was very little powdery mildew infection on _P. lanceolata_ in 2019, and generally small epidemics in 2020 (Fig. 1.2a,c). For this species, there were no significant site type x month effects on infection prevalence (in 2019: LR $\chi^2 = 4.00$, df = 6, $P = 0.68$; in 2020: LR $\chi^2 = 10.34$, df = 8, $P = 0.24$), nor significant main effects of site type (in 2019: LR $\chi^2 = 2.43$, df = 2, $P = 0.30$; in 2020: LR $\chi^2 = 3.36$, df = 2, $P = 0.19$). There was a significant main effect of month in 2020 only (in 2019: LR $\chi^2 = 3.92$, df = 3, $P = 0.27$; in 2020: LR $\chi^2 = 47.64$, df = 4, $P < 0.0001$).

There were large powdery mildew epidemics on _P. rugelii_ in both years, and epidemics started earlier in suburban and urban populations (Fig. 1.2b,d). In both years, _P. rugelii_ infection prevalence was significantly affected by site type (in 2019: LR $\chi^2 = 8.89$, df = 2, $P = 0.012$; in 2020: LR $\chi^2 = 22.00$, df = 4, $P < 0.0001$) and month (in 2019: LR $\chi^2 = 22.57$, df = 3, $P < 0.001$; in 2020: LR $\chi^2 = 70.42$, df = 4, $P < 0.001$), but there were no site type x month interactions (in 2019: LR $\chi^2 = 4.39$, df = 6, $P = 0.62$; in 2020: LR $\chi^2 = 3.90$, df = 6, $P = 0.87$). For 2019, post-hoc testing revealed significantly greater infection prevalence in urban than rural sites (suburban-rural: $P = 0.067$, urban-rural: $P = 0.037$; Fig. 1.2b). In 2020, there was significantly greater
infection prevalence in both suburban and urban relative to rural sites (suburban-rural: \( P = 0.0061 \), urban-rural: \( P = 0.0007 \); Fig. 1.2d).

The infection status of surveyed plant individuals was consistent with that of nearby plants in the population. Specifically, presence/absence of powdery mildew infection on surveyed plants was significantly positively related to the abundance of infected conspecifics within a 1.5-m radius (\( P. lanceolata: \chi^2 = 262.81, \text{df} = 3, P < 0.0001 \); \( P. rugelii: \text{LR} \chi^2 = 1097.6, \text{df} = 3, P < 0.0001 \); Fig. A.2).

Fig. 1.2. Data points represent proportion of plants infected with powdery mildew in each population. Box-and-whisker plots are grouped by site type and survey month in 2019 and 2020.
Field surveys: herbivory

For both species, prevalence of leaf mines depended on the interaction of site type and month ($P. lanceolata$: $\chi^2 = 16.37$, df = 8, $P = 0.037$; $P. rugelii$: $\chi^2 = 55.84$, df = 8, $P < 0.0001$; Fig. 1.3a,b), and the only significant differences between site types occurred in June. For $P. lanceolata$, there was significantly greater prevalence of leaf mines in urban than suburban sites in June (urban-rural: $P = 0.092$, urban-suburban: $P = 0.0083$). For $P. rugelii$, there was greater prevalence of leaf mines in urban than either suburban or rural sites in June (urban-rural: $P = 0.0017$, urban-suburban: $P = 0.0001$).

Chewing-type herbivory also depended on the interaction between site type and month ($P. lanceolata$: $\chi^2 = 66.17$, df = 8, $P < 0.0001$; $P. rugelii$: $\chi^2 = 88.51$, df = 8, $P < 0.0001$; Fig. 1.3c,d). For both species, there was greater prevalence of chewing damage in urban than suburban or rural sites in June ($P. lanceolata$: urban-rural: $P < 0.0001$, urban-suburban: $P < 0.0001$; $P. rugelii$: urban-rural: $P < 0.0001$, urban-suburban: $P < 0.0001$).
Fig. 1.3. Proportion of plants with chewing damage or leaf mines in each population. Box-and-whisker plots are grouped by site type and survey month in 2020.

Field surveys: mowing

For *P. lanceolata*, prevalence of mowing damage on leaves depended on site type ($\chi^2 = 7.50$, df = 2, $P = 0.024$) and month ($\chi^2 = 43.10$, df = 4, $P < 0.0001$), but not their interaction ($\chi^2 = 10.28$, df = 8, $P = 0.25$). For this species, there was marginally greater mowing prevalence in suburban and urban than rural sites (suburban-rural: $P = 0.049$, urban-rural: $P = 0.083$; Fig. 1.4a).

For *P. rugelii*, there was a marginally significant site type x month effect on prevalence of mowing damage (site type x month: $\chi^2 = 13.79$, df = 8, $P = 0.087$; site type: $\chi^2 = 8.56$, df = 2,
This weak interactive effect reflected marginally greater mowing prevalence in suburban and urban relative to rural sites in June (suburban-rural: $P = 0.034$, urban-rural: $P = 0.062$) and significantly greater mowing prevalence in suburban and urban relative to rural sites in the final October/November survey (suburban-rural: $P = 0.0020$, urban-rural: $P = 0.0025$; Fig. 1.4).

Fig. 1.4. Proportion of plants with leaf damage from lawn mower blades in each population. Box-and-whisker plots are grouped by site type and survey month in 2020.
Sentinel experiment: powdery mildew infection and herbivory

All 322 *P. lanceolata* sentinels survived to the end of the experiment, and only one became infected with powdery mildew (Fig. 1.5a). This infected sentinel was placed in Buder South Park within 1.5 m of the only wild infected *P. lanceolata* individual we found after a thorough search at that site (Fig. A.3; note that the one wild infected plant was not among the 50 plants surveyed at that site in July 2020, so infection prevalence of wild plants was recorded as zero for that site-date; Fig. 1.5a). No *P. lanceolata* sentinels became infected in Tilles Park, which did have a small number of infected wild plants at that time (Fig. 1.5a), likely because the sentinels were located almost 6 m from the nearest wild infected plant (Fig. A.3).

Many *P. lanceolata* sentinels experienced herbivory during their week in the field; however, there were no differences between site types for severity (proportion of leaves damaged) of either leaf mines ($\chi^2 = 4.17$, df = 2, $P = 0.12$) or chewing ($\chi^2 = 3.82$, df = 2, $P = 0.15$). Prevalences of leaf mines and chewing (proportion of plants affected) were not correlated between sentinels and wild *P. lanceolata* in the same sites during July 2020 (leaf mines: $r = -0.03$, $P = 0.92$, Fig. 1.5c; chewing: $r = -0.14$, $P = 0.62$, Fig. 1.5e).

All 36 *P. rugelii* sentinels survived to the end of the experiment. Eight of these became infected with powdery mildew in urban sites (Clifton Park and Tilles Park), three became infected in a single suburban site (Kirkwood Park) and none became infected in rural sites (Fig. 1.5b). Although this trend was consistent with the greater prevalence of infection observed in more urban populations of wild *P. rugelii* in 2020 (Fig. 1.2d), there was no significant correlation between prevalence of infection on sentinels and wild plants surveyed in those sites.
during July (P = 0.95, Fig. 1.5b). Instead, the proportion of infected *P. rugelii* sentinels increased with proximity to wild infected plants within the site (P = 0.048, Fig. A.3).

Only a single *P. rugelii* sentinel acquired a leaf mine during the field exposure. *Plantago rugelii* sentinels experienced chewing-type herbivory in most field sites (Fig. 1.5f), and severity of chewing damage did not differ between site types ($\chi^2 = 1.25$, df = 2, P = 0.53). Prevalence of herbivory on *P. rugelii* sentinels was not correlated with that on wild conspecifics in those sites during July 2020 (leaf mines: $r = 0.15$, P = 0.71, Fig. 1.5d; chewing: $r = -0.52$, P = 0.15, Fig. 1.5f).
Fig. 1.5. Proportion of sentinels with (a, b) powdery mildew infection, (c, d) leaf mines, and (e, f) chewing damage in relation to the proportion of wild conspecifics with the same type of damage in each site in July 2020. There are 15 sites plotted in each panel, but 12 points with no mildew on either wild or sentinel plants (i.e., overlapping at the origin) in panel (a).
Infection assay

All *P. rugelii* individuals from populations across urbanization gradient were susceptible to both powdery mildew lines collected from urban and suburban locales (Table 1.1).

<table>
<thead>
<tr>
<th>Pathogen Population Site Type</th>
<th>Susceptibility to Infection</th>
<th>Host Population Site Type</th>
</tr>
</thead>
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<tr>
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<td>Susceptible</td>
<td>Urban</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
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</tr>
<tr>
<td>Suburban</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>0</td>
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</tbody>
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Table 1.1. Number of *P. rugelii* individuals which are susceptible/resistance to *Gol. sordidus* strains from sites across urbanization gradient.

Discussion

We found that the timing of seasonal prevalence of multiple types of foliar damage for wild herbaceous plants changed along an urbanization gradient. A consistent trend between years was that powdery mildew epidemics on *P. rugelii* started earlier and achieved greater infection prevalence in more urban sites consistently between years. Correspondingly, *P. rugelii* sentinels only became infected in suburban and urban sites. However, there was less mildew on *P. lanceolata*, including for sentinels placed into the field sites. There was no significant effect of urbanization on infection prevalence for *P. lanceolata*. Urbanization was associated with accelerated early-summer herbivory on both plant species but was not associated with herbivory of wild or sentinel plants later in summer. Finally, prevalence of mowing damage on leaves was generally greater in urban and suburban than rural sites.

Urbanization was associated with earlier and overall larger powdery mildew epidemics on *P. rugelii*. There are several non-mutually exclusive possible reasons for that trend. Two of
those possible reasons involve temperature. First, mildew survival is decreased by exposure to freezing air temperatures (Penczykowski et al. 2015). Therefore, the UHI may have increased mildew overwinter survival. Second, warmer temperatures during the growing season can promote mildew growth and transmission. Because all the field sites were parks and nature areas, the magnitude of the UHI experienced by our focal plants might have been tempered by the presence of trees and other vegetation. However, soil temperatures in the pots of sentinel plants increased from rural to suburban to urban sites (Fig. A.4), consistent with long-term trends in this region (Fig. 1.1b). Thus, as for powdery mildew on oaks (van Dijk et al. 2022), warmer temperatures may have contributed to the increased infection prevalence of \( P. \ rugelii \) in suburban and urban populations.

Greater rates of spore arrival to more highly connected urban sites may result in increased mildew prevalence with urbanization. Dispersal along roadside populations could allow mildew to spread between sites over the growing season (Numminen and Laine 2020) with denser urban road networks bringing more mildew spores. However, arriving spores are only successful at infecting if they land on susceptible plant genotypes, and \( Plantago \) populations can vary in their susceptibility to arriving mildew strains (Laine 2004, Jousimo et al. 2014, Höckerstedt et al. 2018). Greater resistance of rural \( P. \ rugelii \) to mildew could also have decreased their prevalence of infection, even if rates of spore arrival were equally high across the land use gradient. However, in our experimental infection assay, the urban, suburban, and rural plant lines tested were all susceptible to both the suburban and urban mildew lines. This suggests that the greater infection prevalence in more urban field populations was not due to urban plant genotypes being generally more susceptible to mildew than rural genotypes. Instead, it is likely that there is
greater spore arrival to the more urban sites, reflecting their greater connectivity by roads and traffic. The fact that none of the *P. rugelii* sentinel plants became infected in rural sites is consistent with low spore arrival in those sites, though we note that sample sizes of *P. rugelii* sentinels were small.

At the same time, there were lower rates of infection on wild *P. lanceolata* compared to *P. rugelii* growing in the same sites, as well as near-zero prevalence of infection among the >300 *P. lanceolata* sentinels placed across the urbanization gradient. These results suggest species-specificity of the pathogen strains, and lower regional abundance of spores able to infect *P. lanceolata*. Aerial spore sampling and genotyping of mildew infections collected from both *Plantago* species across the urbanization gradient will provide further insights into the connectivity of pathogen populations within and between land use types.

We hypothesized that early-summer prevalence of insect herbivory would be elevated in urban populations, due to UHI accelerating insect emergence and foraging activity in spring. Indeed, both types of herbivory damage were greatest on urban *Plantago* in our earliest survey month (June). However, we further hypothesized that urban sites would continue to experience greater herbivory over the summer, and that herbivory damage on sentinel plants would similarly increase with urbanization. These hypotheses for later-summer patterns are not supported by our data. Instead, the prevalence of leaf mines became similar across site types, peaking at a moderate level in July-August. Prevalence of chewing damage was sustained at a high level across site types for the remainder of the growing season. Rates of herbivory on sentinels in July neither followed a trend with urbanization nor correlated with prevalence of herbivory in the wild populations at that time. Generally, our results are consistent with herbarium evidence that
increases in winter temperature are a strong driver of increases in insect herbivory (Meineke et al. 2019). Additional experiments will be needed to tease apart effects of winter and spring climate variables, artificial light at night, and other abiotic and biotic facets of urbanization as contemporary drivers of increased early-summer herbivory on Plantago hosts.

As urban areas continue to grow in size and prominence worldwide, there is a societal need for understanding effects of urban environmental factors on plants (United Nations, 2018). Urban vegetation provides ecosystem services that mitigate heat, air pollution, and flooding (Heidt and Neef 2008). Access to green space is also linked to mental and physical health benefits for humans, including decreased heart rate, increased physical activity, and improved mood (Kondo et al. 2018). The abundance, health, and connectivity of native and introduced plant species are also key in supporting diversity of urban fauna (Johnson and Munshi-South, 2017). Thus, it is critical to resolve the mechanisms by which urban environments impact their fitness. In this study, we showed how prevalence of disease, herbivory, and mowing damage varied through time across an urbanization gradient. Our findings point to several mechanisms by which changes in land use may impact plant population growth. While this study offers an in-depth examination of an urbanization gradient, the generality of these effects should be evaluated through similar studies replicated across additional urban areas.

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Chapter Two:

Effects of microclimate on disease prevalence across an urbanization gradient
Abstract

Increased temperatures associated with urbanization (the “urban heat island” effect) have been shown to impact a wide range of traits across diverse taxa. At the same time, temperature varies at fine spatial scales within habitats due to factors including shade from shrubs, trees, and built structures. Patches of shade may function as microclimate refugia that allow species to occur in habitats where high temperatures and/or exposure to ultraviolet radiation would otherwise be prohibitive. However, the importance of shaded microhabitats for interactions between species across urbanized landscapes remains poorly understood. Weedy plants and their foliar pathogens are a tractable system for studying how multiple scales of climatic variation influence infection prevalence. Powdery mildew pathogens are particularly well suited to this work, as these fungi can be visibly diagnosed on leaf surfaces. We studied effects of shaded microclimates on rates of powdery mildew infection on *Plantago* host species in (1) “pandemic pivot” surveys in which undergraduate students recorded shade and infection status of thousands of plants along road verges in urban and suburban residential neighborhoods, (2) monthly surveys of plant populations in 22 parks along an urbanization gradient, and (3) a manipulative field experiment directly testing effects of shade on growth and transmission of powdery mildew. Together, our field survey results show strong positive effects of shade on mildew infection in wild *Plantago* populations across urban, suburban, and rural habitats. Our experiment suggests that this relationship is causal, where microclimate conditions associated with shade promote pathogen growth. These findings highlight the importance of taking microclimate heterogeneity into account when establishing links between macroclimate or land use context and prevalence of fungal plant pathogens.
Introduction

As temperatures increase globally, there is a pressing need to understand how different spatial scales of climate variation will impact both distributions of individual species and interactions between species. For example, over much of the world, urbanization has produced "heat islands" that contribute to temperature heterogeneity at the landscape scale (Imhoff et al. 2010, Zhou et al. 2017). Urban areas are hotter than surrounding suburban and rural areas for reasons including decreased tree canopy cover, greater coverage with impervious surfaces that absorb and emit heat, and increased heat generation by vehicles and machinery (Zhou et al. 2017). Increased temperatures in cities have been shown to impact a wide range of traits across diverse taxa (Johnson et al. 2018, Pisman et al. 2020, Santangelo et al. 2022). At the same time, temperature heterogeneity occurs at fine scales within habitats due to local environmental factors including shade from natural and build features (Liao et al. 2021, Chen et al. 2022). The impacts of fine-scale microclimate variation on species interactions are poorly understood, and it is unclear if such effects vary across degrees of urbanization.

Species distributions are determined in part by their climatic niche requirements including temperature, ultraviolet (UV) radiation, precipitation, and relative humidity. Coarse-scale climate data (e.g., the 1-km spatial resolution of WorldClim 2 (Fick and Hijmans 2017)) are valuable for identifying constraints on species distributions at the macroscale. However, such datasets average over fine-scale climate (microclimate) variation and thus lose pockets of tolerable habitats ("microrefugia") in seemingly intolerable climates (Suggitt et al. 2011, Lenoir et al. 2017). For example, small scale variation in temperature can provide refuge for
temperature-sensitive organisms, allowing for persistence through adverse climatic conditions, or short, extreme weather events (Suggitt et al. 2011, Lenoir et al. 2017, Bramer et al. 2018).

Fungal pathogens on aboveground plant tissues are generally quite exposed to ambient climatic conditions. Pathogen germination, sporulation, and transmission between host plants each occur within a range of temperature, precipitation, relative humidity, and UV conditions (Chaloner et al. 2020). Thermal tolerance of plant pathogens depend on the physiological responses of both the pathogen and the plant host's resistance response (Wang et al. 2009, Desaint et al. 2021). For most fungal plant pathogens, the optimal thermal range across life stages is ca. 20-25 °C (Chaloner et al. 2020). Optimal precipitation and humidity regimes are highly variable among species and life stages (Chaloner et al. 2020). Many fungal species require free water for germination, while germination of powdery mildew spores (ascomycete fungi in order Erysiphales) is inhibited by water (Glawe 2008). At the same time, powdery mildews (and many other fungi) require moderately high relative humidity (Guzman-Plazola et al. 2003). Most fungi are also sensitive to UV radiation (Braga et al. 2015). While fungal species vary in their response to UV radiation, direct exposure to solar radiation can kill conidia (asexual spores) of most fungal species within a few hours (Braga et al. 2015). Temperature, moisture, and UV exposure may (co-)vary at a fine scale within plant populations, and even across parts of the same plant individual. Thus, identifying effects of microclimate variation on plant diseases can improve predictions for when and where pathogens may persist across larger scale spatial and temporal climate variation.

This study focuses on the effects of within-population heterogeneity in shade on powdery mildew pathogens of herbaceous plants across an urbanization gradient and across months of the
growing season. Shade has been found to promote growth of powdery mildews in crop systems. For example, disease severity of grape leaf powdery mildew, *Uncinula necator*, increased by 49–75% on shaded leaves compared to leaves in full sun (Austin and Wilcox 2012). In that system, increased temperatures and UV-B radiation interactively decrease powdery mildew growth in sunlit conditions (Willocquet et al. 1996, Austin and Wilcox 2012). While there is some heterogeneity in shade within agricultural settings, there may be even more in wild plant populations due to diversity of plant heights (including trees) and built structures (including fences and buildings). In a network of ca. 4000 populations of *Plantago lanceolata* in the Åland Islands of southwest Finland, populations with greater overall shade cover were less likely to have any powdery mildew (Jousimo et al. 2014). However, within populations where mildew was present, susceptible plant lines placed into microhabitats with lower temperatures and higher humidity were more likely to become infected (Penczykowski et al. 2018). Effects of microclimate were only detected towards the beginning of the epidemics and not later in the summer season. This suggests that the importance of microclimate for pathogen growth and transmission may vary with phase of epidemics (e.g., germination and establishment of initial infections vs. within-population transmission) or with seasonal variation in climatic conditions.

Despite a rapidly growing body of literature in the field of urban ecology and evolution (Grimm et al. 2008, Schell et al. 2020, Des Roches et al. 2021), there has been little empirical research on variation in plant disease risk across levels of urbanization (Egerer et al., 2020; Fox et al., preprint; van Dijk et al., 2022). Moreover, it is unknown how the magnitude of microclimate effects on plant disease changes across land use types and seasons. Here, we determine the role of microclimate variation – specifically, due to shade – on disease prevalence.
across an urbanization gradient. We conducted surveys of powdery mildew infection on
*Plantago* host plants in suburban and urban road verges as well as in parks and nature centers
across an urbanization gradient. Across all habitat types, we expected to find greater prevalence
of powdery mildew infection on plants in the shade compared to full sun. Due to the urban heat
island effect, we expected shade to have a stronger positive effect on mildew prevalence in more
urban populations. However, shaded and sunlit locations within a habitat may also differ in
factors other than microclimate that contribute to disparities in infection risk. For example,
effects of trees and built structures on air currents and particle transport may alter the probability
of fungal spore arrival to a plant in their shadow (Calonnec et al. 2013). Plants directly under
shade-producing structures (e.g., trees, buildings, and fences) may also experience less
disturbance including from lawn mowers. Therefore, we also performed a manipulative
experiment to directly test effects of shade on growth and transmission of powdery mildew.

**Methods**

**Study system**

This study focused on three herbaceous plants, *Plantago lanceolata* (ribwort plantain),
*Plantago major* (common plantain), and *Plantago rugelii* (blackseed plantain). *Plantago* are
short-lived, rosette-forming perennials that grow abundantly in mowed, grazed, and trodden
habitats such as lawns, parks, pastures, and roadsides (Kuiper and Bos 2012). Due to their
presence across many land use types, *Plantago* are tractable model organisms for studying plant–
pathogen and plant–herbivore interactions across land use gradients (Penczykowski and Sieg 2021).
*Plantago lanceolata* and *P. major* are native to Eurasia but global in distribution, and *P.*
rugelii is endemic to eastern North America. *Plantago major* is rare in our focal geographic region and is thus absent from our observational surveys (Yatskievych and Steyermark 1999, Penczykowski and Sieg 2021).

The most globally and locally common foliar fungal pathogens of *Plantago* are two specialist powdery mildews in the order Erysiphales: *Podosphaera plantaginis* (Castagne; U. Braun and S. Takamatsu) and *Golovinomyces sordidus* (L. Junell) V.P. Heluta (Braun and Cook 2012). Both species are obligate pathogens that extract nutrients from their host's epidermal tissue (Bushnell 2002). Chains of asexual spores produced from mycelia on the leaf surface give infected leaves a white, dusty appearance. The spores are passively transmitted via wind, and more than 90% land within 2 m of the source plant (Tack et al., 2014). Occasional long-distance spore transport allows for pathogen persistence at the regional scale (Ovaskainen and Laine 2006). Powdery mildews overwinter via sexual resting structures that release spores when conditions are favorable in spring (Tack and Laine 2014).

**Road verge surveys**

To compare the frequency of powdery mildew infection on plants in the sun and shade, we performed observational surveys of *Plantago* species along road verges (strips of vegetation between roads and sidewalks) and in the lawns of parks in St. Louis City and County, Missouri (Fig. 2.1a). Five trained surveyors collected data between 16 June and 24 July 2020. Surveys were concentrated in suburban and urban neighborhoods near the Washington University in St. Louis (WashU) campus due to transportation constraints during the first summer of the COVID-19 pandemic (Fig. 2.1b). Each survey involved visual inspection of up to 50 randomly selected
individuals of *Plantago lanceolata* and *P. rugelii*. Start and end locations of each survey were marked on a shared Google Earth project to avoid spatial overlap. We arbitrarily chose a first focal plant of either species and marked the survey start locations using GPS-enabled smart phones. We recorded whether each focal plant was in the sun or shade at the time of the survey, and whether the plant was infected with powdery mildew (presence/absence). As covariates, we recorded the presence/absence of lawnmower damage (leaves cut cleanly across by mower blade) and common types of herbivory (leaf mines and chewing damage). To ensure that the infection status of the focal plants were representative of neighboring individuals, we also estimated the total and infected number of conspecifics within a 1.5 m radius of the focal plant (ordered categorical variable in bins of 0, 1-10, 11-50, 51-100, or 101+ plants). We then walked a few paces in a predetermined direction (i.e., continuing unidirectionally along a roadside or within a park lawn) and arbitrarily selected the next focal plant. Focal plants were always at least 1 m from the previously selected conspecific in the survey. We continued until 50 plants of each species had been surveyed, and then recorded the end location and time. Fewer than 50 plants were surveyed if a *Plantago* species was locally rare or if surveys were cut short due to inclement weather or physical barriers (e.g., construction). We performed 68 surveys of *Plantago rugelii* (65% of these with n = 50) and 60 surveys of *P. lanceolata* (33% of these with n = 50).
Fig. 2.1. Google Earth satellite view of the focal urbanization gradient in St. Louis, Missouri, USA. *Road verge surveys*: Start locations are represented by orange triangles. *Urbanization surveys*: Urban sites are plotted as purple diamonds, suburban as green squares, and rural as yellow circles. (a) View of full region extent (all survey sites). (b) Zoomed-in view of region where road verge surveys were concentrated (showing those survey locations only).

All statistical analyses were performed in R (R Core Team 2020). For each plant species, we analyzed the probability that a focal plant was infected using generalized linear mixed models (GLMMs; package 'lme4') with binomial error distributions and logit link functions. We tested effects of the explanatory factors shade (yes/no), mowing damage (yes/no), and herbivory damage (yes/no for both chewing damage and leaf mines). Unique survey identity was included as a random effect. We first fit models with all possible interactions, and then simplified the models through stepwise deletion of non-significant interactions terms (Crawley 2007). For significance testing of fixed effects terms, we used Type III sums of squares if there were significant interaction terms, in all other cases we used Type II sums of squares (Anova function in package 'car'). To assess whether there were spatial trends among survey locations, we also fit generalized additive mixed models (GAMMs; package 'gamm4') structured as above, but with
additional splines of latitude and longitude of survey start locations. However, the GAMMs did not perform significantly better than the GLMMs (model comparisons for \( P. \ rugelii: \ P = 0.14 \) and \( P. \ lanceolata: \ P = 0.99 \)), so we report only the GLMM results. We used generalized linear models (GLMs) to analyze the relationship between infection status of the focal surveyed plants and the number of infected plants in the surrounding microhabitat (1.5 m radius), with latitude and longitude of survey start locations included as fixed effects.

**Urbanization surveys**

To investigate how the relationship between shade and powdery mildew varied with urbanization, we analyzed data from monthly surveys of *Plantago* populations across 22 sites spanning from the urban City of St. Louis to rural Shaw Nature Reserve in Gray Summit, Missouri (Fig. 2.1a). We classified eight sites within St. Louis City as “urban”, eight sites in St. Louis County east of Missouri Route 141 as "suburban", and six sites west of Missouri Route 141 as “rural”, based on a principal component analysis of land cover variables (Fox et al., *preprint*). Survey methods were identical to the road verge surveys, with the exception that we surveyed plants along a meandering (non-linear) walk throughout each site. Monthly surveys were performed in July, August, September, and late October through early November 2020.

As for the road verge surveys, we used GLMMs to analyze the binomial response variable of plant infection status. The models included month of survey, site type (urban, suburban, and rural), shade (yes/no), mowing damage (yes/no), herbivory damage (yes/no for either chewing damage or leaf mines), and their interactions as fixed effects. Site identity was modelled as a random effect. Again, we first fit statistical models to include all possible
interactions and then simplified the models through stepwise deletion of non-significant interaction terms. As for the road verge surveys, we used GLMs to model infection status of the focal surveyed plants with respect to the number of infected conspecifics and latitude and longitude of the site.

Shade experiment

We performed a manipulative experiment to measure effects of shade on powdery mildew growth and transmission in July 2021. Seeds of seven maternal lines of *P. lanceolata* and six maternal lines of *P. major* seeds were sown on 29-30 April 2021 in the WashU Jeanette Goldfarb Plant Growth Facility. Poor germination success of *P. major* yielded a small sample size of this species. After germination, seedlings were moved to a hoop house at Tyson Research Center (Eureka, Missouri) to become heat acclimated. On 20-21 May, seedlings were transplanted into 4.5-inch pots of soil (BM6 All-Purpose) and covered with pollination bags (PBS International) to prevent infection prior to the start of the experiment.

Plants were placed in shallow trays at locations directly under (shade) and midway between (sun) four large solar panels in an otherwise open, mowed field at Tyson Research Center (Fig. C.1). First, we placed a single tray in each location that was assigned to the "inoculated, watered" treatment; this tray contained four *P. lanceolata* and four or five *P. major* individuals. For both species, replicates of the maternal lines were distributed among trays as evenly as possible. On 7 July, we used sterilized paint brushes to inoculate plants in these first trays by gently brushing one leaf per plant with powdery mildew spores from lab-inoculated source plants. Plants were inoculated with species-specific strains of *Golovinomyces sordidus* that had been previously isolated from wild plants in the St. Louis region and propagated on leaf
tissue in the lab following standard methods (Nicot et al. 2002). The remaining *P. lanceolata* plants remained covered with pollination bags in the hoop house. The next day, we evenly divided these remaining uninoculated *P. lanceolata* among three additional trays in each shade and sun location (8-9 plants per tray). We assessed whether these uninoculated plants became infected over the following weeks of the experiment.

The inoculated plants were watered regularly by filling the trays with an inch of water every other day. Two trays of uninoculated plants per location were similarly watered. The third tray of uninoculated plants had drainage holes; this treatment allowed us to test for plant responses to both the thermal environment and amount of precipitation experienced in each shade and sun site. We monitored microclimates by placing temperature loggers (HOBO MX2201) at a depth of 1 cm below the soil surface in at least one pot per sun and shade location (Fig. C.1). We assessed the infection status of each plant on 15 July, 23 July and 6 August 2021.

*Plantago lanceolata* infection status through time was analyzed as a binomial response variable in a GLMM with random effect of plant individual nested within tray identity. We tested the fixed effects of date, microclimate (placement in shade or sun), inoculation and watering treatment, and plant maternal line. Due to the small sample size of *P. major* in this experiment, it was not possible to fit a GLMM. Infection status of *P. major* was analyzed for the final timepoint of the experiment using a GLM testing effects of shade and maternal line only, since all *P. major* were in the "inoculated, watered" treatment.
Results

Road verge surveys

Powdery mildew infection prevalence was much greater on *P. rugelii* than *P. lanceolata* in our surveys (Fig. 2.2). Infection was more often observed in the shade than in the sun for both plant species; this effect of shade was highly significant for *P. rugelii* but non-significant for *P. lanceolata* (Table 2.1, Fig. 2.2). The presence of damage on leaves from either mowing or herbivory was not related to infection status for either species (Table 2.1). For both species, infection on a focal individual was significantly associated with the presence of additional infected conspecifics in the immediate vicinity (P < 0.0001 for both species; Fig. C.2).

Table 2.1. Analysis of deviance tables from GLMMs of powdery mildew infection on *Plantago* host plants in road verge surveys and urbanization surveys.

<table>
<thead>
<tr>
<th></th>
<th>Likelihood Ratio $\chi^2$</th>
<th>d.f.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Road verge surveys: <em>Plantago lanceolata</em></strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>0.16</td>
<td>1</td>
<td>0.69</td>
</tr>
<tr>
<td>Mowing</td>
<td>3.29</td>
<td>1</td>
<td>0.070</td>
</tr>
<tr>
<td>Herbivory</td>
<td>0.19</td>
<td>1</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Road verge surveys: <em>Plantago rugelii</em></strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>120.92</td>
<td>1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mowing</td>
<td>0.56</td>
<td>1</td>
<td>0.46</td>
</tr>
<tr>
<td>Herbivory</td>
<td>2.06</td>
<td>1</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Urbanization surveys: <em>Plantago lanceolata</em></strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td>75.95</td>
<td>3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Shade</td>
<td>23.22</td>
<td>1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Site type</td>
<td>3.62</td>
<td>2</td>
<td>0.16</td>
</tr>
<tr>
<td>Mowing</td>
<td>7.43</td>
<td>1</td>
<td>0.006</td>
</tr>
<tr>
<td>Herbivory</td>
<td>0.14</td>
<td>1</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Urbanization surveys: <em>Plantago rugelii</em></strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td>21.14</td>
<td>3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Shade</td>
<td>44.71</td>
<td>1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Site type</td>
<td>24.50</td>
<td>2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mowing</td>
<td>1.44</td>
<td>1</td>
<td>0.23</td>
</tr>
<tr>
<td>Herbivory</td>
<td>19.39</td>
<td>1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Month × Shade</td>
<td>9.72</td>
<td>3</td>
<td>0.02</td>
</tr>
<tr>
<td>Month × Site type</td>
<td>74.05</td>
<td>6</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Urbanization surveys

The effect of shade on the infection status of wild plants in parks across the urbanization gradient depended on host species, level of urbanization (site type), and month that the survey took place (Fig. 2.3). Infection on *P. lanceolata* was positively related to shade and increased in later months of the season, and was negatively related to mowing damage (Table 2.1). There was no significant effect of site type or herbivory damage on probability of *P. lanceolata* infection (Table 2.1). For *P. rugelii*, infection depended on the interaction between month and shade and the interaction between month and site type (Fig. 2.3, Table 2.1). Post-hoc Tukey tests revealed significantly greater probability of mildew in the shade than sun only during the later three survey months (July: *P* = 0.12, August: *P* = 0.002, September: *P* < 0.0001, October/November: *P*
The interaction between month and site type reflected a greater increase in mildew prevalence through time in the more urban sites (Fig. 2.3). In addition, there was a significant positive relationship between herbivory damage and infection on *P. rugelii* (Table 2.1). For both species, infection on a focal individual was significantly associated with the presence of additional infected conspecifics in the immediate vicinity (P < 0.0001 for both species; Fig. C.3).

**Fig. 2.3.** Urbanization surveys: Proportion of each *Plantago* host species infected with powdery mildew in shaded and sunlit microhabitats within urban, suburban, and rural sites. The same sites were surveyed monthly between July and October (or November) 2020.
**Shade experiment**

There were strong positive effects of shade on growth of powdery mildew on both host species (Table 2.2, Fig. 2.4). Most inoculated plants in shaded locations (under the solar panels) became visibly infected in the first week of the experiment (Fig. 2.4). No mildew grew on inoculated *P. major* in sunny locations. Two inoculated *P. lanceolata* plants in sunny locations developed infection on one leaf each by the second week; however, these mildew lesions were no longer visible in the third week. With *P. lanceolata*, we additionally tested the effect of shade and watering treatment on transmission and subsequent growth of mildew on nearby plants. By the second week, there was evidence of transmission to initially uninoculated plants in the watered trays in the shade only. By the third week, there was a small amount of mildew growth on uninoculated plants in the unwatered trays in the shade. Daytime temperatures in the experimental pots in the sun were consistently much higher than in the shade (Fig. C.4).

**Table 2.2. Shade experiment:** Analysis of deviance table from a GLMM of powdery mildew infection on *P. lanceolata* (with random effect of plant identity nested in tray identity). We used a GLM to analyze infection on *P. major* on the last date of the experiment.

<table>
<thead>
<tr>
<th></th>
<th>Likelihood Ratio $\chi^2$</th>
<th>d.f.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plantago lanceolata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>20.59</td>
<td>2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Shade</td>
<td>19.70</td>
<td>1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>24.07</td>
<td>2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Maternal line</td>
<td>12.40</td>
<td>6</td>
<td>0.054</td>
</tr>
<tr>
<td><strong>Plantago major</strong> (final timepoint of experiment)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>66.67</td>
<td>1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Maternal line</td>
<td>22.77</td>
<td>4</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
**Fig. 2.4.** *Shade experiment*: Proportion of each *Plantago* host species that became infected with powdery mildew in shaded (under solar panels) and sunlit (between solar panels) microhabitats during the three weeks of the experiment in July 2021. Treatment combinations: inoculated/watered, uninoculated/watered, uninoculated/not watered. *Plantago major* were only included in the inoculated/watered treatment due to low sample size of this species.

**Discussion**

We found strong positive effects of shade on powdery mildew in wild plant populations in urban, suburban, and rural habitats. Correspondingly, our shade manipulation experiment
showed a clear positive effect of shade on the success of powdery mildew infection on both *P. major* and *P. lanceolata*. Shade has consistently been shown to promote the development of powdery mildew infection across different host plant species in agricultural systems (Austin and Wilcox 2012). Direct sunlight in warm climates may heat the leaf surface to a temperature that is lethal to the fungus (Chaloner et al. 2020). Moreover, UV radiation has been shown to inhibit powdery mildew, where plants exposed to sunlight with UV radiation filtered out experienced a 20-40% increase in disease prevalence compared to plants subjected to both sunlight and UV radiation (Austin and Wilcox 2012). Light can also affect virulence in pathogens. For example, pathogens can be less efficient at attaching and rooting to plants in the light due to light-responsive immune functions in the plant host (Roden and Ingle 2009).

For both species, the prevalence of infection advanced over time. This is consistent with the seasonality of powdery mildew in this region, due to decreasing temperatures in late summer/early autumn (Chapter 1). The increased effect of shade in comparison to sun in the mid-summer months is thus likely due to shade acting as microrefugia for powdery mildew spores during the hottest months across the urbanization gradient, with the effect decreasing as the sunlit temperatures decrease towards more tolerable levels.

Contrary to our expectations, the effect of shade on disease prevalence was not more pronounced in urban habitats. Despite this, there is an increased prevalence of powdery mildew infection in urban habitats for *P. rugelii*. Meaning, factors other than temperature and UV exposure are driving increased disease prevalence in urban habitats. A possible factor at play includes increased arrival of spores into urban habitats due to the greater connectivity of urban parks. The increased abundance of roadways which characterize our focal urban parks may
facilitate the transport of spores within and between sites (Numminen and Laine 2020). Increased abiotic stress from pollution and elevated temperature stress may also make urban plants more susceptible to infection. Additionally, it is possible that *Plantago* demonstrates variation in resistance traits across the urbanization gradient, with increased resistance in more rural sites resulting in the infection patterns that we see here.

While previous studies suggest that powdery mildew should be enhanced by shade, effects of shade on plant–pathogen interactions may be scale-dependent. Specifically, in a study of a *Plantago lanceolata* metapopulation in Finland, populations with greater shade coverage (e.g., from forested borders of the habitat patch) had lower likelihood of powdery mildew presence in that population (Jousimo et al. 2014). Such results could indicate that the effect of shade varies with baseline climate in the region, where shade negatively affects pathogen growth in cooler climates but improves growth in hotter climates. Alternatively, such results need not involve a direct effect of shaded microclimates on powdery mildew growth. Instead, landscape features that provide shade (e.g., forests) may block dispersal of mildew spores. A forested landscape might also reflect a more rural setting with less connectivity between pathogen populations (Numminen and Laine 2020).

Understanding how plant–pathogen interactions respond to climate variation is essential for predicting consequences of climate change on wild and cultivated plants. As climate change increases the frequency of extreme heat worldwide, plants in unshaded microhabitats may be especially prone to negative effects of heat stress (Mittler et al. 2012). At the same time, plants that are protected from extreme heat by shade may be vulnerable to fungal pathogens. Thus, predicting the net effects of climate change on plant populations requires resolving the direct and
pathogen-mediated effects of warming, including effects of heat stress on plant immune responses (Wang et al. 2009). Quantifying effects of microclimate on pathogen success also has direct relevance to agriculture. For example, the practice of growing crops beneath solar panels – agrivoltaics – is proving to be an innovative solution for decreasing agricultural water usage and increasing productivity of farms (Barron-Gafford et al. 2019). However, the results of our experiment suggest that the shaded microclimates under solar panels may come with increased risk of some fungal diseases.

In urban settings, plant communities provide an array of ecosystem services including flood and heat mitigation in addition to mental and physical benefits for humans (Kondo et al. 2018). Moreover, urban agriculture provides food and connection to nature, as well as offering numerous economic and environmental benefits (Pearson et al. 2010). Yet very few studies to date have examined how climatic variability in cities impacts prevalence of plant diseases (Egerer et al., 2020). Our study highlights the strong effects of microclimate heterogeneity on patterns of infection prevalence across urban, suburban, and rural land use types. Resolving the role of microclimate effects (e.g., due to patches of shade) within larger-scale climatic trends (e.g., urban heat island effects) can inform strategies for predicting and managing plant diseases in a warming world.

Acknowledgements

We thank Mahal Bugay, Philippa Tanford, and Shayna Rosenbloom for assistance with field work. Mike Dyer and Kim Medley provided greenhouse and hoop house support on the Washington University in St. Louis campus and at Tyson Research Center (TRC), respectively. Susan Flowers provided undergraduate mentoring support through TRC. We thank staff at St.
Louis City Department of Parks, Recreation, and Forestry; Forest Park Forever; Tower Grove Park; Webster Groves Parks and Recreation Department; St. Louis County Parks and Recreation; Kirkwood Parks and Recreation Department; Missouri Department of Conservation; TRC; Missouri State Parks; and Missouri Botanical Garden’s Shaw Nature Reserve for access to field sites.
Chapter Three:
Local adaptation of a fungal pathogen to temperature over a latitudinal gradient
Abstract

Climate change and infectious disease pose simultaneous threats to the health of organisms. Thus, there is a need for understanding how host–pathogen systems respond to climate change, including warmer mean temperatures. One way to assess the potential for pathogens to respond to future climate change is through investigation of phenotypic variation in pathogen thermal performance and tests of pathogen local adaptation to current temperature regimes. Here, we quantified growth of a fungal plant pathogen at several different temperatures and tested whether pathogen strains were locally adapted to temperature or to host genotypes along a latitudinal gradient. To do this, we collected seeds of the host plant Plantago rugelii and isolated strains of its specialist powdery mildew pathogen Golovinomyces sordidus from five locations along a latitudinal transect from southern Mississippi to northern Wisconsin, USA. We then placed sympatric and allopatric pairings of pathogen and host genotypes into seven temperature treatments spanning an a priori estimate of the thermal range of powdery mildew. We found that pathogen strains from more southern/warmer sites had warmer thermal optima, and those from more northern/cooler sites had cooler thermal optima. This pattern is consistent with powdery mildew strains being locally adapted to temperature. However, there was no evidence of pathogen local adaptation to sympatric host genotypes. Our results suggest that wild plant pathogens harbor substantial existing among-strain variation in thermal tolerance. This variation could facilitate pathogen adaptation to a warming world.
**Introduction**

Climate change and infectious disease present simultaneous threats to the health of plants and wildlife worldwide (Chakraborty et al. 2000). Thus, there is an urgent societal need to understand how host–pathogen systems respond to climate change, including warmer mean temperatures (Harvell et al. 2002, Altizer et al. 2013, Chakraborty 2013, Burdon and Zhan 2020). Climate warming is expected to alter the geographic and seasonal occurrence of many diseases, through effects on abundance and physiology of hosts and pathogens (Rohr et al. 2011, Altizer et al. 2013, Claar and Wood 2020, Cohen et al. 2020, Jeger 2022). However, our ability to predict how disease dynamics will change with future warming is limited by our knowledge of the capacity for pathogens to adapt to changing climatic conditions. Moreover, pathogens face selective pressures from hosts as well as from climatic factors. Therefore, it is essential to establish whether pathogen adaptation to climate regimes is robust across standing variation in host resistance.

Within a species, pathogen strains can vary in their thermal tolerance and optima (Kowalski and Bartnik 2010, Voyles et al. 2017). Where temperatures are below optimum for growth of a pathogen on its host, warming should increase disease risk (Cohen et al. 2020). By the same logic, warming should decrease disease if temperatures are already at or above optimum for pathogen growth on the host (Faticov et al. 2022). Pathogens can evolve to become locally adapted to their abiotic environment if selective pressures act on standing genetic variation to select for individuals most suited to those environmental conditions (Kawecki and Ebert 2004). Pathogen local adaptation to environments can be detected as genotype-by-environment (G x E) interactions, with better performance in local than foreign environments. In
some systems, pathogens are adapted to their local temperature regimes (Laine 2008, Stefansson et al. 2013), with thermal optima that are a good match to mean temperatures in their local environment (Mariette et al. 2016). However, fungal pathogens do not always adapt to increases in temperature (Schampera et al. 2022).

At the same time, many host–pathogen systems exhibit specificity as genotype-by-genotype (G x G) interactions. In such systems, strong selection for pathogen infectivity and host resistance causes genetically variable pathogens and their hosts to engage in reciprocal adaptation and counter-adaptation. Given enough time to coevolve, pathogen populations can become locally adapted to their host populations, meaning that infections are more likely in sympatric (local) host-pathogen pairings than allopatric (novel) pairings (Lively and Jokela 1996, Kawecki and Ebert 2004, Höckerstedt et al. 2018). Local adaptation is most likely to occur when pathogen populations have high levels of genetic diversity, and thus high potential for novel genotypes that can overcome host resistance (Höckerstedt et al. 2018). Typically this requires the pathogen to have short generation times, large populations, high mutation rates, and moderate gene flow, all which aid in increasing genetic diversity in a population (Gandon et al. 1996, Höckerstedt et al. 2018). Indeed, many pathogens are found to be more infective on their sympatric (local) than allopatric (foreign) hosts (Lively and Jokela 1996, Thrall et al. 2002, Kawecki and Ebert 2004, Laine et al. 2011, Koskella 2014). Yet, this is not always the case, even for specialist pathogens that should be under strong selection to infect local hosts (Höckerstedt et al. 2018).

Importantly, the performance of pathogen life history traits can depend on interactions between pathogen genotype, host genotype, and environmental conditions including temperature
(Wolinska and King 2009, Penczykowski et al. 2016). With so-called genotype-by-genotype-by-environment (G x G x E) interactions, a pathogen that is adapted to local host genotypes under a given climate regime might not be locally adapted to its hosts under warming. Similarly, conclusions about pathogen local adaptation to climate can depend on host genotype. Furthermore, increasing temperatures may lead to temporal shifts in host development and susceptibility to infection. If the duration of host vulnerability is reduced by climate change, pathogens may be under selection for shifts in their thermal tolerance or for increased virulence in order to capitalize on the diminishing period of host vulnerability (St. Leger 2021). Increased virulence, however, can come at a cost to other pathogen fitness traits such as spore production (Laine and Barrès 2013). For multiple different fungal pathogens, virulent strains have been found to produce less spores than avirulent strains (Thrall and Burdon 2003, Bahri et al. 2009, Fraile et al. 2011). Yet the fitness costs of virulence may be offset by competitive advantages under certain temperatures. For example, a study of two virulence loci in a stem canker fungus of a crop plant (oilseed rape) found that one locus incurred a generally higher cost of virulence (i.e., greater loss of fitness in isolate with virulent relative to avirulent allele at that locus) compared to the other locus (Huang et al. 2010). However, the higher cost of virulence for that locus was offset by a fitness advantage for that isolate under warmer temperatures (Huang et al. 2010). Given that the relative fitness of pathogen isolates informs deployment of resistance genes in agricultural systems, this example underscores the importance of understanding how host-pathogen genotypic interactions are altered by temperature.

Here, we tested for local adaptation of pathogens to thermal regimes and host genotypes over a latitudinal gradient spanning large variation in mean annual temperature. We used an
experimentally tractable system comprised of the herbaceous host plant *Plantago rugelii* and its *Plantago*-specific powdery mildew, *Golovinomyces sordidus*, collected along a latitudinal gradient in North America (Penczykowski and Sieg 2021). We expected that pathogen performance (measured as spore production) would vary with temperature according to unimodal thermal performance curves, such that a thermal optimum could be estimated for each pathogen strain. We hypothesized that pathogen genotypes would exhibit local adaptation to temperature, where pathogen strains from more southern locations would have warmer thermal optima and strains from more northern locations would have cooler thermal optima. Additionally, we hypothesized that pathogen genotypes would be locally adapted to host populations, with more growth on sympatric than allopatric host genotypes.

**Materials and Methods**

**Study system**

Our focal host plant is the rosette-forming herb *Plantago rugelii* (blackseed plantain). This short-lived perennial grows commonly in pastures and human-disturbed landscapes (e.g., along paths or roads and in mowed open areas) over much of eastern North America (Penczykowski and Sieg 2021). Our focal pathogen is the specialist powdery mildew fungus *Golovinomyces sordidus* (L. Junell) V.P. Heluta (Braun and Cook 2012). Powdery mildews (order Erysiphales) are obligate pathogens which extract nutrients from the epidermal tissue of their host. Powdery mildew infections are visually conspicuous due to the chains of asexual spores produced from mycelium on the leaf surface which give infected leaves a characteristic white, dusty appearance. Spores are transmitted passively via wind (Tack and Laine 2014). More than 90% of spores land within 2 meters of their host plant; however, rare instances of long-
distance spore transport allow pathogens to spread over a regional scale (Ovaskainen and Laine 2006, Tack and Laine 2014). Mildews survive winter by producing protective sexual resting structures (chasmothecia) which release ascospores when conditions become favorable in the spring (Tack and Laine 2014).

Field populations

Plantago seeds and powdery mildew spores used in this study were collected from field populations spanning a latitudinal gradient in the central US (Fig. 3.1). We collected P. rugelii seeds from healthy-looking plants in each of five populations: McComb, MS ("Far South", FS), Memphis, TN ("Near South", NS); St. Louis, MO ("Central", C); Morrison, IL ("Near North", NN); and Altoona, WI ("Far North", FN); a span of over 1,500 km (Fig. 3.1). Leaves infected with Golovinomyces sordidus were collected from the same populations as the seeds, with the exception of the Central host and pathogen genotypes, which were collected 18 km apart.

We used NOAA climate data from weather stations nearest our field populations to characterize average daily temperature during the typical powdery mildew growing season (summer-autumn) between 2012-2022 (Fig. 3.1; data from NOAA Climate Data Online). The peak average daily temperatures at our field sites ranged from 29.2 °C in the Far South to 26.2 °C in the Near North (Fig. 3.1).
Fig. 3.1. Locations and average temperatures of sampled host and pathogen populations. **Left panel:** Populations sampled for *Plantago rugelii* (host) and *Golovinomyces sordidus* (pathogen) genotypes used in our experiment. **Right panel:** Mean (solid line) and standard error (ribbon) in average daily temperature between June 1 and October 31 over the 10-year period from 2012-2022 at weather stations (labelled with city names) nearest our sampled populations. Temperature data from NOAA (NOAA and NCEI 2023).

*Plant and mildew preparation*

Plants used in this experiment were grown from seeds collected from wild *Plantago* populations between 2020-2022. We used a single maternal line per population, where each maternal line consists of a single seed spike from a mother plant. *Plantago rugelii* seeds require cold stratification to increase germination success (C. L. Goodson, unpublished). Therefore, seeds were placed between layers of wet filter paper in Petri dishes in a 6-7 °C incubator for two weeks in August 2022. Seeds were then moved to a 20 °C incubator for 10 days to allow
germination. In a greenhouse, seedlings were initially established in FPX B soil (ProMix), and
two weeks later transplanted into individual 4” pots in BM6 All-Purpose soil (Berger). Each pot
was covered with an autoclaved, spore-proof pollination bag (PBS International, model 3D.55)
to avoid unintentional powdery mildew infection in the greenhouse.

Mildew lines were collected in 2020-2022 by detaching infected leaves from wild host
plants using ethanol-sterilized forceps. Infected leaves were placed into Petri dishes containing
wetted filter paper. Upon return to the lab, spores from the field-collected leaves were brushed
onto healthy leaves detached from greenhouse-grown plants using a sterilized paintbrush. We
isolated pure clonal lines ("strains") by transferring a single chain of asexual spores from an
infected to a healthy leaf for three successive generations (~10 days each) (Nicot et al. 2002).
The mildew strains were maintained in growth chambers at 20 °C, 16:8 hours light:dark, and
70% relative humidity (Nicot et al. 2002).

*Experimental setup*

Individual leaves were removed from the source plants, cut into 2-cm fragments and
surface-sterilized using 70% ethanol. Sterilized leaf fragments were placed in Petri dishes, so
that each dish contained one leaf piece from each of the five host maternal lines (Fig. D.1). On
10-November-2022, each dish was inoculated with a single mildew strain, such that each plate
contained one sympatric pairing and four allopatric pairings (Fig. D.1). To inoculate, we used a
single sterilized paintbrush hair to transfer six chains of spores from source mildew lesions onto
the center of each leaf piece. The plates of inoculated leaves were placed in growth chambers at
each of seven temperature treatments, with three replicate plates of each mildew strain at each
temperature (n = 525 leaf pieces total). We chose the growth chamber temperatures of 7, 12, 16, 20, 24, 28, and 33 °C to span the typical thermal range for powdery mildew sporulation, where the average thermal minimum (T_{\text{min}}), thermal maximum (T_{\text{max}}), and thermal optimum (T_{\text{opt}}) estimated for several other mildew species are T_{\text{min}} = 11.9 °C, T_{\text{max}} = 31.2 °C, and T_{\text{opt}} = 21.6 °C (Chaloner et al. 2020). Chambers were kept at 16:8 hours light:dark and 60-70% humidity throughout the experiment to promote mildew growth.

**Assessment of pathogen performance**

Inoculated leaves were examined for progression of asexual growth under a dissecting microscope every other day for 14 days post-inoculation (DPI). Due to a complete lack of mildew growth in the 33 °C treatment, leaves in that treatment were no longer examined after 10 DPI. We quantified mildew growth using a modified five-level categorical index of mildew development ("Bevan score"), where 0 = no growth, 1 = mycelium only, 2 = mycelium and sparse sporulation visible only under a dissecting microscope, 3 = abundant sporulation and lesion size < 0.5 cm$^2$, and 4 = abundant sporulation and lesion size > 0.5 cm$^2$ (Bevan et al. 1993, Numminen and Laine 2020).

**Statistical analyses**

All statistical analyses were performed in R version 4.1.0 (R Core Team 2021). To analyze the number of days until sporulation for each unique leaf piece, we used a Cox proportional hazard model (function coxph() in 'survival' package; Therneau 2023). We modeled the days until sporulation in response to host genotype, pathogen genotype, temperature
treatment, and their interactions. Additionally, we tested for a correlation between minimum
days until sporulation and maximum Bevan score across replicates for each mildew × host
pairing that successfully achieved sporulation (i.e., for the n =153 leaf pieces that achieved
Bevan score ≥ 2) using the function corTest() in 'MASS' package (Venables and Ripley 2002).

A cumulative link model (CLM) was used to analyze the ordered categorical response
variable of maximum Bevan score for each leaf in response to host genotype, pathogen genotype,
temperature treatment, and their interactions at 14 DPI (function clm() in 'ordinal' package
(Christensen 2022)). First, a model containing all interactions was fit, then reduced models
lacking one or more interactions were fit for comparison (Table 3.3). Models were compared
using Akaike’s information criterion (AIC), and the best fitting model was selected using
function aictab() from the 'AICcmodavg' package (Mazerolle 2020).

For each mildew strain, Thermal Performance Curves (TPCs) were fit to the maximum
Bevan score (at 14 DPI) among all replicates of all host genotypes in each temperature treatment.
We compared the fits of multiple models commonly used for fitting fungal TPCs (Dumur et al.
1990, Angilletta 2006, Mastrodimos et al. 2019, Omuse et al. 2022). Models were fit using the
'rTPC' package pipeline which uses the 'nls.multstart' package (Padfield and Matheson 2020,
Padfield and O’Sullivan 2021). Models were compared using AIC, and the best fitting model
was selected using function aictab() from the 'AICcmodavg' package (Mazerolle 2020).

For the best-fitting model, we performed bootstrapping (n = 999 resamplings) to generate
95% confidence intervals for each curve and to estimate average temperature optima (Padfield
and O’Sullivan 2021). Then we tested whether thermal optima were significantly negatively
related to latitude (i.e., cooler thermal optima for more northern mildew strains), using a
bootstrapped linear regression approach (Shocket et al. 2018). We did this by sampling one of the 999 bootstrapped estimates of thermal optima for each of the five mildew strains and fitting a linear regression between those five bootstrapped estimates and their corresponding latitudes of origin. This produced 999 slopes, and we calculated the fraction of those slopes that were less than zero (null hypothesis for this one-sided hypothesis test: > 5% of slopes greater than zero).

Results

Time to sporulation

Temperature had a significant effect on time to sporulation (P = 0.004), with generally faster sporulation at warmer temperatures (up to 24 °C; Table 3.1 and Table 3.2; Fig. 3.2). Very little sporulation occurred at the lowest (7 °C) or second-highest (28 °C) temperatures, and no sporulation occurred at the highest temperature of 33 °C (Fig. 3.2). Time to sporulation also depended on host genotype and mildew genotype, but not on their interaction (Table 3.2). There were also no significant interactions between either host or mildew genotype and temperature (Table 3.2). For leaves on which mildew successfully sporulated, there was a significant correlation between days until sporulation and the maximum Bevan score achieved (t = -11.48, df = 151, P < 0.0001).

Table 3.1. Comparison of Cox proportional odds models explaining minimum days until sporulation. The best performing model is bolded.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>AICc</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 5 Temperature + Host genotype + Mildew genotype</td>
<td>1848.74</td>
<td>0.00</td>
</tr>
<tr>
<td>Model 2 Temperature × Mildew genotype + Host genotype</td>
<td>1854.91</td>
<td>6.17</td>
</tr>
<tr>
<td>Model 3 Temperature + Mildew genotype × Host genotype</td>
<td>1867.86</td>
<td>19.11</td>
</tr>
<tr>
<td>Model 4 Temperature + Host genotype × Mildew genotype</td>
<td>1867.86</td>
<td>19.11</td>
</tr>
<tr>
<td>Model 1 Temperature × Mildew genotype × Host genotype</td>
<td>1915.22</td>
<td>66.47</td>
</tr>
</tbody>
</table>
Table 3.2. Analysis of deviance table for days until sporulation for each individual leaf.

<table>
<thead>
<tr>
<th></th>
<th>D.F</th>
<th>Chi-sq</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host genotype</td>
<td>4</td>
<td>17.93</td>
<td>0.001</td>
</tr>
<tr>
<td>Mildew genotype</td>
<td>4</td>
<td>10.78</td>
<td>0.03</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>8.28</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Fig. 3.2. Days until pathogen sporulation for each host and pathogen genotype pairing in each temperature treatment. The minimum number of days between inoculation and sporulation among three replicate leaves for each host-pathogen-temperature combination is shown here, while each individual leaf is included in the corresponding statistical model (Table 3.2).

Maximum Bevan score

There was a significant interactive effect of temperature treatment and mildew genotype on the maximum amount of mildew growth, as well as a main effect of mildew genotype (Tables 3.3 and 3.4; Fig. 3.3). However, there were no significant main or interactive effects involving host genotype (Table 3.4; Fig. 3.3). All but the Far South strain achieved some growth at the lowest temperature (7 °C). By contrast, only the Far South strain grew at 28 °C. No strains grew at 33 °C.
Table 3.3. Comparison of CLM models explaining maximum Bevan score at 14 DPI. The best performing model is bolded.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>AICc</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 2: Temperature × Mildew genotype + Host genotype</td>
<td>1077.82</td>
<td>0.00</td>
</tr>
<tr>
<td>Model 4: Temperature + Host genotype × Mildew genotype</td>
<td>1078.53</td>
<td>0.72</td>
</tr>
<tr>
<td>Model 3: Temperature + Mildew genotype × Host genotype</td>
<td>1098.06</td>
<td>20.25</td>
</tr>
<tr>
<td>Model 1: Temperature × Mildew genotype × Host genotype</td>
<td>1142.87</td>
<td>65.06</td>
</tr>
</tbody>
</table>

Table 3.4. Analysis of deviance table for CLM model of maximum Bevan score for each individual leaf.

<table>
<thead>
<tr>
<th></th>
<th>D.F.</th>
<th>Chi-sq</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host genotype</td>
<td>4</td>
<td>4.61</td>
<td>0.33</td>
</tr>
<tr>
<td>Mildew genotype</td>
<td>4</td>
<td>139.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>3.55</td>
<td>0.06</td>
</tr>
<tr>
<td>Mildew × Temperature</td>
<td>4</td>
<td>20.19</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Fig. 3.3. Maximum amount of asexual pathogen growth (Bevan score) for each host and pathogen genotype pairing in each temperature treatment. The maximum value among three replicate leaves for each host-pathogen-temperature combination is shown here, while each individual leaf is included in the corresponding statistical model (Table 3.4).
**Thermal Performance Curves**

The thermal responses of all five mildew strains were best described by a Gaussian model (Table 3.5). The average thermal optima of the mildew genotypes were: Far South = 20.6 °C, Near South = 18.4 °C, Central = 18.1 °C, Near North 16.7 °C, Far North = 17.1 °C (Fig. 3.4). These thermal optima were significantly negatively related to latitude (median slope = -0.25; p = 0.001 based on 998/999 bootstrapped linear regressions having negative slopes; Fig. 3.5).

**Table 3.5.** AIC model comparisons for TPCs. The best performing model is bolded.

<table>
<thead>
<tr>
<th>Pathogen genotype</th>
<th>Model</th>
<th>AICc</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Far South</td>
<td>Gaussian</td>
<td>41.46</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Brière</td>
<td>77.74</td>
<td>36.28</td>
</tr>
<tr>
<td></td>
<td>Ratkowsky</td>
<td>79.27</td>
<td>37.81</td>
</tr>
<tr>
<td></td>
<td>Weibull</td>
<td>82.35</td>
<td>40.89</td>
</tr>
<tr>
<td>Near South</td>
<td>Gaussian</td>
<td>44.40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Weibull</td>
<td>82.16</td>
<td>37.76</td>
</tr>
<tr>
<td></td>
<td>Ratkowsky</td>
<td>83.89</td>
<td>39.49</td>
</tr>
<tr>
<td></td>
<td>Brière</td>
<td>91.15</td>
<td>45.76</td>
</tr>
<tr>
<td>Central</td>
<td>Gaussian</td>
<td>42.22</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Weibull</td>
<td>80.65</td>
<td>38.43</td>
</tr>
<tr>
<td></td>
<td>Ratkowsky</td>
<td>81.70</td>
<td>39.48</td>
</tr>
<tr>
<td></td>
<td>Brière</td>
<td>91.97</td>
<td>49.75</td>
</tr>
<tr>
<td>Near North</td>
<td>Gaussian</td>
<td>41.96</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Weibull</td>
<td>78.97</td>
<td>37.00</td>
</tr>
<tr>
<td></td>
<td>Ratkowsky</td>
<td>81.81</td>
<td>39.85</td>
</tr>
<tr>
<td></td>
<td>Brière</td>
<td>92.95</td>
<td>50.98</td>
</tr>
<tr>
<td>Far North</td>
<td>Gaussian</td>
<td>39.74</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ratkowsky</td>
<td>79.24</td>
<td>39.51</td>
</tr>
<tr>
<td></td>
<td>Weibull</td>
<td>79.87</td>
<td>40.13</td>
</tr>
<tr>
<td></td>
<td>Brière</td>
<td>90.66</td>
<td>50.93</td>
</tr>
</tbody>
</table>
Fig. 3.4. Thermal performance curves for each mildew genotype. Gaussian models (curves) were fit to pathogen growth data measured at each of seven temperatures (points).Ribbons give bootstrapped 95% confidence intervals and a dashed vertical line indicates the thermal optimum for each pathogen genotype.

Fig. 3.5. Evidence of pathogen local adaptation to temperature, using latitude as a proxy for local thermal regime. **Left panel:** Linear regression (black line) fit to the estimated thermal optimum for each pathogen genotype (color-coded points), with 999 bootstrapped linear regressions (gray lines). **Right panel:** Histogram of slopes of the 999 bootstrapped linear regressions.
Discussion

Predicting consequences of climate change for infectious diseases remains a formidable challenge in most host-pathogen systems. Even when the many facets of climate change are simplified to focus on warming, there are still complex pathways by which warming can impact all three corners of the disease triangle. Here, we performed a laboratory experiment to specifically test for interactions among host genotypes, pathogen genotypes, and temperatures, using Plantago host plant lines and powdery mildew pathogen strains collected from populations along a latitudinal gradient spanning wide variation in mean temperature during the growing season. We applied the temperature treatment to detached leaf pieces immediately after inoculation with the powdery mildew fungus; therefore, our study did not address longer-term temperature effects on growth or susceptibility of the plants themselves. However, we were able to quantify how pathogen strains varied in their thermal performance and to test for local adaptation of pathogen strains to temperature regimes and host genotypes.

As expected, each pathogen genotype had a unimodal response to temperature, with slower growth at cooler temperatures, an increase in growth over the middle range of temperatures, and a sharp decline in growth in the hottest treatments. The temperature range used in our experiment encompassed the upper thermal limits for mildew growth, as no mildew strains grew at 33 °C (and only one grew at 28 °C). Based on thermal constraints documented for other powdery mildew species, we predicted that our lowest temperature treatment would be too low for mildew growth (Chaloner et al. 2020). However, for four of the five mildew strains, we observed some mildew growth (though not necessarily development through to sporulation) at our lowest temperature treatment. Thus, we have less confidence in the ability of our fitted
thermal performance curve models to estimate the lower thermal limits for growth of these pathogens. Future experiments with additional colder temperature treatments are needed to assess among-strain variation in lower thermal limits.

In support of our first hypothesis, we found evidence of pathogen local adaptation to regional temperatures. Strains from more southern/warmer populations had higher thermal optima, and strains from more northern/cooler populations had lower thermal optima. There was a 3.9 °C difference between the highest and lowest thermal optima (Far South and Near North, respectively). Notably, the Far South mildew strain was the only one to successfully grow at the highest survivable temperature (28 °C), and also the only one that failed to establish in the coldest treatment (7 °C). While this pattern might be driven by natural selection for thermal optima closer to average temperatures, it is also possible that the observed differences in thermal performance are the legacy of other neutral or adaptive processes. Future analysis of population genetic structure and genotype-environment associations will be helpful for resolving the causes of the observed pattern of local adaptation.

Contrary to our second hypothesis, there was no evidence of local adaptation of pathogen strains to their sympatric host lines. Indeed, there was not even a significant main effect of host genotype nor interaction between host genotype and mildew genotype (nor was there a three-way interaction between host genotype, mildew genotype, and temperature). Speed of sporulation did vary significantly between host genotypes as well as between mildew genotypes, but there was also no interaction between host and mildew genotype for this pathogen life history trait. The lack of a pattern of local adaptation is perhaps not surprising, given that host-pathogen coevolution is a dynamic process, and a given pathogen population could be locally adapted to
their hosts in one year, but not the next (e.g., due to parasite-mediated selection on hosts or stochastic overwintering or dispersal events). Moreover, due to constraints on mildew and *Plantago* seed availability, we used host lines and pathogen strains that had been collected from field populations over the span of a few years (i.e., our sympatric host and mildew pairings were not always contemporary). However, the lack of a significant interaction between host and mildew genotypes is surprising, given that powdery mildews tend to show genotypic specificity in their interaction with a congeneric host, *Plantago lanceolata*, in its native range of Europe (Laine 2004). Further research on genotypic and phenotypic variation of *Plantago rugelii* in eastern North America (where it is endemic) will be necessary for explaining the apparent lack of specificity observed here.

Plant disease epidemics have the potential to cause significant ecological, agricultural, and economic damage – with disease outbreaks being one of the foremost challenges to achieving global food security (Velásquez et al. 2018). The environmental conditions in which a host and its pathogens interact has a significant impact on the outcome of the relationship. Understanding and predicting impacts of climate change on plant-pathogen interactions is therefore a huge societal challenge. Here, we documented among-strain variability in thermal performance of a wild plant pathogen and a pattern of pathogen local adaptation to temperature along a latitudinal gradient. For pathogens with high potential for dispersal -- including wind-dispersed fungi such as powdery mildews -- this existing variation in thermal tolerance could facilitate adaptation to future warming.
Acknowledgements

We thank Cheyenne Morris, Lily Goldberg, and Allison Rea for assistance with lab and greenhouse work. Mike Dyer provided greenhouse support. We thank Marta Shocket for advice on statistical analysis of bootstrapped parameter estimates.
Conclusions and Future Directions

Plant disease epidemics have the potential to cause significant ecological, agricultural, and economic damage and are one of the foremost challenges to achieving global food security (Velásquez et al. 2018). Amid global climate shifts and expanding urbanization, it is increasingly important to understand the effects of human mediated changes on plant systems. In this thesis I investigate how environmental and climatic variation at various spatial scales impact the ecological and evolutionary relationship of a plant host – natural enemy system. Specifically, I investigate the relationships between Plantago and specialized fungal pathogens and generalist herbivores. This work contributes to the developing field of urban ecology by joining a small body of literature focused on the effects of urbanization and temperature change on plant disease/pest dynamics (van Dijk et al. 2022). Below, I outline each Chapter and comment on future directions for this work.

Chapter One

Chapter One uses the St. Louis metropolitan area to observationally quantify differences in the timing and prevalence of powdery mildew infection, herbivory damage, and mowing across the urbanization gradient. To do this I conducted monthly surveys of 22 populations of Plantago lanceolata and P. rugelii in parks in summer-autumn 2019 and 2020. Results showed that early-summer herbivory on both plant species was accelerated in urban sites. I also found that mildew epidemics on P. rugelii started earlier and reached a higher prevalence in more urban sites. However, there were generally low levels of infection on P. lanceolata, and no significant differences in epidemic dynamics across the urbanization gradient. Additionally, I conducted a
field and a laboratory experiment to investigate any variability in susceptibility to disease in this
system. First, I placed potted “sentinel” plants into field populations to directly measure infection
and herbivory rates while controlling for host genotype. I found consistent results: *P. rugelii*
sentinels only became infected in suburban and urban sites, and there was less infection on *P.
lanceolata* sentinels, suggesting low availability of pathogen genotypes that are able to infect
*P. lanceolata*. Lastly, I performed an infection assay to experimentally test the susceptibility of *P.
rugelii* genotypes from across the urbanization gradient. I found equal susceptibility of urban,
suburban, and rural plant genotypes to powdery mildew, suggesting that the patterns I observed
across the urbanization gradient were not driven by differences in host resistance.

Chapter Two

Chapter Two explores the interaction of Urban Heat Island effect and within-site
microclimate variability on the prevalence of powdery mildew infections. I hypothesized that
shaded microclimates would have higher prevalences of mildew infection due to lower UV
levels and temperatures than sunlit areas. Additionally, I hypothesized that shade would be more
important for mildew growth in more urban areas due to the combined stress of sunlit
temperatures and increased temperatures from Urban Heat Island Effect. This Chapter uses data
collected from monthly surveys in the St. Louis metropolitan area, as well as fine scale
observational surveys within St. Louis City. Together, these field survey results show strong
positive effects of shade on mildew infection in wild *Plantago* populations across urban,
suburban, and rural habitats. To verify that this pattern is due to microclimate differences, I
conducted a manipulative field experiment to directly quantify the effect of shade and sun
exposure on mildew development. Microclimate conditions associated with shade promoted pathogen growth, suggesting a causal relationship between shade and infection success. Contrary to the second hypothesis however, the field surveys showed that there was no significant interaction between urbanization level and shade presence, meaning other factors are driving the patterns we see of increased disease development in more urban areas.

Chapter Three

Chapter Three investigates variation in the performance of different powdery mildew strains, and tests whether the pathogens are locally adapted to temperature or their sympatric *P. rugelii* host plants along a latitudinal gradient. To do this, I performed a laboratory inoculation experiment using *P. rugelii* host lines and powdery mildew strains sampled from sites spanning a ~600-km latitudinal gradient. I placed sympatric and allopatric pairings of pathogen and host genotypes into seven temperature treatments representing an approximation of powdery mildew’s thermal tolerance range. Powdery mildew strains were found to be locally adapted to temperature, with higher thermal optima for strains from more southern/warmer sites, and lower thermal optima for strains from more northern/cooler sites. However, there was no evidence of pathogen local adaptation to sympatric hosts. With nearly 4 °C difference between thermal optima of northern and southern mildew strains, these results point to substantial existing variation in pathogen thermal tolerance phenotypes that, given the high dispersal abilities of wind-dispersed powdery mildew spores, may facilitate future adaptation to climate change.
Synthesis

Results from all three chapters can help explain the patterns of infection observed across the urbanization gradient in Chapter One, in which *P. rugelii* experienced earlier and larger powdery mildew epidemics in more urban environments. One possible explanation for this pattern is that rural plant genotypes have greater resistance to powdery mildew compared to urban plants. However, in Chapter One I show experimentally that plant lines collected from across the urbanization gradient are equally susceptible to infection. Thus, it is likely that the observed differences in infection prevalence are instead due to other factors, which could include environmental effects and differences in exposure to pathogen spores.

In Chapters One and Two, I found opposing relationships between temperature and infection at different scales. There were larger epidemics in more urban/warmer populations, while plants in cooler, shaded microhabitats are significantly more likely to be infected. Multiple additional studies will be required to resolve this paradox. For example, one unexplored hypothesis is that urban sites may have warmer winters which promote overwintering success of powdery mildew. This could result in more persistence of infections within urban sites across years. Testing this hypothesis will require future genotyping of powdery mildew samples that I collected in all populations in autumn 2019 and 2020 (the years featured in Chapters One and Two) as well as in autumn 2021 and 2022. Additionally, future experiments could track overwinter survival of infections on plants experimentally infected across the urbanization gradient. Whether the mildew overwinters in place or disperses into populations early in spring, warmer spring temperatures in more urban sites could accelerate early-season growth of powdery mildew, leading to larger epidemics overall.
Once mildew is established, cooling effects of shade may allow infections to persist when temperatures exceed the upper thermal limit for mildew growth in summer. Notably, temperatures in the St. Louis region in summer are frequently above the upper thermal limits for growth that I documented in Chapter Three. The Chapter Two field experiment manipulating shade demonstrated that shade itself promotes mildew growth, even after controlling for host genotype, water availability, and exposure to mildew spores. This experiment was essential because the observation of greater infection prevalence in the shade in our field surveys could also have been driven by other factors correlated with the microhabitat location. For example, plants in shade may have increased rates of encounter with pathogen spores due to altered wind turbulence caused by the tree or other structure casting shade. Plants in shade may also be located at the base of a tree, fence, or building, that is routinely missed by lawn mowers. This reduced frequency of disturbance from mowing could allow infected leaves to persist for longer, promoting more sporulation and fine-scale transmission in the shade. Finally, there could also be genetic differences between plants in different microhabitats that lead to variable infection rates. Shade itself is also not a single variable, as it involves lowered temperature and UV exposure. As both high temperatures and UV are known to reduce survival of exposed fungi, both of these facets of shade could be underpinning our observational and experimental findings. Future experiments individually manipulating temperature, light, and UV will be required to untangle their separate contributions.

Evolutionary forces may also have contributed to the pattern of greater infection prevalence in more urban populations. In Chapter Two, I found that shade was not increasingly important in the warmer urban areas, despite baseline temperatures being warmer in more urban
areas. This could mean that powdery mildew strains have adapted to temperature across the
urbanization gradient, where urban mildew strains have higher thermal optima. In Chapter Three,
I show that *P. rugelli* are locally adapted to temperature across a much larger latitudinal scale. To
determine if pathogen genotypes are locally adapted to temperature over the smaller scale of the
urbanization gradient, an experiment similar to that in Chapter Three could be conducted using
host and pathogen genotypes collected from across the urbanization gradient.

Another possible driver of larger epidemics in more urban sites could be increased
population connectivity with greater urbanization. The urban sites in this study are characterized
by their proximity to a high density of roadways. Roadways – specifically air movement due to
car traffic – have previously been found to aid in the spread of powdery mildew spores
(Numminen and Laine 2020). For roadside weeds such as *Plantago*, even short-distance
dispersal along roadside host plants could result in substantial dispersal between urban habitats
over the course of a summer. Contrastingly, the only other study exploring the effect of
urbanization on plant disease found nonsignificant and negative relationships between
connectivity and infection prevalence between years (van Dijk et al. 2022). Future directions
should include aerial spore sampling and genotyping of mildew infections across the
urbanization gradient to provide insights into the connectivity of pathogen populations within
and between land use types. It should be noted that these connectivity patterns may not apply to
other modes of disease transmission. While wind-dispersed pathogens may benefit from roads
for spore dispersal, modes of disease transmission that are not dependent on air movement, such
as vector-borne diseases of mammals, may be hindered by roadways (Oxley et al. 1974, Long et
al. 2010).
Finally, other unmeasured abiotic stress associated with urban environments may be driving the trends we see. Abiotic stress such as exposure to long- and short-term extreme temperatures and drought may impact plant responses to pathogen challenge (Desaint et al. 2021, Sunarti et al. 2022). Plants exposed to exceedingly hot microclimates may display increased susceptibility to powdery mildew exposure. Increased susceptibility to infection in urban and fully sunlit conditions could explain the patterns seen in Chapters One and Two. An avenue for future exploration includes experimentally studying the effect of various long- and short-term abiotic stresses on a plant’s susceptibility to infection in both lab and field conditions. However, as demonstrated in Chapter Three, powdery mildew must also be able to survive environmental stress to successfully infect a plant. Therefore, the realized amount of disease will depend on the impact of the given stressor on both host and pathogen.

**Differences between co-occurring host species**

Infection prevalence on *P. lanceolata* was low in comparison to that for co-occurring *P. rugelii* across the urbanization gradient. Even at the within-site level, there were lower rates of infection on wild *P. lanceolata* compared to *P. rugelii*, despite the fact that both species are known to be infected by *Golovinomyces sordidus* in the St. Louis region (Goodson et al., unpublished). The results from Chapter One suggest species-specificity of the pathogen strains, and lower regional abundance of spores available to infect *P. lanceolata*. Genotyping of mildew infections on both host species will reveal the degree of species specificity in this region. Additionally, an infection assay testing the susceptibility of *P. lanceolata* lines from across the
urbanization gradient to powdery mildew strains (i.e., as in the Chapter One inoculation assay) would be informative.

**Replication**

We are still far from understanding the effect of urbanization on species interactions. The generalizability of the findings in this thesis are limited by the lack of geographic replication, as only a single transect was studied. However, the observational urbanization surveys featured in Chapters One and Two have continued annually since 2019 and are planned to continue for many years. Continuing these surveys over an extended timeframe in the same region will give us a deeper understanding of how *Plantago*-powdery mildew dynamics change across the focal urbanization gradient, growing seasons, and years. Future studies including additional transects in the St. Louis region (e.g., a North-South transect to complement the existing East-West transect) and transects across other metropolitan areas will be critical to understand the generalizability of the mechanisms underlying our results. Repeating studies similar to those in Chapters One and Two in additional cities of varying climates will inform how effects of urbanization (including but not limited to the Urban Heat Island effect) vary across different baseline climates. Previously, the effect of urbanization on plant reproductive phenology has been shown to depend on regional climate (Li et al. 2019). Specifically, acceleration of phenology due to urban warming was more pronounced in regions with cooler baseline regions (Li et al. 2019). Similarly, the pattern of increased disease prevalence in more urban areas seen here may be magnified in colder cities due to the Urban Heat Island effect raising ambient
temperatures closer to the pathogen’s thermal optima. Contrasting, cities in warmer climatic regions may become too hot for growth of fungal pathogens like powdery mildews.

*Application to climate change*

As effects of temporal climate change are difficult to observe, spatial gradients in temperature are often used as a proxy to study ecological processes across climate variation (De Frenne et al. 2013). My thesis shows that plant disease dynamics change dramatically over an urbanization gradient that is correlated with increased temperatures. However, because many other abiotic and biotic factors also change with urbanization, the link to climate itself must be investigated further. Moreover, my studies highlighted a paradox wherein disease risk can increase with temperature at one spatial scale (i.e., larger epidemics in more urban populations that are warmer) but decrease with increasing temperatures at another spatial scale (i.e., less disease in the sun compared to shade throughout the urbanization gradient). Taken together, these results illustrate that predictive models of climate change impacts on disease must account for fine-scale microclimate heterogeneity. Finally, my thesis provides evidence of wide variation in thermal optima among strains of a single pathogen species, and current local adaptation of pathogen strains to temperature along a latitudinal gradient. These results suggest that pathogens may be able to adapt to shifting temperatures under continued climate change.

To help to determine the generalizability of the findings laid out here, but it would help to establish how host-pathogen dynamics may change given the surrounding climate. It is possible that the pattern seen here, of increased disease prevalence with increasingly urban areas, may be magnified in colder cities due to Urban Heat Island effect raising ambient temperatures to those
closer to the pathogen’s thermal optima. Contrastingly, cities with generally warmer climates may become too hot to establish mildew population.

Spatial temperature gradients can also be used as proxies for temporal temperature shifts, thus these results can be applied to possible future patterns of climate change (De Frenne et al. 2013). Jointly, these results show how climatic clines impact the ecological and evolutionary processes of a plant-enemy system. Increased urban temperature likely play a role in the earlier and greater powdery mildew epidemics on *P. rugelii*, indicting possible future increased fungal pathogen epidemics, given continued climate change. Additionally, these results show the ability of pathogens to adapt to their climates across a regional scale.
## Appendices

### Appendix A: Supplement to Chapter One

Table A.1. Site names, locations (in Missouri, USA), management, site type classifications, and survey years in our study.

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Site name</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Municipality</th>
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<th>Site type</th>
<th>Years</th>
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<td>City</td>
<td>Type</td>
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<td>2019-20</td>
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**Site managers:**

- a. Missouri Botanical Garden;
- b. Missouri State Parks, a division of the Missouri Department of Natural Resources;
- c. St. Louis County Parks and Recreation;
- d. Tyson Research Center, Washington University in St. Louis;
- e. Missouri Department of Conservation;
- f. Kirkwood Parks and Recreation Department;
- g. Webster Groves Parks and Recreation Department;
- h. St. Louis City Department of Parks, Recreation, and Forestry;
- i. Forest Park Forever;
- j. Tower Grove Park
Table A.2. Plant lines used in infection assay, for both mildew lines.

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<td><strong>Total urban plant lines</strong></td>
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<td><strong>Total of all site types</strong></td>
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Fig. A.1. Total area (m$^2$) surveyed in each field site.
Fig. A.2. Binary infection status of focal surveyed plant (y-axis) vs. categorical abundance of infected conspecifics within a 1.5 m radius (x-axis categories: "0" = 0, “1” = 1-10, “2” = 11-50, and “3” = 51-100 infected conspecifics). The plotted data are from all field sites and survey dates, beginning in July 2020. Size of black circles represents the total number of focal plants (n) with overlapping data points.
**Fig. A.3.** Distance (m) from trays of sentinel plants to the nearest infected conspecific in each field site in July 2020. The radius of our search for mildew extended to 25 m from the trays of sentinels. In multiple sites, there were no wild infected plants within that search radius. We assigned those sites the maximum value of 25 m for the purposes of this plot and accompanying statistical analyses. Thus, the overlapping points at x = 25 m include sites where the actual distance to infected plants may have been much greater.
Fig. A.4. Temperature (°C) readings of HOBO MX2201 dataloggers in pots of sentinel plants within rural (yellow), suburban (green), and urban (purple) sites. Vertical grid lines at date label tick marks indicate midnight (00:00), and vertical lines between date labels indicate noon (12:00). Dataloggers were initially placed 1 cm below the soil surface in each pot; however, exceptionally high and inconsistent daytime temperature readings suggest that the dataloggers became exposed to direct sunlight. As such, daytime temperatures should not be interpreted. During nights, when readings were not impacted by direct sunlight, there is a clear pattern of increased nighttime temperature in more urban sites (consistent with the greater average monthly temperature in more urban sites shown in main text Fig. 1b).
Appendix B:

Chapter One, Reproductive Phenology Data

Here, I provide discuss additional data collected during urbanization surveys in 2019 and 2020. These data were originally going to be included in Chapter One, however it was decided that with additional experimentation (outlined in Appendix B, Discussion) that these data would be best suited for a separate manuscript.

Introduction

Urban heat island effect (UHI) is the phenomena in which cities have higher temperatures than surrounding rural areas. This is due to the retention of solar radiation by built materials, and the release of heat from vehicles and other machinery (Zhou et al. 2017). These increased temperatures can impact plant development; in a study of herbarium records, 24% of herbaceous and woody plant species flowered earlier in urban environments (Neil et al. 2010). Whether UHI advances plant phenology depends on the baseline climate in the region; a study using a large database of >22 million plant phenological observations from the US and Europe found that leaf out and flowering date advanced with city size, but only in regions with cool or cold winters (Li et al. 2019).

We observationally examined the phenology of plant reproduction across a 56-km land use gradient in the metropolitan area of St. Louis, Missouri, US (Fig. B.1, Table A.1). We conducted monthly field surveys of wild Plantago lanceolata (ribwort plantain) and P. rugelii (blackseed plantain) populations co-occurring in 22 sites summer–autumn 2019 and 2020. We hypothesized that urbanization would be associated with earlier flowering and seed production in the field surveys.
Materials and Methods

Study system

This study focused on two co-occurring perennial weedy species, *Plantago lanceolata* (ribwort plantain) and *Plantago rugelii* (blackseed plantain). These small herbs grow in open-canopy pastures or human-disturbed landscapes (e.g., lawns and roadsides), and are tractable model organisms for studies of plant–pathogen and plant–herbivore interactions across land use gradients (Penczykowski and Sieg 2021). *Plantago lanceolata* is native to Eurasia but cosmopolitan in its distribution, and *P. rugelii* is endemic to eastern North America (Penczykowski and Sieg 2021). Both species are found abundantly in the St. Louis region.

Focal populations

We surveyed 22 sites (parks and nature reserves) with *Plantago* populations, extending southwest from downtown St. Louis City to Grey Summit, MO along the I-44 interstate highway corridor (Fig. B.1). The study began with 19 sites in July 2019, two additional sites were added at Shaw Nature Reserve, Grey Summit, MO in August and September 2019, and a site was added at Forest Park, St. Louis, MO in June 2020. Sites were previously classified as “urban”, “suburban”, or “rural” based on a principal component analysis (PCA) of environmental and spatial variables performed with the 'prcomp' function in R version 3.6.2 (R Core Team, 2019) (See Chapter One).
**Fig. B.1.** Google Earth satellite view of the focal urbanization gradient in St. Louis, Missouri, USA. Urban sites are plotted as purple diamonds (n = 8), suburban as green squares (n = 8), and rural as yellow circles (n = 6).

*Field survey methods*

We performed monthly surveys in the focal populations from July to October 2019 (four surveys) and from June to October/November 2020 (five surveys). This time frame represents the majority of the growing season of the plants. In each survey, we collected data for 50 individuals each of *P. lanceolata* and *P. rugelii*. To sample *Plantago* we selected a random individual of either species, then following a meandering path, selected another random individual that was >1 m from the previously surveyed conspecific. The average sizes of the surveyed areas were 1004, 1714, and 2699 m$^2$ for rural, suburban, and urban sites, respectively (Fig. B.1; sizes estimated from polygons in Google Earth).
For each selected plant, we recorded the status of the most mature flower stem on the plant, if present, using the following ordered categories: (0) stem present but flowers/seeds removed by mowing, (1) budding, (2) flowering, (3) immature seeds, (4) mature seeds, or (5) seeds dispersed. Seeds were categorized as immature if the seed capsules were green and plump, and mature if they were brown and papery.

Statistical analyses

All statistics were performed in R version 3.6.2 (R Core Team, 2019). Field survey data were analyzed separately for each species and year. In models testing effects of survey month (ordered factor), site type (urban, suburban, or rural), and their interaction on a given response variable, we included site (population) identity as a random effect. We performed post-hoc Tukey's tests of contrasts between site types within each month if there was at least a marginally significant (P < 0.10) site type x month interaction, or between site types averaged across months if there was a significant (P < 0.05) main effect of site type but no interaction (package 'emmeans'; Lenth, 2022). We report all site type contrasts with P < 0.10, reserving the word "significant" for P < 0.05.

We analyzed plant reproductive development stage as an ordered categorical response variable (levels 0–5, as explained above in Field survey methods) in cumulative link mixed models (package 'ordinal'; Christensen, 2019).
Results

Field surveys: plant reproductive phenology

In both 2019 and 2020, *P. lanceolata* populations had begun flowering in all site types before our first survey, the frequency of flowering peaked in July, and frequency of mature and/or dispersed seeds peaked in August (Fig. B.2a,c). For *P. lanceolata* in 2019, parameters could not be uniquely determined for the model of reproductive development through time when October data were included. Excluding October 2019 from the analysis, the effect of site type on *P. lanceolata* reproductive development (as an ordered categorical variable) varied through time in both years (site type x month interaction in 2019: Likelihood Ratio [LR] $\chi^2 = 20.40$, df = 4, $P = 0.0004$; in 2020: LR $\chi^2 = 51.52$, df = 8, $P < 0.0001$). Post-hoc Tukey's tests showed that, in 2019, maturity was marginally lower in urban and/or suburban than rural sites in July (urban-rural: $P = 0.050$, suburban-rural: $P = 0.068$) and August (suburban-rural: $P = 0.060$), with no other differences between site types within a given month ($P > 0.10$ for all unreported pairwise contrasts; Fig. B.2a). In 2020, *P. lanceolata* reproductive development was marginally lower in urban than rural sites in August ($P = 0.079$) and significantly lower in urban than either suburban or rural sites in September (urban-rural: $P = 0.036$, urban-suburban: $P = 0.008$; Fig. B.2c).

In both years, flowering continued at a higher rate into August for *P. rugelii* compared to *P. lanceolata*, and overall rates of seed production and maturation were also much higher for *P. rugelii* (Fig. B.2). The effect of site type on *P. rugelii* reproductive development varied through time in both years (site type x month interaction in 2019: LR $\chi^2 = 115.72$, df = 6, $P < 0.0001$; in 2020: LR $\chi^2 = 128.98$, df = 8, $P < 0.0001$; Fig. B.2b,d). Post-hoc tests revealed that *P. rugelii* development was significantly lower in suburban than either rural ($P = 0.0092$) or urban ($P =
0.0002) sites in September 2019 but increased with level of urbanization in October 2019 (suburban-rural: P = 0.0001, urban-rural: P < 0.0001, urban-suburban: P = 0.009; Fig. B.2b). Our June 2020 survey captured significantly earlier *P. rugelii* development with increasing levels of urbanization (suburban-rural: P = 0.018, urban-rural: P < 0.0001, urban-suburban: P = 0.0011; Fig. B.2d). Maturity of *P. rugelii* was greater in urban than rural sites in both August and September 2020 (P = 0.025 and P = 0.043, respectively), and greater in both suburban and urban than rural sites in October/November 2020 (P < 0.0001 for both contrasts).

**Fig. B.2.** Relative frequency of each stage of plant reproductive development for each site type and survey month in 2019 and 2020 (Rur = rural, Sub = suburban, Urb = urban).
Discussion

The timing of key reproductive events for wild herbaceous plants changed along an urbanization gradient. The co-occurring focal plant species responded differently in terms of their reproductive phenology. Specifically, urbanization was associated with earlier flowering and more seed production for \textit{P. rugelii}, but less seed maturation for \textit{P. lanceolata}. These species-specific effects on reproductive phenology were broadly consistent between the two years.

Reproductive development of \textit{P. rugelii} was accelerated along the urbanization gradient. However, there were neutral-to-negative associations with urbanization on reproductive development of \textit{P. lanceolata}. One possible explanation for this difference involves the differential impact of mowing on flower stems of these species. \textit{Plantago rugelii} flowers are produced along most of the length of the flower stem, starting below the height of mower blades. If the upper portion of the flower spike had been removed by mowing, we recorded the maturity of the flowers or seeds that remained. By contrast, \textit{P. lanceolata} produces taller stems with flowers produced only at the tip, where they are highly susceptible to complete removal by lawn mowing. Indeed, stems with completely removed flower spikes were more common for \textit{P. lanceolata} than \textit{P. rugelii} (Fig. B.2). Therefore, more frequent mowing in suburban and urban sites may have reduced the abundance of flower and seed spikes on \textit{P. lanceolata} but not affected the proportion of \textit{P. rugelii} individuals with flowers or seeds. How variation in frequency and/or height of mowing impacts plant reproductive phenology across urbanization gradients is an open question. Given the consistently shorter length of the flowering season of \textit{P. lanceolata} compared to \textit{P. rugelii}, their different responses to urbanization may also be
explained by differences in their responses to environmental cues including temperature and light. Experimental tests of these possible underlying mechanisms are needed to clarify why urbanization has species-specific effects on reproduction. Moreover, for some plants, later flowering and senescence in urban sites is adaptive (Lambrecht et al. 2016). Thus, future experiments testing the genetic basis for differences in phenology between Plantago populations will contribute to our understanding of how plant reproductive traits evolve in cities.

Further exploration of these patterns will involve a common garden greenhouse experiment observing phenological development. For this experiment, many lines of Plantago collected from across the urbanization gradient will be grown and the number of days until (1) budding, (2) flowering, (3) immature seeds, (4) mature seeds, or (5) seeds dispersed will be recorded. This experiment will help determine if there are widespread differences in reproductive phenology between sites, or if the observed patterns are due to another factor.

Acknowledgements

We thank Whitney Anthonysamy, Beth Biro, and Solny Adalsteinsson for help selecting field sites and Michelle Pollowitz, Armando Sánchez-Conde, Philippa Tanford, and Shayna Rosenbloom for assistance with field work. Carrie Goodson provided greenhouse and lab support. Mike Dyer and Kim Medley provided greenhouse and hoop house support on the Washington University in St. Louis campus and at Tyson Research Center (TRC), respectively. Susan Flowers provided undergraduate mentoring support through TRC. Bill Winston assisted with acquisition of land use data. We thank staff at St. Louis City Department of Parks, Recreation, and Forestry; Forest Park Forever; Tower Grove Park; Webster Groves Parks and
Recreation Department; St. Louis County Parks and Recreation; Kirkwood Parks and Recreation Department; Missouri Department of Conservation; TRC; Missouri State Parks; and Missouri Botanical Garden’s Shaw Nature Reserve for access to field sites.

Author Contributions

QNF, MJB, and RMP conceived of the study design. QNF, MJB, EG, OSS, and KNF performed field surveys. RMP provided logistical support for the field surveys. QNF and RMP performed statistical analyses. QNF wrote the first draft, and all authors contributed to subsequent drafts of the manuscript.
Appendix C:

Supplement to Chapter Two

Fig. C.1. Diagram (top; not to scale) and photograph (bottom) of the shade experiment under an array of solar panels at Tyson Research Center. In the diagram, rectangles represent trays of plants. The trays in the “inoculated, watered” treatment (red) contained four *Plantago lanceolata* and four or five *P. major* individuals. Trays in the uninoculated treatments contained 8-9 *P. lanceolata* each. The solar panels were separated by 5 m each. Cages were installed over the trays to prevent herbivory by deer or other mammals. Trays of uninoculated plants were rotated around the central (inoculated) tray every other day.
Fig. C.2. Road verge surveys: Infection status of focal surveyed plant vs. categorical abundance of infected conspecifics within a 1.5 m radius (abundance categories: "0" = 0, "1" = 1-10, “2” = 11-50, and “3” = 51-100 infected conspecifics). The plotted data are from all sites and survey dates, beginning in July 2020. Size of black circles represents the total number of focal plants (n) with overlapping data points.
Fig. C.3. Urbanization surveys: Infection status of focal surveyed plant vs. categorical abundance of infected conspecifics within a 1.5 m radius (abundance categories: "0" = 0, “1” = 1-10, “2” = 11-50, “3” = 51-100, and "4" = 100+ infected conspecifics). The plotted data are from all sites and survey dates, beginning in July 2020. Size of black circles represents the total number of focal plants (n) with overlapping data points.
Appendix D:

Supplement to Chapter Three

Fig. D.1. **Left Panel:** Format for allopatric and sympatric inoculation pairings. X and y-axis range from the most southern site (1, McComb, Mississippi) to the most northern site (5, northern Wisconsin). “X” represents a pairing between a plant sourced from the host population on the x axis and an isolate sourced from the pathogen population on the y-axis. The color of the “X” represents the identity of the pathogen population, and the color of the vertical bar represents the identity of the host population. Each row represents the powdery mildew x *P. rugelii* genotype combinations in each Petri dish. **Right Panel:** Layout of *P. rugelii* genotypes in each Petri dish. Leaves in each Petri dish were inoculated with a single powdery mildew strain.
Curriculum Vitae
Graduation: May 2023

Education

2019 - 2023  **PhD Candidate in Ecology, Evolution, & Population Biology.** GPA: 3.96
Washington University in St. Louis (WUSTL) | Missouri
Defense date: March 2023
Advisor: Dr. Rachel M. Penczykowski | rpenczykowski@wustl.edu

2015 - 2019  **B.A. in Biology, GPA: 3.43**
Franklin & Marshall College (F&M) | Lancaster, PA
University of Otago | Dunedin, New Zealand | Spring 2018

Experience

**Doctoral Research / Washington University in St. Louis / June 2019 – Spring 2023**

*Wild Plant–Pathogen Interactions Across Gradients of Urbanization and Latitude*
Experimentally and observationally investigated how increased temperatures impact the ecology and evolution of plants and their parasites.
- Developed and conducted experimental design for a four-year observational survey, three field-based manipulative experiments, and two laboratory experiments.
- Analyzed and visualized data using statistical methods (GLMM, ANOVA, PCA, etc.) in R programming language.
- Composed manuscripts on novel research results which are to be published in peer reviewed journals.
- Mentored and managed teams of up to five researchers.
- Presented research as an invited speaker at Truman State University and at the Ecological Society of America’s and the Ecology & Evolution of Infectious Diseases’ annual meeting poster sessions.

**Mentored Teaching Experience / Washington University in St. Louis / Spring 2021 & 2022**
- Designed and delivered a lecture on disease and parasite management in agricultural systems to a class of 25 for the course “Disease Ecology”.
- Lead discussion sections and designed assignments based on assessment of primary literature.
- Provided timely and substantial feedback on assignments and offered weekly office hours.

**Independent Study / Franklin & Marshall College / Jan 2017 – May 2019**

*Dendroecological analysis of recruitment and growth by dominant tree species during 75 years of old field forest succession in Indiana*
- Observationally investigated tree growth rates to understand differing growth histories in relation to climate and neighborhood competition.
- Analyzed and visualized data using statistical methods in SPSS.
- Developed proposal for and received $1,000 Leser Grant to conduct field research.
- Presented research poster at college wide research symposium.

**Laboratory Teaching Assistant / Franklin & Marshall College / Jan – May 2017 & 2019**
- Assisted students with lab procedures, experimental design, statistical analysis, graphing, computer software, report writing, and field work for the course “Principles of Evolution, Ecology and Heredity”.

**Hackman Scholar / Franklin & Marshall College / May – Dec 2016**

**Secondary Crater-Initiated Debris Flow on the Moon**
- Developed and documented a system to utilize NASA’s Integrated System for Imagers and Spectrometers and the GNU Data Language to process satellite images from the Lunar Reconnaissance Orbiter Camera and integrate with topographical data.

**Talks and Posters**

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<tr>
<td>2023</td>
<td>Speaker at Urban Biodiversity Symposium in St. Louis</td>
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<tr>
<td>2021</td>
<td>Invited speaker for Biology seminar series at Truman State University</td>
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**Leadership**

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<td>2022</td>
<td>Graduate student representatives for division wide committee to restructure of graduate curriculum</td>
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**Publications & Manuscripts**

- **In revision**
  - Fox, Q. N., K. N. Farah, O. S. Shaw, M. Pollowitz, A. Sanchez-Conde, C. L. Goodson, and R. M. Penczykowski. Effects of microclimate on disease prevalence across an urbanization gradient. In revision for *Ecology*.

- **In review**
  - Robinson, M. L. and HerbVar consortium [>100 authors including Q. N. Fox]. Plant size, latitude, and phylogeny explain variability in global herbivory. In review at *Nature*.

- **Preprint**
  - Fox, Q. N., M. J. Bugay, E. Grant, O. S. Shaw, K. N. Farah, R. M. Penczykowski. Phenology of plant reproduction, foliar infection, and herbivory change along an urbanization gradient. DOI: https://doi.org/10.1101/2022.03.22.485313

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**Grants, Honors, & Awards**

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**Science Outreach**

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<td>Jackson Park Elementary School, University City, MO; Science Fair Judge</td>
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<td>2022</td>
<td>Growing Together School Food Garden Network Event Brittany Woods Middle School, University City, MO; Answered questions from community members about common plant diseases.</td>
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<tr>
<td>2019</td>
<td>Market Fresh Science at Ferguson Farmers Market, Ferguson, MO; Taught attendees about disease ecology dynamics through a hands-on game.</td>
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