Effects of microclimate on disease prevalence across an urbanization gradient

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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, climatic conditions vary 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. Overall, infection prevalence increased with urbanization despite a negative association between urbanization and tree cover at the landscape scale. These findings highlight the importance of taking microclimate heterogeneity into account when establishing links between macroclimate or land use context and prevalence of disease.

Pandemic Pivot

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 have examined how environmental changes along urbanization gradients or climatic variability within cities impact prevalence of plant diseases (Egerer et al. 2020). As we completed field surveys to help fill that research gap during summer 2020, our research team experienced first-hand the physical and mental benefits of access to urban greenspaces. In order to adhere to necessary precautions during the first summer of the COVID-19 pandemic, we pivoted from our originally planned research (involving carpool travel and time spent in laboratory and field station spaces) to this safe, field-based Pandemic Pivot project. Specifically, the undergraduate authors on this paper went on daily socially-distanced walks to complete the road verge surveys and become acquainted with local flora. The first-author of this study, a graduate student at the time, was also able to safely survey parks across the urbanization gradient while maintaining social distancing. All of us gained greater appreciation for opportunities to step away from computer-based work to conduct research on plants growing abundantly in our own urban and suburban neighborhoods.

Introduction

While urban areas take up a relatively small amount of total global land use, the rate of urban expansion is dramatically increasing (Güneralp et al. 2020). Human reliance on urban land is also increasing, as the majority of the world's human population resides in urban areas (55% in 2018) and this percentage is expected to grow to 68% by 2050 (United Nations 2019).

Consequently, there is increasing need to understand how environmental changes associated with urbanization impact the species interactions that underlie ecosystem functions. Such understanding is critical for managing urbanized land to promote ecosystem services (Felson and Ellison 2021). Importantly, urbanization is a process that produces not only landscape-level changes, but also a high degree of heterogeneity in natural and built features at local scales within and between urbanized habitats (Cadenasso et al. 2007). Habitat heterogeneity within urban settings has the potential to impact timing, likelihood, and outcome of the myriad species interactions that contribute to ecosystem functioning (e.g., pollination (Theodorou et al. 2022), herbivory (Egerer et al. 2017, Braem et al. 2023), predation (Kotze et al. 2022), and parasitism (Murdock et al. 2017, van Dijk et al. 2022)). However, a major challenge for understanding how urbanization alters species interactions is to determine how effects of environmental factors vary across spatial scales, from landscapes to microhabitats.

Urbanization involves dramatic changes to abiotic and biotic facets of environments (Grimm et al. 2008, Johnson and Munshi-South 2017, Schell et al. 2020). One of the most apparent abiotic changes is increased temperature. 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 less tree canopy cover, more coverage with impervious surfaces that absorb and emit heat, and more heat generation by vehicles and machinery (Zhou et al. 2017). Increased temperatures in cities impact a wide range of traits across diverse taxa (Johnson and Munshi-South 2017, Pisman et al. 2020). At the same time, climatic conditions vary over fine scales within habitats, including due to shade cast by natural and built features (Liao et al. 2021, Chen et al. 2022). Patches of shade can function as microclimatic refugia ("microrefugia") that allow species to occur in habitats where climatic conditions are otherwise intolerable (Suggitt et al. 2011, Lenoir et al. 2017, Murdock et al. 2017, Haba and McBride 2022). Climatic variation can alter the phenology and abundances of individual species and resulting probability that they encounter each other (Bale et al. 2002, Murdock et al. 2017). Physiological responses of organisms that govern the strength of species interactions are also thermally dependent (Rall et al. 2012). Thus, urban heat islands and within-habitat microclimate heterogeneity are likely to contribute to spatiotemporal differences in species interactions across urbanized landscapes.

Here we focus on the effects of urban environmental heterogeneity on host-pathogen interactions. A meta-analysis of >100 published studies revealed that wildlife face greater health risks in urban areas, including due to directly transmitted parasites (Murray et al. 2019). By contrast, there has been little empirical research on plant-pathogen interactions across levels of urbanization (exceptions include: (Egerer et al. 2020, van Dijk et al. 2022)). Many environmental changes associated with urbanization could impact rates of infection in either animal or plant hosts. Urban heat may increase or decrease disease prevalence, depending on regional climate, magnitude of the temperature increase, and thermal sensitivity of host and pathogen life history traits as well as host defense responses (Wang et al. 2009, Shocket et al. 2018, Mordecai et al. 2019, Desaint et al. 2021, Gsell et al. 2023). Yet temperature is not the only climatic factor relevant to disease. Pathogen success can further depend on exposure to precipitation (Glawe 2008, Jousimo et al. 2014), humidity (Guzman-Plazola et al. 2003, Altizer et al. 2006), wind (Calonnec et al. 2013), and solar radiation (Braga et al. 2015, Shaw et al. 2020). Like temperature, these climatic factors vary with heterogeneity in built infrastructure and vegetation across urban landscapes (Lai et al. 2019). Effects of microclimate on pathogen growth and transmission can in turn vary over the course of epidemics or seasons (Penczykowski et al.

2018). Furthermore, heterogeneity in factors such as pollution, disturbance, habitat connectivity, and biodiversity may additionally influence patterns of disease across levels of urbanization.

We investigated effects of urbanization and microclimate on plant fungal infection across a land use gradient in the St. Louis metropolitan area (Missouri, USA; Fig. 1). In road verges and parks, we surveyed populations of common weedy plants in the genus *Plantago*. We hypothesized that urbanization would be associated with larger epidemics of powdery mildew (Urbanization Hypothesis), potentially due to warmer temperatures accelerating pathogen growth in spring and/or greater pathogen dispersal between more highly connected urban populations. Powdery mildew growth is typically inhibited by high temperatures and ultraviolet (UV) radiation (Austin and Wilcox 2012, Chaloner et al. 2020). Therefore, we expected greater prevalence of infection on plants in the shade compared to full sun (Shade Hypothesis), with the positive effect of shade fading in cooler autumn months (Shade-Time Hypothesis). Due to the urban heat island effect, we expected shade to have a stronger positive effect on mildew prevalence in more urban populations (Urban-Shade Hypothesis). However, shaded and sunlit locations within a habitat may also differ in other factors 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 adjacent to shade-producing structures (e.g., trees, buildings, and fences) may also experience less disturbance from lawn mowing, such that infected leaves persist longer and are more likely to be observed. To account for the possibility that unmowed plants within a population would be more likely to exhibit disease, we included individual-level mowing damage as a covariate in our analyses of infection. Then, to determine whether mowing was a driver of either landscape-scale relationships between urbanization and disease or local-scale relationships between shade and disease, we separately assessed prevalence of mowing itself with respect to land use type and shade. Specifically, we tested hypotheses that prevalence of mowing would increase with urbanization due to greater intensity of lawn management in urban and suburban parks (Urbanization Hypothesis for Mowing) and that at fine spatial scales mowing would be less common in shade due to cautious mowing around trees and other structures (Shade Hypothesis for Mowing). Finally, we performed a manipulative field experiment to directly test effects of shade on growth and transmission of powdery mildew.

Methods

Study system

This study focused on three herbaceous host plants: *Plantago lanceolata* (ribwort plantain), *P. major* (common plantain), and *P. 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 widespread occurrence, *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 globally distributed, and *P. rugelii* is endemic to eastern North America. *Plantago major* is relatively rare in Missouri and thus absent from our observational surveys (Yatskievych and Steyermark 1999, Penczykowski and Sieg 2021).

The most common foliar pathogens of *Plantago* are two specialist powdery mildew fungi 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 are obligate pathogens that extract nutrients from host epidermal tissue (Glawe 2008). Chains of asexual spores produced from mycelia on the leaf surface give infected leaves a white, dusty appearance. Spores are passively transmitted by wind, and more than 90% land within 2 m of their source (Tack et al., 2014). Occasional long-distance dispersal allows 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 (Glawe 2008).

Road verge surveys

To compare the frequency of powdery mildew infection 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. 1a). Five 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 campus due to transportation constraints during the first summer of the COVID-19 pandemic (Fig. 1b). Each survey involved visual inspection of up to 50 randomly selected individuals of *P. 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 the 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 or not it was infected with mildew. We also recorded presence/absence of mowing damage (leaves cut cleanly across) and common types of herbivory (leaf mines and chewing damage; these were combined for analysis and included as a covariate). To assess if infection statuses of focal plants were representative of neighboring individuals in the same microhabitat, we estimated the number of infected conspecifics within a 1.5 m radius of each focal plant (ordered categorical variable in bins of 0, 1-10, 11-50, 51-100, or 101+ plants). We then walked a few paces, continuing unidirectionally, and arbitrarily selected the next focal plant. Focal plants were always at least 1 m from the previously surveyed conspecific. We continued until 50 plants of each species had been surveyed. Fewer than 50 plants were surveyed if a *Plantago* species was locally rare or if surveys were cut short due to inclement weather or barriers (e.g., construction). We performed 48 surveys of *P. lanceolata* (total = 1092 plants) and 59 surveys of *P. rugelii* (total = 2736 plants).

All statistical analyses were conducted in R version 4.1.0 (R Core Team 2020). Separate models were fit to the two plant species because we expected that differences in pathogen susceptibility and plant morphology could lead to different rates of infection and mowing damage on the two species. For road verge surveys, the following analyses of binary infection data and binary mowing data from focal plants were performed using generalized linear mixed models (GLMMs; package 'lme4', version 1.1.27.1) with binomial error distributions (logit link functions) and random effects of unique survey identity. We modeled mildew infection status with respect to shade (yes/no), mowing damage (yes/no), and herbivory damage (yes/no). Separately, we analyzed the correspondence between infection status of a focal surveyed plant and abundance of infected conspecifics in the surrounding microhabitat. Finally, we tested if the probability of mowing damage differed between sun and shade microhabitats.

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 56 km from the urban City of St. Louis to rural Shaw Nature Reserve in Gray Summit, Missouri (Fig. 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”. These classifications were consistent with separation of sites along the first axis of a principal component analysis (PCA) of environmental and spatial variables performed with the 'prcomp' function (package ‘stats’, version 4.1.0) (Fig. 1c). The PCA included average temperature from June-October (Temp), nighttime radiance (NightLight), percent impervious area (%Imp), percent tree cover (%Tree), and intersection density of walkable roads (IntDen; walkable roads defined as having speed limits between 8-55 miles per hour). Temperatures were long-term (1970-2000) averages at these sites during the focal months of our study (WorldClim 2; Fick & Hijmans, 2017). Results were qualitatively similar when mean temperatures from June-October 2020 were used instead (Appendix S1: Fig. S1). Nighttime radiance came from the NASA Black Marble data product for December 2020 (NASA Worldview). Details of how we estimated %Imp, %Tree, and IntDen are in Appendix S1. The three urban sites closest to the suburban grouping in the PCA (sites #18, 19, 20) are located in large parks featuring high percentage tree cover within an otherwise urban setting in St. Louis City (Fig. 1c; Appendix S1: Table S1).

Survey methods were as in the road verges, except that we surveyed plants along a meandering (non-linear) walk throughout each site. Sites were surveyed monthly in July, August, September, and late October or early November 2020. In the following analyses, binary infection data and binary mowing data from focal plants were analyzed using GLMMs with binomial error distributions (logit link functions) and random effects of site identity. To test effects of urbanization, shade, and time on probability of mildew infection, we compared among eight models for each host species using Akaike Information Criterion corrected for small sample sizes

(AICc) via the ‘aictab’ function (package ‘AICcmodavg’, version 2.3.1). Candidate models included fixed effects of month (four-level categorical variable), site type (urban, suburban, or rural), shade (yes/no), mowing damage (yes/no), herbivory damage (yes/no), and up to three hypothesized two-way interactions (site type × month, shade × month, and shade × site type). Separately, we analyzed the infection status of focal plants with respect to abundance of nearby infected conspecifics. To evaluate differences in mowing damage between microhabitats and across the urbanization gradient, we again used AICc to compare eight models per host species (Appendix S1: Table S3). Candidate models included month, site type, shade, and their possible two-way interactions. In models with significant interactions, post-hoc Tukey tests were used to examine treatment contrasts, using function 'emmeans' (package 'emmeans', version 1.8.3).

Shade experiment

We performed a manipulative experiment to measure effects of shade on powdery mildew growth and transmission. 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 Washington University 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 in locations directly under (shade) or between (sun) four large solar panels in an otherwise open, mowed field at Tyson Research Center (Fig. 2). Replicates of maternal lines were distributed among trays. We created eight blocks each containing four trays. Within each block, one tray was assigned to an "inoculated, watered" treatment, two trays were assigned to an "uninoculated, watered" treatment, and one tray was assigned to an "uninoculated, unwatered" treatment. Blocks were then randomly assigned to either the shade or sun microclimate treatment, so that all three inoculation/watering treatments were nested within each sun or shade treatment. 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. On 7 July, we used sterilized paint brushes to inoculate plants in these first trays by gently brushing one leaf per plant with spores from lab-inoculated source plants. We used species-specific strains of *Golovinomyces sordidus* that had been isolated from *Plantago* in the St. Louis region and propagated following standard methods (Nicot et al. 2002). The next day, we placed the trays of "uninoculated, watered" and "uninoculated, unwatered" plants into each shade and sun location (9-10 plants per tray; all *P. lanceolata*). We assessed whether these uninoculated *P. lanceolata* became infected over the following weeks.

The inoculated plants were watered regularly by filling the trays with an inch of water every other day. The "uninoculated, watered" plants were similarly watered. The trays of "uninoculated, unwatered" plants had drainage holes; this treatment allowed us to test plant–mildew 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) in the pots at a depth of 1 cm below the soil surface (Fig. 2 and Appendix S1: Fig. S2). We assessed the infection status of each plant on 15 July, 23 July, and 6 August 2021.

We analyzed the binary infection status of each plant on the final day of the experiment using generalized linear models (‘glm’ function, package ‘stats’, version 4.1.0) with binomial error distribution (logit link function). Separate models were fit to inoculated *P. lanceolata* (n =

32), inoculated *P. major* (n = 34), and uninoculated *P. lanceolata* (n = 186 alive at end of experiment). In all three models, we tested effects of microclimate treatment (shade or sun) and maternal line. For uninoculated *P. lanceolata*, we additionally tested effects of watering treatment.

Results

Road verge surveys

In road verges, powdery mildew prevalence on *P. rugelii* was 11.5 times that on *P. lanceolata* (15.85% vs. 1.37% infected overall; Fig. 3). For both species, infection on a focal plant was significantly associated with additional infected conspecifics in the immediate vicinity, and uninfected focal plants were almost always surrounded by uninfected conspecifics (P < 0.0001 for both species; Appendix S1: Fig. S3). Infection was more often observed in shade than sun (Fig. 3). This effect of shade was non-significant for *P. lanceolata* (β = 0.27 95% CI [-1.03, 1.57], P = 0.69; mildew only observed in sun during three surveys and in shade during six surveys) but highly significant for *P. rugelii* (β = 1.88 [1.55, 2.22], P < 0.0001). Infection on *P. lanceolata* was weakly negatively associated with mowing (β = -1.49 [-3.11, 0.12], P = 0.07) but unrelated to herbivory (β = -0.26 [-1.45, 0.93], P = 0.67). There were no effects of mowing (β = -0.12 [-0.43, 0.19], P = 0.46) or herbivory (β = -0.24 [-0.57, 0.09], P = 0.15) on *P. rugelii* infection. There was no effect of shade on mowing for *P. lanceolata* (β = -0.22 [-0.61, 0.18], P = 0.28), but *P. rugelii* in shade had significantly less mowing damage (β = -0.64 [-0.88, -0.39], P < 0.0001; Appendix S1: Fig. S4).

Urbanization surveys

Across the urbanization gradient, powdery mildew prevalence on *P. rugelii* was 4.7 times that on *P. lanceolata* in the final month of surveys (46.61% vs. 9.91% , averaged across sites and microhabitats; Fig. 4). Infection on focal surveyed plants was positively related to abundance of nearby infected conspecifics (P < 0.0001 for both species; Appendix S1: Fig. S5).

Of the models fitted to *P. lanceolata* mildew data, the top one included only main effects (Table 1; Fig. 4). Infection was more likely in shade (β = 1.39 [0.83, 1.96], P < 0.001). Infections also increased in later months (Sept relative to July baseline: β = 2.53 [1.49, 3.57], P < 0.001; Oct/Nov relative to July: β = 3.09 [2.06, 4.12], P < 0.001). There was somewhat more disease in urban than rural populations of *P. lanceolata*, but no significant contrasts among site types for this species (urban-rural contrast: β = 2.35 [-0.10, 4.80], P = 0.14; suburban-rural: β = 1.33 [-1.22, 3.88], P = 0.56), urban-suburban: β = 1.03 [-1.01, 3.07], P = 0.59). Mildew was less likely on plants with mowing damage (β = -0.51 [-0.88, -0.14], P = 0.006). However, there was no significant relationship between herbivory and mildew (β = 0.08, [-0.35, 0.52], P = 0.71). The next best model included a shade × site type interaction, where shade effects were stronger in rural and urban than suburban sites (shade-sun contrast in rural: β = 1.47, [0.19, 2.75], P = 0.025; suburban: β = 0.28 [-0.99, 1.55], P = 0.66; urban: β = 1.64 [0.91, 2.37], P < 0.0001).

The top model of *P. rugelii* infections included all two-way interactions (Table 1, Fig. 4). Overall, there was greater mildew prevalence in urban than rural (β = 3.19 [1.95, 4.43], P < 0.001) and suburban than rural sites (β = 2.10 [0.85, 3.35], P = 0.003), while the contrast between suburban and urban sites was not significant (β = 1.09 [-0.010, 2.19], P = 0.13). The site type × month interaction reflected a greater increase in disease through time in more urban sites (Fig. 4). Differences between site types were largest at the end of the season (Oct/Nov contrasts of urban-rural: β = 4.98 [3.64, 6.32], P < 0.0001; suburban-rural: β = 3.33 [1.99, 4.67], P <

0.0001; urban-suburban: β = 1.65 [0.51, 2.79], P = 0.013). There was an overall positive effect of shade on mildew (β = 0.84 [0.23, 1.45], P = 0.007). This shade effect also varied through time. Yet, averaged across other factors, there was more mildew in shade than sun in all months, and the shade effect did not fade in autumn (shade-sun contrast in July: β = 0.42 [0.02, 0.81], P = 0.04; Aug: β = 0.85 [0.38, 1.32], P = 0.0004; Sept: β = 1.14 [0.81, 1.47], P < 0.0001; Oct/Nov: β = 0.69 [0.33, 1.04], P = 0.0002). The shade effect was strongest in rural sites (shade-sun contrast in rural: β = 1.20 [0.68, 1.72], P < 0.0001; suburban: β = 0.50 [0.21, 0.80], P = 0.0008; urban: β = 0.62 [0.34, 0.89], P < 0.0001). Infection on *P. rugelii* was positively associated with herbivory (β = 0.58 [0.32, 0.83], P < 0.0001) but not related to mowing (β = -0.10 [-0.27, 0.07], P = 0.25).

The best model of mowing on *P. lanceolata* included site type × month and shade × month interactions (Appendix S1: Table S2 and Fig. S6). The site type × month effect reflected more mowing in suburban and urban than rural sites in September (urban-rural: β = 1.53 [0.75, 2.31], P = 0.0003; suburban-rural: β = 1.60 [0.78, 2.42], P = 0.0004; urban-suburban: β = -0.07 [-0.82, 0.69], P = 0.98). The shade × month interaction was driven by marginally more mowing in shade than sun in July (β = 0.32 [-0.01, 0.65], P = 0.059) and August (β = 0.28 [-0.03, 0.59], P = 0.082) but less mowing in shade than sun in September (β = -0.59 [-0.93, -0.26], P = 0.0006) and marginally less in shade than sun in October/November (β = -0.30 [-0.62, 0.01], P = 0.060).

Prevalence of mowing on *P. rugelii* also depended on interactions of site type × month and shade × month (Appendix S1: Table S2 and Fig. S6). Specifically, there was more mowing in suburban and urban than rural sites in October/November (urban-rural: β = 1.72 [0.80, 2.65], P = 0.0008; suburban-rural: β = 1.63 [0.70, 2.56], P = 0.0017; urban-suburban: β = 0.09 [-0.73, 0.92], P = 0.97). There was more mowing in shade than sun in July (β = 0.63 [0.36, 0.91], P <

0.0001) but less mowing in shade in August (β = -0.32 [-0.62, -0.02], P = 0.035), September (β = -0.33 [-0.61, -0.04], P = 0.025), and October/November (β = -0.36 [-0.64, -0.08], P = 0.013).

Shade experiment

Shade strongly promoted growth of powdery mildew on inoculated plants in our field experiment (Appendix S1: Table S3). Over the course of the experiment, mildew developed on only one out of 16 inoculated *P. lanceolata* placed in the sun but on 10 out of 16 placed in the shade (Fig. 5). For *P. major*, no mildew developed on any of the 17 inoculated plants in the sun while 12 out of 17 inoculated plants developed infection in the shade (Fig. 5). The lack of infections in the sun treatment created the statistical problem of complete separation of the predictor variable (sun/shade) by the outcome variable (infection), such that parameters could not be reliably estimated (Albert and Anderson 1984). Most inoculated plants in shade became visibly infected already in the first week (Fig. 5). One inoculated *P. lanceolata* individual in the sun developed infection on a single leaf by the second week; however, this mildew lesion was no longer visible in the third week (Fig. 5). For *P. lanceolata*, we also tested effects of shade and watering treatment on transmission of mildew to nearby uninoculated plants (Appendix S1: Table S3). No mildew developed on any of the 118 uninoculated *P. lanceolata* in the sun, but mildew spread to 28 of the 119 uninoculated plants in the shade (Fig. 5). Among the uninoculated *P. lanceolata* that became infected by the second and third weeks of the experiment, more infection developed in the watered than unwatered treatment (Fig. 5). Daytime temperatures (6am-7pm) in the sun were typically much higher than in shade (mean ± SE among pots: 26.4 ± 0.38 °C in shade and 31.0 ± 1.04 °C in sun; Appendix S1: Fig. S2).

Discussion

We found that more urban plant populations had larger powdery mildew epidemics (support for Urbanization Hypothesis). This was especially the case for *Plantago rugelii*, which had overall much greater rates of infection than co-occurring *P. lanceolata*. At a finer spatial scale, shaded microhabitats were positively associated with mildew infection (support for Shade Hypothesis in road verge surveys, urbanization surveys, and shade experiment). Shade did not have a stronger positive effect on disease in more urban sites (no support for Urban-Shade Hypothesis). We also did not find a decrease in the effect of shade as temperatures cooled in autumn (no support for Shade-Time Hypothesis). Together, the positive urbanization-disease and shade-disease relationships present a paradox: because urbanization is associated with less tree cover (Fig. 1c), disease is negatively related to tree cover at the landscape scale but positively related to tree cover at the within-population scale. There are several non-mutually-exclusive possible explanations of that paradox, which we discuss below.

Our results may be explained by effects of climate variation at different spatial scales and times of year. In geographic regions where baseline climate is cooler than optimal for pathogen survival or growth, urban warming should promote disease. Indeed, in several European cities, warmer urban temperatures are suggested to drive positive effects of urbanization on powdery mildew on oak trees (van Dijk et al. 2022). Yet urban heat island effects on disease may vary across seasons. Mildew survival is decreased by exposure to freezing temperatures (Penczykowski et al. 2015). Therefore, a year-round urban heat island effect could promote mildew overwintering. On the other hand, cities can have colder temperature minima than non-urban areas in winter (Thompson et al. 2016), so mildew survival may further depend on the presence of microhabitats that are protected from freezing. Warmer spring temperatures in urban areas have been shown to advance the seasonal phenology of many organisms (Meineke et al. 2019, Li et al. 2019). Similarly, warmer spring temperatures may have accelerated early-season mildew growth and transmission in our urban populations. However, across our focal region, summer temperatures in full sun often exceed the range of upper thermal limits for powdery mildews, even in our rural sites (Chaloner et al. 2020). Across the urbanization gradient, plants in full sun may also have been exposed to equally high levels of damaging UV radiation during summer (Austin and Wilcox 2012). Thus, the microclimate afforded by shade in terms of cooler temperatures and less UV exposure (as well as higher relative humidity; (Guzman-Plazola et al. 2003)) may have been as critical to mildew success in rural areas as in urban areas. This would explain why we found a positive shade effect across land use types (i.e., no support for Urban-Shade Hypothesis). Another possible reason why the shade effect was not stronger in urban than rural sites is that we surveyed parks and nature areas, where the magnitude of the urban heat island effect may have been tempered by large patches of greenspace (i.e., "urban cool island effect"; (Liao et al. 2023)). Contrary to our prediction, the effect of shade on infection did not fade in autumn (i.e., no support for Shade-Time Hypothesis). This could indicate that the benefits of shaded microrefugia for mildew during the hottest months carry over into the cooler months.

Other potential explanations for our finding of larger epidemics in more urban populations involve differences in population connectivity and/or host susceptibility to disease. *Plantago* grow in relatively open-canopy habitats with frequent mowing or other disturbance (Kuiper and Bos 2012, Penczykowski and Sieg 2021). Therefore, forested rural areas may support fewer or less well-connected populations of these host species compared to urban areas that are crisscrossed with roads and dotted with mowed lawns. In general, greater host population connectivity can promote spread of disease (Margosian et al. 2009, Bolte et al. 2023). A role of forests in disrupting host connectivity or blocking wind-dispersed spores may explain why populations of *P. lanceolata* with more shade cover (e.g., by forested borders) had lower probability of mildew presence in a metapopulation in Finland (Jousimo et al. 2014). In that same system, mildew dispersal was shown to be facilitated by roads, especially busier roads (Numminen and Laine 2020). In urban settings, patchy trees and buildings may not block spores as effectively as larger forest tracts. Meanwhile, dense networks of roadsides and mowed patches of vegetation may be highly connected habitats for weedy hosts like *Plantago*. Movement of humans and vehicles into and through cities may then increase spore arrival into those habitats. Importantly, pathogen spores that arrive to a host population are only successful if they land on susceptible host genotypes. Wild plant populations can vary substantially in their mean susceptibility to mildew, including due to selection from past epidemics (Thrall et al. 2012, Jousimo et al. 2014). In our system, greater resistance of rural *P. rugelii* to mildew could explain their lower disease prevalence, even if rates of spore arrival were equal across the land use gradient. Future analysis of spatial structure in pathogen genotypes and tests of host resistance will be needed to determine the extent to which connectivity and resistance explain the observed increase in disease with urbanization. Future studies will also clarify if species-specificity of mildew strains underlies the low rates of disease on *P. lanceolata* in our field surveys, even where co-occurring *P. rugelii* had high infection prevalence.

Variation in mowing within and between populations may also have influenced disease patterns. Effects of mowing on wild plant pathosystems have rarely been investigated despite the potential for mowing to influence disease through multiple mechanisms including wounding stress (Inguagiato et al. 2009), removal of infected tissue, and dispersal of pathogens (Nutter et al. 1982). At the level of individual plants, we found that mowing damage was negatively related to infection on *P. lanceolata* but not significantly associated with infection on *P. rugelii*. Differences in plant morphology could help explain this difference. *Plantago lanceolata* has a relatively upright growth form, and might typically lose a larger fraction of leaf area (including a larger fraction of infected leaf area) when mowed. By contrast, *P. rugelii* leaves tend to be flatter to the ground, such that a larger fraction of each leaf (including infected leaves) may remain after mowing. Prevalence of mowing damage itself increased with urbanization in some months (Urbanization Hypothesis for Mowing), and in no months did rural sites have the most mowing. Given the negative relationship between mowing and infection status of *P. lanceolata* at the individual plant level, the urbanization hypothesis for mowing (more urban, more mowing) seems to run counter to the urbanization hypothesis for mildew (more urban, more infection). However, it is possible that mowing has opposing effects on disease by simultaneously removing infected leaf tissue and aiding spore dispersal through the population (Nutter et al. 1982). The direction of the relationship between shade and mowing varied between months and survey types (mixed support for Shade Hypothesis for Mowing). As predicted, there was less mowing in shade than sun in the June-July road verge surveys. However, across the urbanization gradient, mowing was generally more prevalent in shade than sun in July, and less prevalent in shade than sun in later months. Thus, the observed patterns of more disease in shade are not simply due to less mowing in those microhabitats. Furthermore, shade had a strong positive effect on disease even in the absence of mowing in our manipulative field experiment.

Our study highlights strong associations of urbanization and microclimate heterogeneity with patterns of infection prevalence. Resolving the role of microclimate effects within larger-scale climate and land use trends can inform strategies for predicting and managing plant diseases on a warming and increasingly urbanized planet. As the frequency of extreme heat increases worldwide, plants in unshaded microhabitats may be prone to negative effects of heat stress (Mittler et al. 2012). At the same time, plants protected from heat stress by shade may be vulnerable to fungal diseases. These tradeoffs are relevant not only to wild plant populations, but also have agricultural implications. For example, the practice of growing crops beneath solar panels – agrivoltaics – is proving to be an innovative solution for decreasing water usage and increasing productivity of farms (Barron-Gafford et al. 2019). Our experimental results suggest that microclimates under solar panels may promote risk of some plant diseases. Overall, our findings highlight the importance of accounting for microclimate heterogeneity when establishing links between macroclimate or land use context and frequency of species interactions, including prevalence of pathogen infection.

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Author Contributions: QNF and RMP designed the study. OSS, ASC, MP, and KNF performed road verge surveys and OSS curated those data. QNF, KNF, OSS, ASC, and MP conducted urbanization surveys. QNF, KNF, and CG carried out the shade experiment. RMP

provided support for the surveys and experiment. QNF, MP, and RMP analyzed the data. QNF, MP, and RMP wrote the first draft, and all authors contributed to subsequent manuscript drafts.

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Table 1. Comparison of candidate generalized linear mixed models predicting powdery mildew infection in the urbanization surveys. Models were fit separately to the two host species. Dashes ("-") indicate models that either did not converge or were unidentifiable and thus not included in model selection. All models included mowing and herbivory as fixed effects and site identity as a random effect. Models were compared using AICc. The number of estimated coefficients is given by k, and the Akaike weight is given by w_i. Models within two ΔAICc are considered to be equally well supported by the data; hence, the top two best models for each *Plantago* species are bolded.

Host species	Model terms (in addition to Mowing + Herbivory)	k	AICc	ΔAICc	w_i
P. lanceolata	**Shade + Month + Site type**	**10**	**945.40**	**0.00**	**0.55**
P. lanceolata	**Shade × Site type + Month**	**12**	**946.40**	**1.00**	**0.34**
P. lanceolata	Shade × Month + Site type	13	949.46	4.05	0.07
P. lanceolata	Shade × Month + Shade × Site type	15	950.73	5.32	0.04
P. lanceolata	Shade × Month + Shade × Site type + Month × Site type	21	-	-	-
P. lanceolata	Shade × Month + Month × Site type	19	-	-	-
P. lanceolata	Shade × Site type + Month × Site type	18	-	-	-
P. lanceolata	Shade + Month × Site type	16	-	-	-
P. rugelii	**Shade × Month + Shade × Site type + Month × Site type**	**21**	**3928.62**	**0.00**	**0.56**
P. rugelii	**Shade × Month + Month × Site type**	**19**	**3930.18**	**1.57**	**0.26**
P. rugelii	Shade × Site type + Month × Site type	18	3931.47	2.86	0.14
P. rugelii	Shade + Month × Site type	16	3833.77	5.16	0.04
P. rugelii	Shade × Month + Shade × Site type	15	3990.06	61.45	0.00
P. rugelii	Shade × Site type + Month	12	3993.56	64.94	0.00
P. rugelii	Shade × Month + Site type	13	4003.94	75.32	0.00
P. rugelii	Shade + Month + Site type	10	4007.59	78.98	0.00

Figures

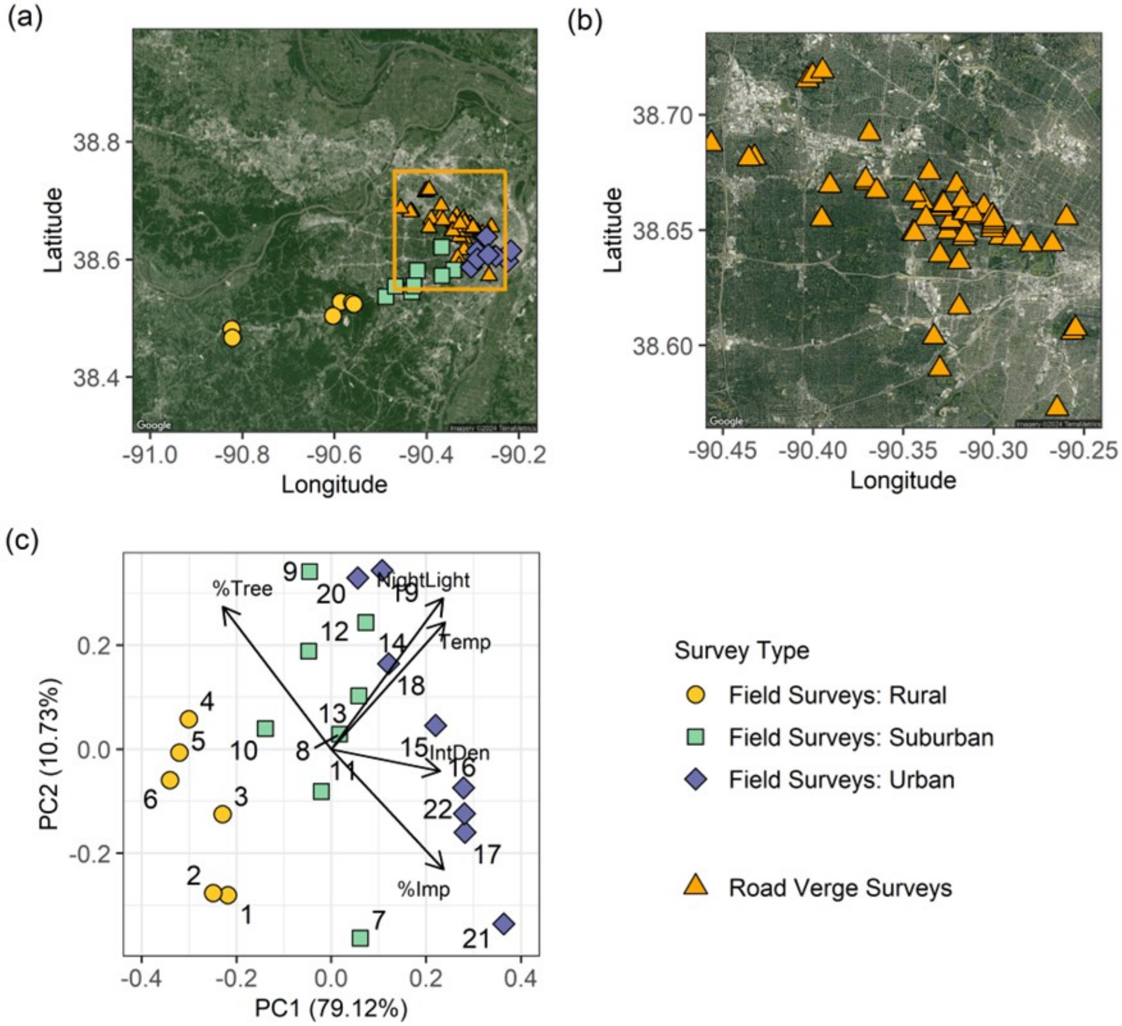


Figure 1.

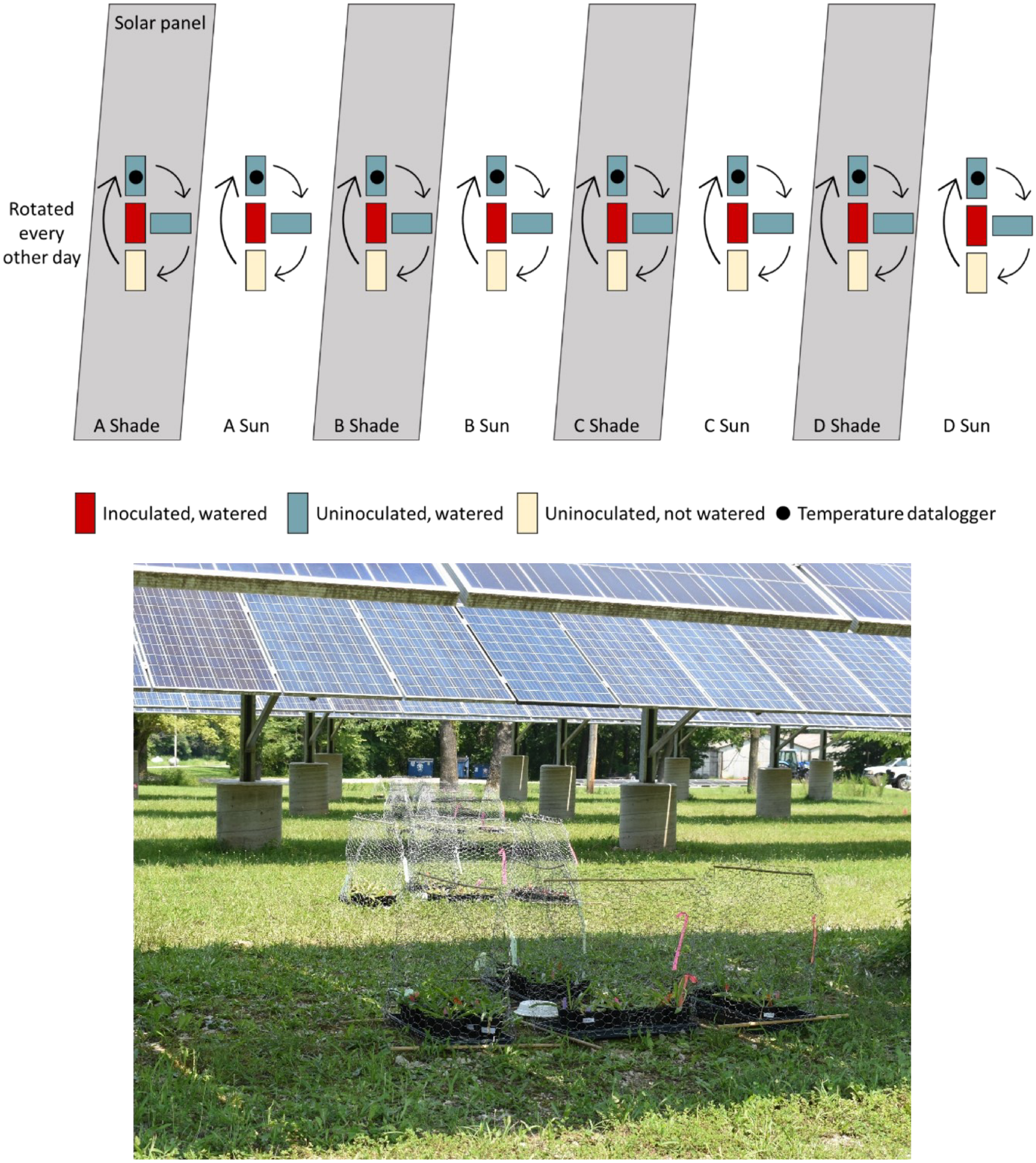

Solar panel
Rotated every other day
A Shade
A Sun
B Shade
B Sun
C Shade
C Sun
D Shade
D Sun
Inoculated, watered
Uninoculated, watered
Uninoculated, not watered
● Temperature datalogger

Figure 2.

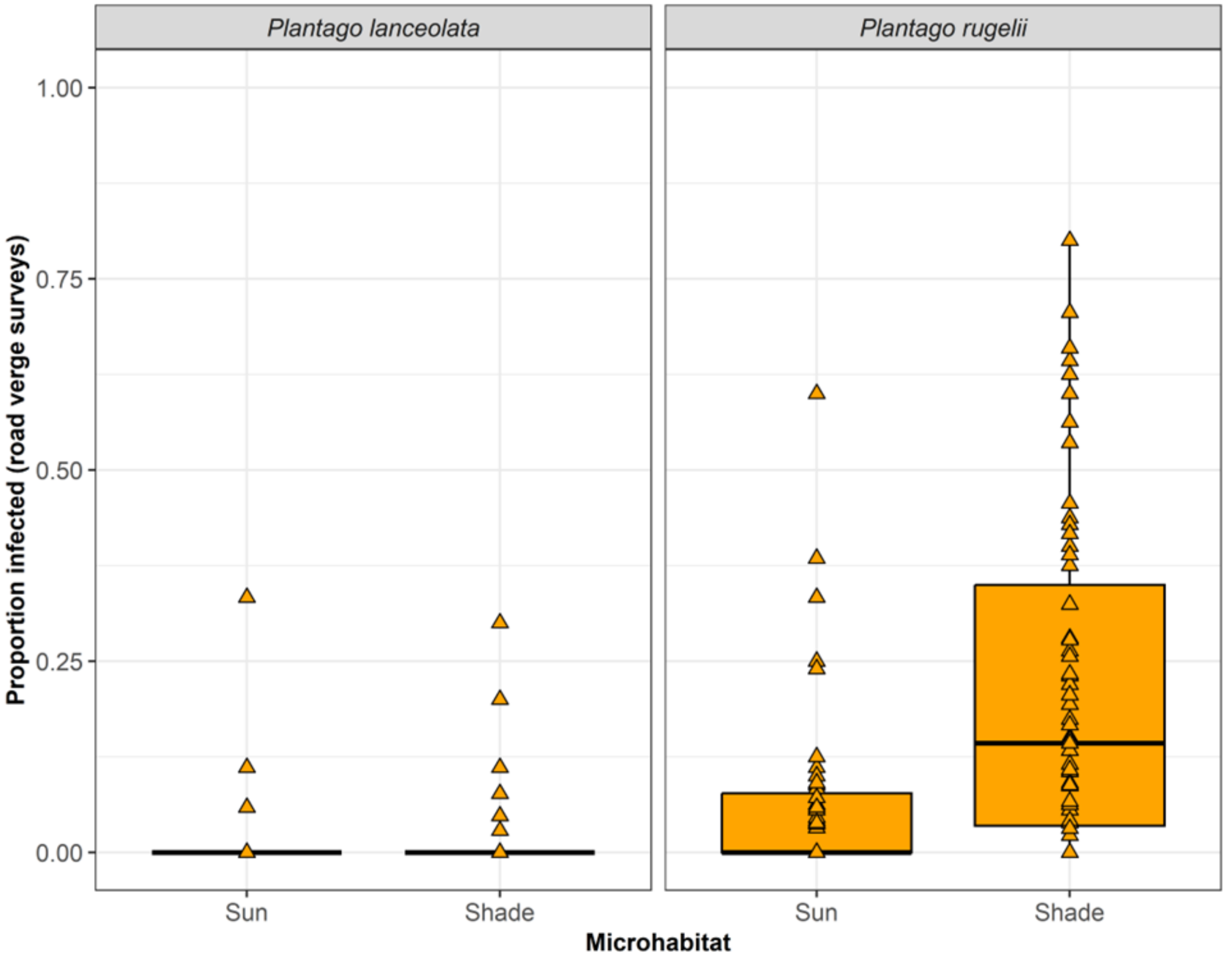
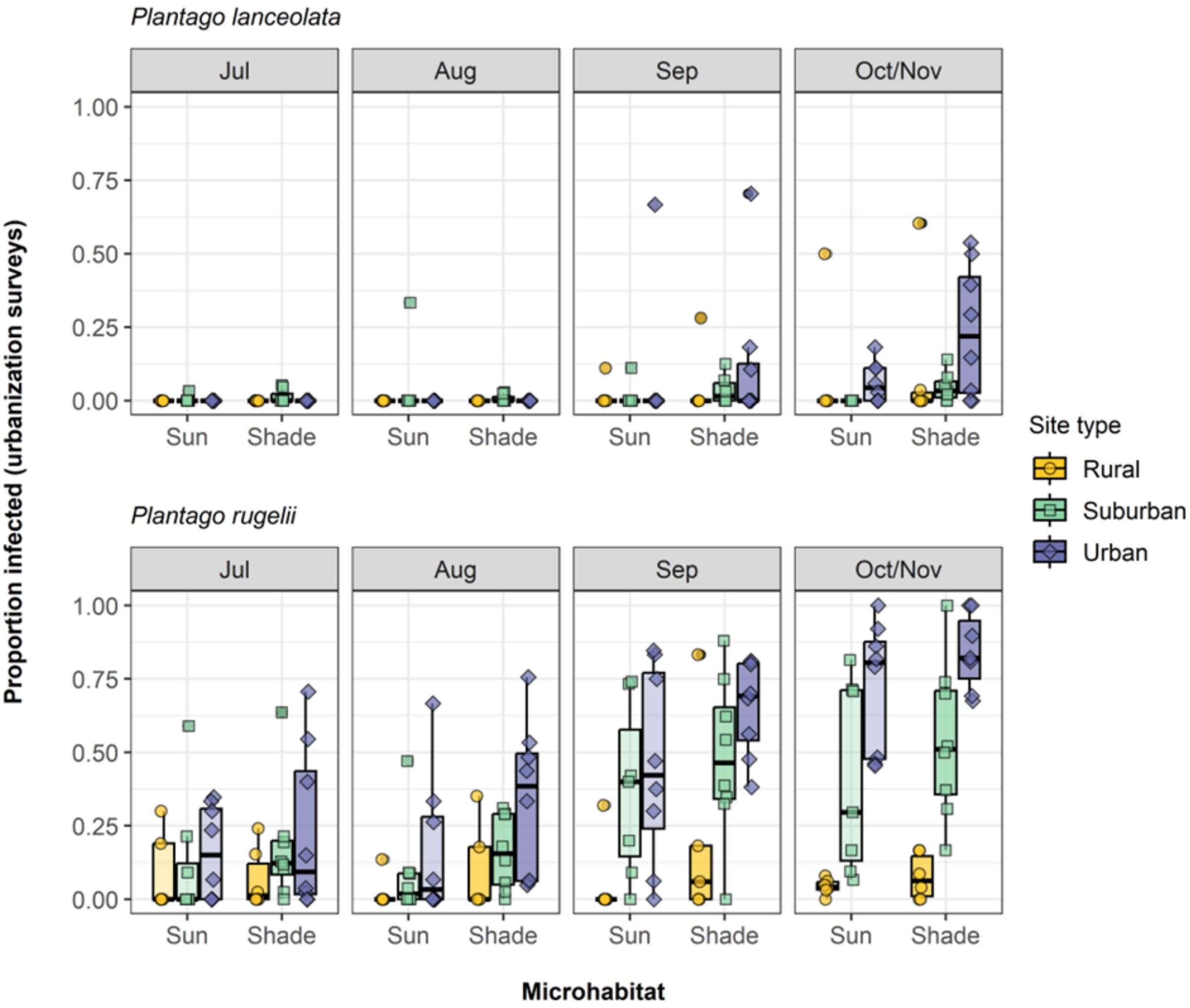

Plantago lanceolata
Plantago rugelii
Proportion infected (road verge surveys)
1.00
0.75
0.50
0.25
0.00
Sun
Shade
Sun
Shade
Microhabitat

Figure 3.



686

687 **Figure 4.**

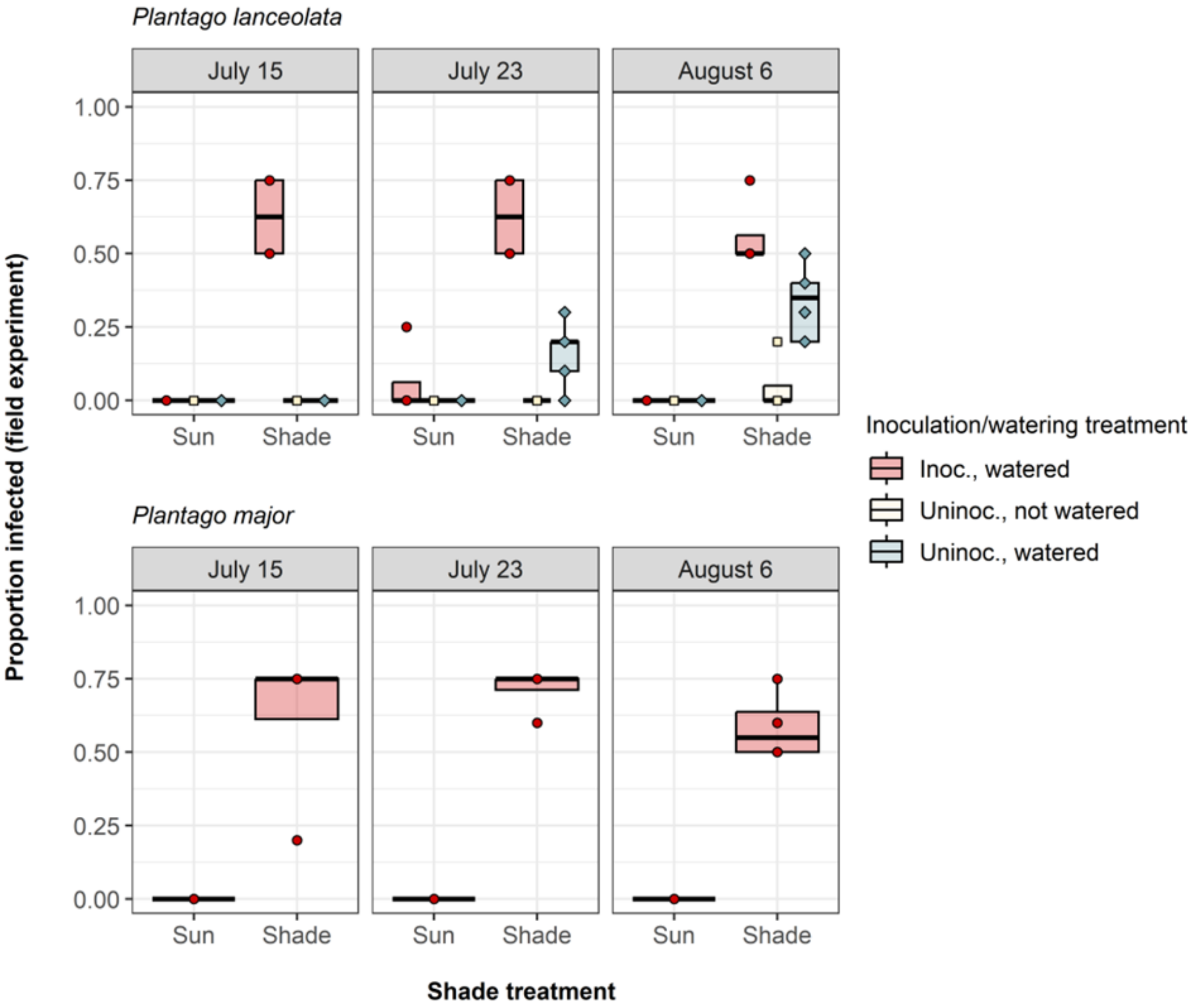

Plantago lanceolata
July 15
July 23
August 6
1.00
0.75
0.50
0.25
0.00
Sun
Shade
Plantago major
Proportion infected (field experiment)
Shade treatment
Inoculation/watering treatment
Inoc., watered
Uninoc., not watered
Uninoc., watered

Figure 5.

Figure Legends

Fig. 1. Maps (Google Earth satellite view) and environmental characteristics of the focal urbanization gradient in St. Louis, Missouri, USA. Road verge surveys: Start locations are shown as 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). Orange box denotes area magnified in Panel B. (b) Zoomed-in view of area where road verge surveys were concentrated (showing those locations only). (c) Biplot of a principal component analysis of environmental variables at the study sites. Black arrows give loadings of percent impervious area (%Imp), percent tree cover (%Tree), intersection density of walkable roads (IntDen), average temperature from June–October (Temp), and nighttime radiance (NightLight). Sites are numbered from west to east (additional site information in Appendix S1: Table S1).

Fig. 2. 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. Photographer: Quinn N. Fox.

Fig. 3. Along road verges, there was greater powdery mildew prevalence in shaded microhabitats. The positive effect of shade on mildew was significant for *Plantago rugelii.* Each point represents a survey of up to 50 individuals in June-July 2020; many points overlap at zero.

Fig. 4. Across the urbanization gradient, there was significantly greater powdery mildew prevalence in shaded microhabitats for both species. On *Plantago rugelii*, mildew epidemics

grew significantly larger in more urban sites. Each point represents a survey of up to 50 individuals. Sites were re-surveyed monthly between July and October (or November) 2020.

Fig. 5. In the field experiment, powdery mildew growth on inoculated plants and subsequent transmission to nearby plants was significantly greater in the shade than sun. *Plantago lanceolata* were divided among three inoculation and watering treatment combinations in both shade and sun microhabitats: inoculated/watered, uninoculated/watered, and uninoculated/not watered. *Plantago major* were all inoculated/watered due to small sample size.