Urbanization alters phenology of plant reproduction, foliar infection, and herbivory

Quinn N. Fox*, Mahal J. Bugay, Eleanor Grant, Olivia Shaw, Keiko Farah,
and Rachel M. Penczykowski

Department of Biology, Washington University in St. Louis, St. Louis, Missouri, 63130, USA

*Corresponding author: qfox@wustl.edu

Abstract

1. Urbanization is associated with numerous environmental changes that may affect the timing of plant reproduction and foliar damage by pathogens, herbivores, and human activities. Yet such effects have not previously been quantified simultaneously in plant populations across levels of urbanization. Thus, it remains unclear how urbanization impacts multiple fitness components of a single plant species. Moreover, it is unclear which effects are consistent between species.

2. We studied 22 populations of two co-occurring *Plantago* species in parks and nature areas spanning rural, suburban, and urban sections of the Greater St. Louis metropolitan area in the central United States. In monthly surveys of *Plantago lanceolata* and *P. rugelii* during summer–fall in two consecutive years, we quantified plant reproductive development and prevalence of powdery mildew infection. In the second year, we additionally quantified prevalence of insect herbivory and mowing damage and placed potted sentinel plants into the field populations to directly measure rates of infection and herbivory.

3. Urbanization was associated with earlier flowering and more seed production for *P. rugelii*, but less seed maturation for *P. lanceolata*. Mildew epidemics on *P. rugelii* started earlier and achieved greater infection prevalence in more urban sites. Correspondingly, sentinels only became infected in suburban and urban sites. By contrast, there was less mildew infection on *P. lanceolata*, including on sentinels, suggesting low availability of pathogen genotypes able to infect this species. Urbanization accelerated early-summer herbivory on both plant species, but did not affect 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.

4. Synthesis: We show that urbanization affects the timing of key reproductive events and multiple types of aboveground damage for wild herbaceous plants. The two plant species

responded differently to urbanization in terms of their reproductive phenology. However, in months when significant effects of urbanization were detected, they were typically in the direction of greater rates of pathogen infection, herbivory, and mowing damage. Overall, these findings suggest multiple mechanisms by which urbanization may impact plant population growth.

Key words: fungal pathogen; global change ecology; human disturbance; plant development and life-history traits; plant phenology; plant-herbivore interactions; plant-pathogen interactions

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). The process of urbanization involves dramatic changes to the environment, typically including increases in habitat fragmentation, frequency of disturbance, coverage by impervious surfaces, temperature, pollution, and introduction of invasive species (Johnson & Munshi-South, 2017). These changes can alter ecological and evolutionary processes that impact the fitness of organisms. For example, a meta-analysis of more than 100 published studies revealed that 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 & Munshi-South, 2017; Rivkin et al., 2019). Notably, only a single 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. Moreover, no studies have simultaneously quantified effects of urbanization on timing of plant reproduction and damage from pathogens, herbivores, and human activities.

One well documented consequence of urbanization is the heat island effect, 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). These increased temperatures can impact plant growth and development. In a study of herbarium records, 24% of herbaceous and woody plant species flowered earlier in urban environments (Neil et al., 2010). However, whether urban heating advances plant phenology depends on baseline climate in the region. For example, flowering date advances with city size only in regions with cool or cold winters, where degree-days are limiting to development (Li et

al., 2019). Moreover, heat stress beyond a plant's thermal optimum can reduce shoot and root growth (Desaint et al., 2021). Quantifying the impact of urban heating on plant growth and development is necessary for understanding consequences of land use change. In addition, studies conducted along spatial temperature gradients can provide insight into how plants will respond to future climate warming (De Frenne et al., 2013).

Urban heating may increase or decrease prevalence of plant diseases, depending on regional climate, magnitude of the urban heat island effect in a given city, and thermal sensitivity of host and pathogen physiological traits. Plant pathogens include fungi, oomycetes, bacteria, and viruses. Here, we focus on fungi because these are the most well studied in wild plant populations (Alexander, 2010; Burdon & 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, urban heating may promote disease. Indeed, urban heat 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 urban heat on disease can further depend on microclimate heterogeneity (Penczykowski et al. 2018), temporal variation in climate (Egerer et al., 2020), and thermal sensitivity of plant defense responses (Desaint et al., 2021; Wang et al., 2009).

In addition to being warmer, green spaces are often more fragmented in urban than rural environments. This fragmentation may cause populations of some plant and pathogen species to be more isolated in urban environments (Dubois & Cheptou, 2017). Alternatively, plant or pathogen species that grow or disperse along roadways may be more highly connected in cities with denser road networks. For example, fungal infection is more likely in populations of ribwort

plantain along roadsides, particularly at hubs of a road network, suggesting that vehicular traffic facilitates spore transport (Numminen & Laine, 2020). Similarly, roads enhance transmission of an oomycete pathogen between populations of Port Orford cedar (Jules et al., 2002). Thus, increasing road density and traffic volume from rural to urban environments could 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). For example, urban warming 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. By contrast, if urban heating causes phenological asynchrony (e.g., insects emerge before their host plants, or after plants have developed morphological or chemical defenses), we would expect less herbivory (Bale et al., 2002). From the few published studies linking urbanization to herbivory, a variety of patterns have been documented (Miles et al., 2019). Positive relationships between urbanization and herbivory have been shown for wolf apple trees in Brazil (Cuevas-Reyes et al., 2013), for red maples in the southeastern United States (Just et al., 2019), and for English oaks in Europe (Moreira et al., 2019). Contrastingly, in an analysis of herbarium samples of three woody species and one understory herb collected over 112 years in the northeastern United States, urbanization was negatively correlated with insect abundance and herbivory (Meineke et al., 2019). Other evidence for changes in herbivory pressure with urbanization comes from clines in production of an antiherbivore chemical defense (hydrogen cyanide, HCN) in white clover (Johnson et al., 2018; Santangelo et al., 2022). Specifically, 75 of 160 cities around the world show clines in white clover HCN production along urban–rural transects, and most clines (62 of 160 cities) are

115 in the direction of decreasing HCN production with urbanization (Santangelo et al., 2022).
116 However, as for plant–pathogen interactions, so few studies have directly quantified rates of
117 herbivory on herbaceous plants across urbanization gradients that the generality of such trends
118 across plant species are unknown.

119 The frequency and intensity of disturbance from human activities including walking and
120 lawn mowing may also increase with urbanization. For herbs growing along paths, roadways, or
121 lawns, human activities may damage or remove leaves or reproductive tissues directly, or affect
122 the probability of damage by pathogens or herbivores. For example, human footsteps increased
123 severity of fungal anthracnose infections on annual bluegrass on a golf course (Roberts &
124 Murphy, 2014). Height of mower blades has also been shown to influence disease severity on
125 golf course grass (Inguagiato et al., 2009; Williams et al., 2001). Yet effects of walking and
126 mowing on plant populations across levels of urbanization remain to be explored.

127 Here, we tested effects of urbanization on phenology of plant reproduction and three
128 types of leaf damage (fungal infection, insect herbivory, and lawn mowing) across a 56-km land
129 use gradient in the Greater St. Louis metropolitan area in Missouri, United States (Fig. 1, Table
130 S1). We did this through monthly field surveys of *Plantago lanceolata* (ribwort plantain) and *P.*
131 *rugelii* (blackseed plantain) populations co-occurring in 22 sites in summer–fall 2019 and 2020.
132 In a separate 2020 field experiment, we placed groups of healthy, pathogen-free "sentinel plants"
133 of these two species into a subset of sites to directly measure rates of infection and herbivory
134 across the urbanization gradient. We hypothesized that urbanization would be associated with:
135 (1) earlier flowering and seed production, (2) earlier and larger powdery mildew epidemics, (3)
136 earlier and overall more prevalent insect herbivory, and (4) 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.

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 they are tractable model organisms for studies of plant–pathogen and plant–herbivore interactions across land use gradients (Penczykowski & Sieg, 2021). *Plantago lanceolata* is native to Eurasia but cosmopolitan in its distribution, and *P. rugelii* is endemic to eastern North America. We did not include the similarly weedy and globally abundant *Plantago major* (common plantain) in our study, because it is rare in our focal geographic region (Penczykowski & Sieg, 2021; Yatskievych & Steyermark, 1999).

Both species produce flower stems bearing spikes of many small, wind-pollinated flowers. Individuals can flower repeatedly over the growing season. The flowers are typically monecious, though *P. lanceolata* are sometimes gynodioecious (Ross 1973). Fertilization of the flowers can only occur via outcrossing for *P. lanceolata* (i.e., it is self-incompatible), while *P. rugelii* can successfully self-pollinate (Hawthorn, 1974; Ross, 1973; Sagar & Harper, 1964). Fruits then develop within seed capsules that can be visually assessed for maturity.

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 & 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 & Laine, 2006). Powdery mildews overwinter via sexual resting structures that release spores when conditions are favorable in spring (Tack & Laine, 2014).

Plantago are also host to numerous invertebrate herbivores (Penczykowski & Sieg, 2021). Here, we focus on two types of distinctive insect herbivory: leaf mines and chewing damage. Leaf mines on *Plantago* are created by larvae of the leaf miner fly *Phytomyza plantaginis* (Penczykowski & 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 & Sieg, 2021).

Focal populations

We investigated the relationships between urbanization and plant phenology, powdery mildew infection, and insect herbivory in a total of 22 sites with co-occurring *P. lanceolata* and *P. rugelii*. These sites were parks and nature areas extending from downtown St. Louis City to the southwest along the I-44 interstate highway corridor (Fig. 1a, Table S1). 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. We added two sites at Shaw Nature Reserve in August and September 2019, and we added a site at Forest Park in June 2020 (Table S1).

We classified the 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 8-55 miles per hour) (Fig. 1b). The temperature data represented long-term (1970-2000) averages at these sites during the focal months of our study (WorldClim 2; Fick & 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). However, some rural sites were located outside the boundaries of that urban-centered dataset. Thus, for rural sites #1, 2, and 3 (Table S1), 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). For these, 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).

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 walk through each population, we stopped every few steps and selected the nearest plant of either species that was at least 1 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. S1; sizes estimated from polygons in Google Earth). Rural survey areas tended to be smaller because those sites were typically clearings 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 status of the most mature flower stem on the plant, if present, using the following ordered categories: (0) no flower stem or 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. We recorded the powdery mildew

229 infection status for each plant as the presence or absence of conspicuous white mycelia and/or
230 conidia on the leaf surface. In 2020, to assess whether the infection statuses of the selected plants
231 were representative of nearby conspecifics, we additionally recorded the numbers of infected
232 conspecifics within a 1.5-m radius of each focal individual, using the following categories: (0) 0,
233 (1) 1-10, (2) 11-50, (3) 51-100, and (4) 101 or more plants. In 2020, we also recorded the
234 presence or absence of two main types of herbivory damage (chewing and leaf mines on leaves)
235 and mowing damage (straight cuts across leaves).
236
237 *Sentinel experiment*
238 In July 2020, we placed healthy, greenhouse-grown "sentinel" plants into 15 of our field
239 survey sites to directly test rates of powdery mildew infection and herbivory across the
240 urbanization gradient. These sites (five each of urban, suburban, and rural) were chosen from the
241 full set of 22 sites based on presence of mildew on at least one of the *Plantago* species in 2019.
242 We selected sites based on that criterion to avoid biasing the outcome of the experiment towards
243 rejection of the null hypothesis that infection prevalence would be the same across site types.
244 Sentinel plants were grown from *P. lanceolata* and *P. rugelii* seeds collected from wild,
245 uninfected plants in St. Louis City and County during July–October 2019. To capture variation in
246 plant genetic background, we planted seeds from six maternal lines of *P. lanceolata*, and five of
247 *P. rugelii*. Each maternal line consisted of a single seed spike from a mother plant. Seeds were
248 sown in BM6 All-Purpose soil (Berger) on 16 May 2020 in the Jeanette Goldfarb Plant Growth
249 Facility at Washington University in St. Louis. All six *P. lanceolata* lines but only three of the *P.*
250 *rugelii* lines successfully germinated. On 1 June, we moved the seedlings to a hoop house at
251 Tyson Research Center (Eureka, Missouri) and transplanted each into a 4.5-in diameter pot. To

252 ensure that sentinels remained uninfected before deployment into the field, we covered each with
253 an autoclaved, spore-proof pollination bag (PBS International, model 3D.55), which we secured
254 around the pot with a silicone band. Three times per week, we placed the sentinels in a shallow
255 pool of water for 10 min, allowing water to soak up through the base of the pot.

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

270 Between 14-16 July 2020, sentinels were retrieved from the field sites in the same order
271 they were deployed. Before removal from the field, we recorded the number of leaves, length
272 and width of largest leaf, and presence/absence of chewing and leaf miner herbivory for each
273 leaf. Then we re-covered each sentinel with a pollination bag and moved them to a common
274 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.

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). 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 (zeros) or subtracted (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.

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. 1b). The pre-classified site types (rural, suburban, and urban) were primarily separated along PC1,

322 which was associated with greater temperature, nighttime radiance, intersection density, and
323 percent impervious area, and with lower percent tree cover (Fig. 1b). Variation within site types
324 occurred primarily along PC2, which was associated with greater temperature, nighttime
325 radiance, and percent tree cover, and with lower percent impervious area (Fig. 1b).
326
327 *Field surveys: plant reproductive phenology*
328 In both 2019 and 2020, *P. lanceolata* populations had begun flowering in all site types
329 before our first survey, the frequency of flowering peaked in July, and frequency of mature
330 and/or dispersed seeds peaked in August (Fig. 2a,c). For *P. lanceolata* in 2019, parameters could
331 not be uniquely determined for the model of reproductive development through time when
332 October data were included. Excluding October 2019 from the analysis, the effect of site type on
333 *P. lanceolata* reproductive development (as an ordered categorical variable) varied through time
334 in both years (site type x month interaction in 2019: Likelihood Ratio [LR] $\chi^2 = 20.40$, df = 4, P
335 = 0.0004; in 2020: LR $\chi^2 = 51.52$, df = 8, P < 0.0001). Post-hoc Tukey's tests showed that, in
336 2019, maturity was marginally lower in urban and/or suburban than rural sites in July (urban-
337 rural: P = 0.050, suburban-rural: P = 0.068) and August (suburban-rural: P = 0.060), with no
338 other differences between site types within a given month (P > 0.10 for all unreported pairwise
339 contrasts; Fig. 2a). In 2020, *P. lanceolata* reproductive development was marginally lower in
340 urban than rural sites in August (P = 0.079) and significantly lower in urban than either suburban
341 or rural sites in September (urban-rural: P = 0.036, urban-suburban: P = 0.008; Fig. 2c).
342 In both years, flowering continued at a higher rate into August for *P. rugelii* compared to
343 *P. lanceolata*, and overall rates of seed production and maturation were also much higher for *P.*
344 *rugelii* (Fig. 2). The effect of site type on *P. rugelii* reproductive development varied through

345 time in both years (site type x month interaction in 2019: LR χ^2 = 115.72, df = 6, P < 0.0001; in
346 2020: LR χ^2 = 128.98, df = 8, P < 0.0001; Fig. 2b,d). Post-hoc tests revealed that *P. rugelii*
347 development was significantly lower in suburban than either rural (P = 0.0092) or urban (P =
348 0.0002) sites in September 2019, but increased with level of urbanization in October 2019
349 (suburban-rural: P = 0.0001, urban-rural: P < 0.0001, urban-suburban: P = 0.009; Fig. 2b). Our
350 June 2020 survey captured significantly earlier *P. rugelii* development with increasing levels of
351 urbanization (suburban-rural: P = 0.018, urban-rural: P < 0.0001, urban-suburban: P = 0.0011;
352 Fig. 2d). Maturity of *P. rugelii* was greater in urban than rural sites in both August and
353 September 2020 (P = 0.025 and P = 0.043, respectively), and greater in both suburban and urban
354 than rural sites in October/November 2020 (P < 0.0001 for both contrasts).
355
356 *Field surveys: powdery mildew infection*
357 There was very little powdery mildew infection on *P. lanceolata* in 2019, and generally
358 small epidemics in 2020 (Fig. 3a,c). For this species, there were no significant site type x month
359 effects on infection prevalence (in 2019: LR χ^2 = 4.00, df = 6, P = 0.68; in 2020: LR χ^2 = 10.34,
360 df = 8, P = 0.24), nor significant main effects of site type (in 2019: LR χ^2 = 2.43, df = 2, P =
361 0.30; in 2020: LR χ^2 = 3.36, df = 2, P = 0.19). There was a significant main effect of month in
362 2020 only (in 2019: LR χ^2 = 3.92, df = 3, P = 0.27; in 2020: LR χ^2 = 47.64, df = 4, P < 0.0001).
363 There were large powdery mildew epidemics on *P. rugelii* in both years, and epidemics
364 started earlier in suburban and urban populations (Fig. 3b,d). In both years, *P. rugelii* infection
365 prevalence was significantly affected by site type (in 2019: LR χ^2 = 8.89, df = 2, P = 0.012; in
366 2020: LR χ^2 = 22.00, df = 4, P < 0.0001) and month (in 2019: LR χ^2 = 22.57, df = 3, P < 0.001;
367 in 2020: LR χ^2 = 70.42, df = 4, P < 0.001), but there were no site type x month interactions (in

368 2019: LR χ^2 = 4.39, df = 6, P = 0.62; in 2020: LR χ^2 = 3.90, df = 6, P = 0.87). For 2019, post-hoc
369 testing revealed significantly greater infection prevalence in urban than rural sites (suburban-
370 rural: P = 0.067, urban-rural: P = 0.037; Fig. 3b). In 2020, there was significantly greater
371 infection prevalence in both suburban and urban relative to rural sites (suburban-rural: P =
372 0.0061, urban-rural: P = 0.0007; Fig. 3d).

373 The infection status of surveyed plant individuals was consistent with that of nearby
374 plants in the population. Specifically, presence/absence of powdery mildew infection on
375 surveyed plants was significantly positively related to the abundance of infected conspecifics
376 within a 1.5-m radius (*P. lanceolata:* χ^2 = 262.81, df = 3, P < 0.0001; *P. rugelii:* LR χ^2 = 1097.6,
377 df = 3, P < 0.0001; Fig. S2).

378

379 *Field surveys: herbivory*

380 For both species, prevalence of leaf mines depended on the interaction of site type and
381 month (*P. lanceolata*: χ^2 = 16.37, df = 8, P = 0.037; *P. rugelii*: χ^2 = 55.84, df = 8, P < 0.0001;
382 Fig. 4a,b), and the only significant differences between site types occurred in June. For *P.*
383 *lanceolata,* there was significantly greater prevalence of leaf mines in urban than suburban sites
384 in June (urban-rural: P = 0.092, urban-suburban: P = 0.0083). For *P. rugelii*, there was greater
385 prevalence of leaf mines in urban than either suburban or rural sites in June (urban-rural: P =
386 0.0017, urban-suburban: P = 0.0001).

387 Chewing-type herbivory also depended on the interaction between site type and month
388 (*P. lanceolata*: χ^2 = 66.17, df = 8, P < 0.0001; *P. rugelii*: χ^2 =88.51, df = 8, P < 0.0001; Fig.
389 4c,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: $P < 0.0001$).

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. 5a).

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, $P = 0.014$; month: $\chi^2 = 23.44$, df = 4, $P = 0.0001$; Fig. 5b). 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. 5b).

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. 6a). This infected sentinel happened to be 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. S3; 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. 6a). No *P. lanceolata* sentinels became infected in Tilles Park, which

413 did have a small number of infected wild plants at that time (Fig. 6a), likely because the sentinels
414 were located almost 6 m from the nearest wild infected plant (Fig. S3).

415 Many *P. lanceolata* sentinels experienced herbivory during their week in the field;
416 however, there were no differences between site types for severity (proportion of leaves
417 damaged) of either leaf mines ($\chi^2 = 4.17$, df = 2, P = 0.12) or chewing ($\chi^2 = 3.82$, df = 2, P =
418 0.15). Prevalences of leaf mines and chewing (proportion of plants affected) were not correlated
419 between sentinels and wild *P. lanceolata* in the same sites during July 2020 (leaf mines: r = -
420 0.03, P = 0.92, Fig. 6c; chewing: r = -0.14, P = 0.62, Fig. 6e).

421 All 36 *P. rugelii* sentinels survived to the end of the experiment. Eight of these became
422 infected with powdery mildew in urban sites (Clifton Park and Tilles Park), three became
423 infected in a single suburban site (Kirkwood Park) and none became infected in rural sites (Fig.
424 6b). Although this trend was consistent with the greater prevalence of infection observed in more
425 urban populations of wild *P. rugelii* in 2020 (Fig. 3d), there was no significant correlation
426 between prevalence of infection on sentinels and wild plants surveyed in those sites during July
427 (P = 0.95, Fig. 6b). Instead, the proportion of infected *P. rugelii* sentinels increased with
428 proximity to wild infected plants within the site (P = 0.048, Fig. S3).

429 Only a single *P. rugelii* sentinel acquired a leaf mine during the field exposure. *Plantago*
430 *rugelii* sentinels experienced chewing-type herbivory in most field sites (Fig. 6f), and severity of
431 chewing damage did not differ between site types ($\chi^2 = 1.25$, df = 2, P = 0.53). Prevalence of
432 herbivory on *P. rugelii* sentinels was not correlated with that on wild conspecifics in those sites
433 during July 2020 (leaf mines: r = 0.15, P = 0.71, Fig. 6d; chewing: r = -0.52, P = 0.15, Fig. 6f).
434

Discussion

We found that urbanization affected the timing of key reproductive events and seasonal prevalence of multiple types of foliar damage for wild herbaceous plants. 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 *P. rugelii*, but less seed maturation for *P. lanceolata*. These species-specific effects on reproductive phenology were broadly consistent between the two years. Another consistent trend between years was that powdery mildew epidemics on *P. rugelii* started earlier and achieved greater infection prevalence in more urban sites. Correspondingly, *P. rugelii* sentinels only became infected in suburban and urban sites. By contrast, 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 accelerated early-summer herbivory on both plant species, but did not affect 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 accelerated reproductive development of *P. rugelii*, consistent with trends for many other species (e.g., Neil et al., 2010; Li et al. 2019). However, there were neutral-to-negative effects of urbanization on reproductive development of *P. lanceolata*. One possible explanation for this difference involves the differential impact of mowing on flower stems of these species. *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, *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 *P. lanceolata* than *P. rugelii* (Fig. 2). Therefore, more frequent mowing in suburban and urban sites may have reduced the abundance of flower and seed spikes on *P. lanceolata* but not affected the proportion of *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 *P. lanceolata* compared to *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.

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 urban heat island effect 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 urban heat island effect 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. S4), consistent with long-term trends in this region (Fig. 1b). Thus, as for powdery mildew on

481 oaks (van Dijk et al., 2022), warmer temperatures may have contributed to the increased
482 infection prevalence of *P. rugelii* in suburban and urban populations.

483 Our observation of increased mildew prevalence with urbanization could also be due to
484 greater rates of spore arrival to more highly connected urban sites. Dispersal along roadside
485 populations could allow mildew to spread between sites over the course of the growing season
486 (Numminen & Laine, 2020). Vehicular traffic can also facilitate transport of particles including
487 seeds (Lippe et al., 2013) and spores (Numminen & Laine, 2020). Hence, denser road networks
488 surrounding urban parks may bring more mildew spores. However, arriving spores are only
489 successful at infecting if they land on susceptible plant genotypes, and *Plantago* populations can
490 vary in their susceptibility to arriving mildew strains (Höckerstedt et al., 2018; Jousimo et al.,
491 2014; Laine, 2004). Thus, greater resistance of rural *P. rugelii* to mildew could also have
492 decreased their prevalence of infection, even if rates of spore arrival were equally high across the
493 land use gradient. The fact that none of the *P. rugelii* sentinel plants became infected in rural
494 sites is consistent with low spore arrival in those sites, though we note that sample sizes of *P.*
495 *rugelii* sentinels were small. At the same time, there were lower rates of infection on wild *P.*
496 *lanceolata* compared to *P. rugelii* growing in the same sites, as well as near-zero prevalence of
497 infection among the >300 *P. lanceolata* sentinels placed across the urbanization gradient. These
498 results suggest species-specificity of the pathogen strains, and lower regional abundance of
499 spores able to infect *P. lanceolata*. Aerial spore sampling and genotyping of mildew infections
500 collected from both *Plantago* species across the urbanization gradient will provide further
501 insights into the connectivity of pathogen populations within and between land use types.

502 We hypothesized that early-summer prevalence of insect herbivory would be elevated in
503 urban populations, due to urban heat island effects 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 course of 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, prevalence of leaf mines became similar across site types, peaking at a moderate level in July-August. Meanwhile, 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. In general, 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). Yet 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 & Neef, 2008). Access to urban 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). Given these important roles of urban plants, it is critical to resolve the mechanisms by which urban environments impact their fitness. In this study, we showed how plant reproductive

527 development and prevalence of disease, herbivory, and mowing damage varied through time
528 across an urbanization gradient. Our study is the first to test for differences in fungal disease
529 prevalence in wild herbaceous plant populations across levels of urbanization. More importantly,
530 because we quantified effects of urbanization on multiple plant traits and sources of damage
531 through the growing season, our findings point to several mechanisms by which changes in land
532 use may impact plant population growth.
533

534 **Author Contributions**

535 QNF, MJB, and RMP conceived of the study design. QNF, MJB, EG, OSS, and KNF
536 performed field surveys. QNF, OSS, and KNF performed the sentinel experiment. RMP provided
537 logistical support for the field surveys and sentinel experiment. QNF and RMP performed
538 statistical analyses. QNF wrote the first draft, and all authors contributed to subsequent drafts of
539 the manuscript.
540

541 **Data Availability**

542 Data and code for analyses will be archived at Dryad upon acceptance of the article.
543

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Figure Legends

Fig. 1. (a) Google Earth satellite view of the focal urbanization gradient into 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 for additional site information).

Fig. 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).

Fig. 3. 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.

Fig. 4. 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.

Fig. 5. 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.

Fig. 6. 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).

Figure 1

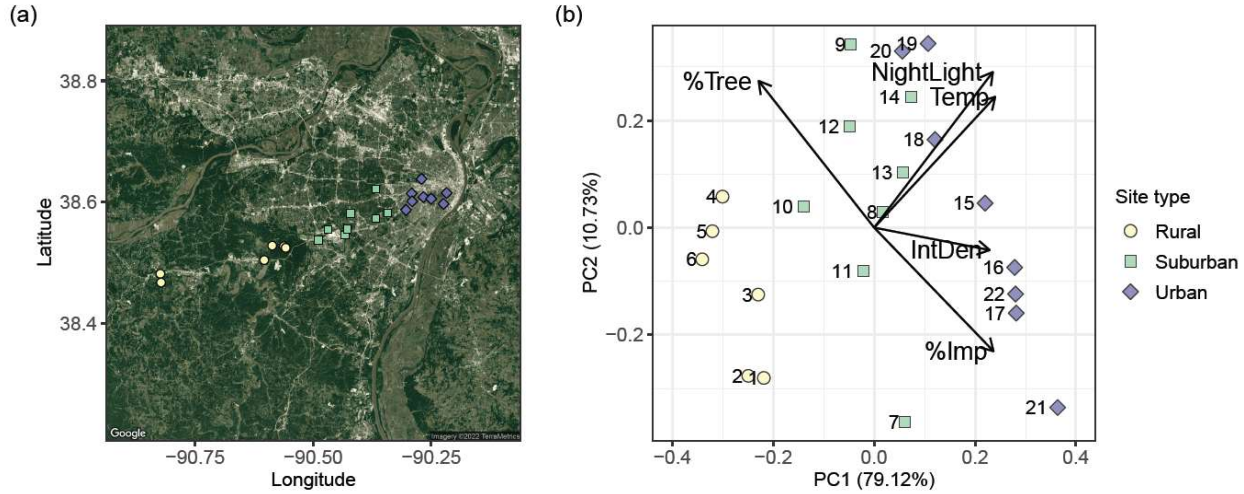

(a)
38.8
38.6
38.4
Latitude
−90.75
−90.50
−90.25
Longitude
Google
(b)
%Tree
NightLight
Temp
IntDen
%Imp
PC2 (10.73%)
PC1 (79.12%)
0.2
0.0
−0.2
−0.4
0.4
Site type
Rural
Suburban
Urban

Figure 2

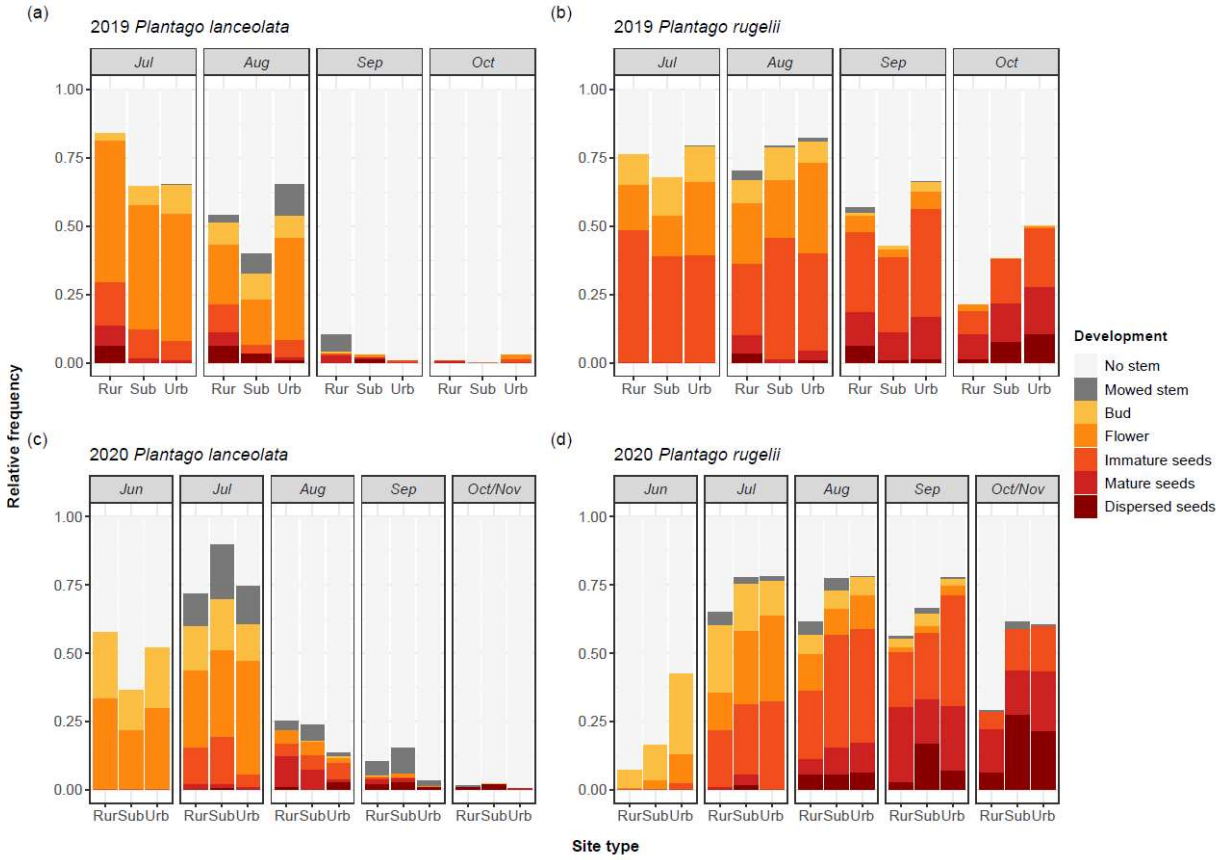

(a)
2019 Plantago lanceolata
Jul
Aug
Sep
Oct
(b)
2019 Plantago rugelii
(c)
2020 Plantago lanceolata
Jun
Oct/Nov
(d)
2020 Plantago rugelii
Relative frequency
1.00
0.75
0.50
0.25
0.00
Rur Sub Urb
Site type
Development
No stem
Mowed stem
Bud
Flower
Immature seeds
Mature seeds
Dispersed seeds

Figure 3

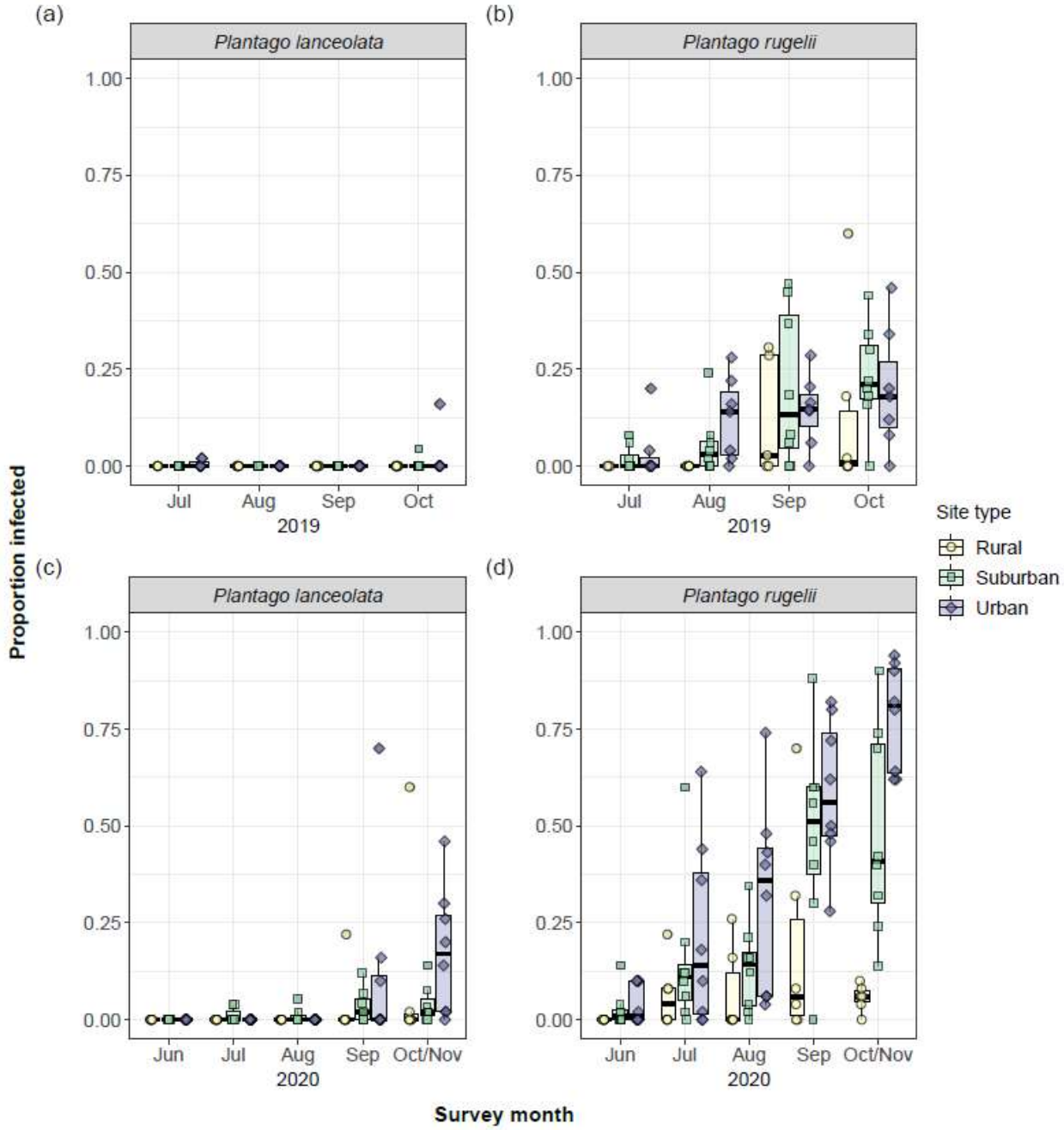

(a)
Plantago lanceolata
(b)
Plantago rugelii
(c)
Plantago lanceolata
(d)
Plantago rugelii
Proportion infected
1.00
0.75
0.50
0.25
0.00
Jul
Aug
Sep
Oct
2019
Jun
Jul
Aug
Sep
Oct/Nov
2020
Survey month
Site type
Rural
Suburban
Urban

Figure 4

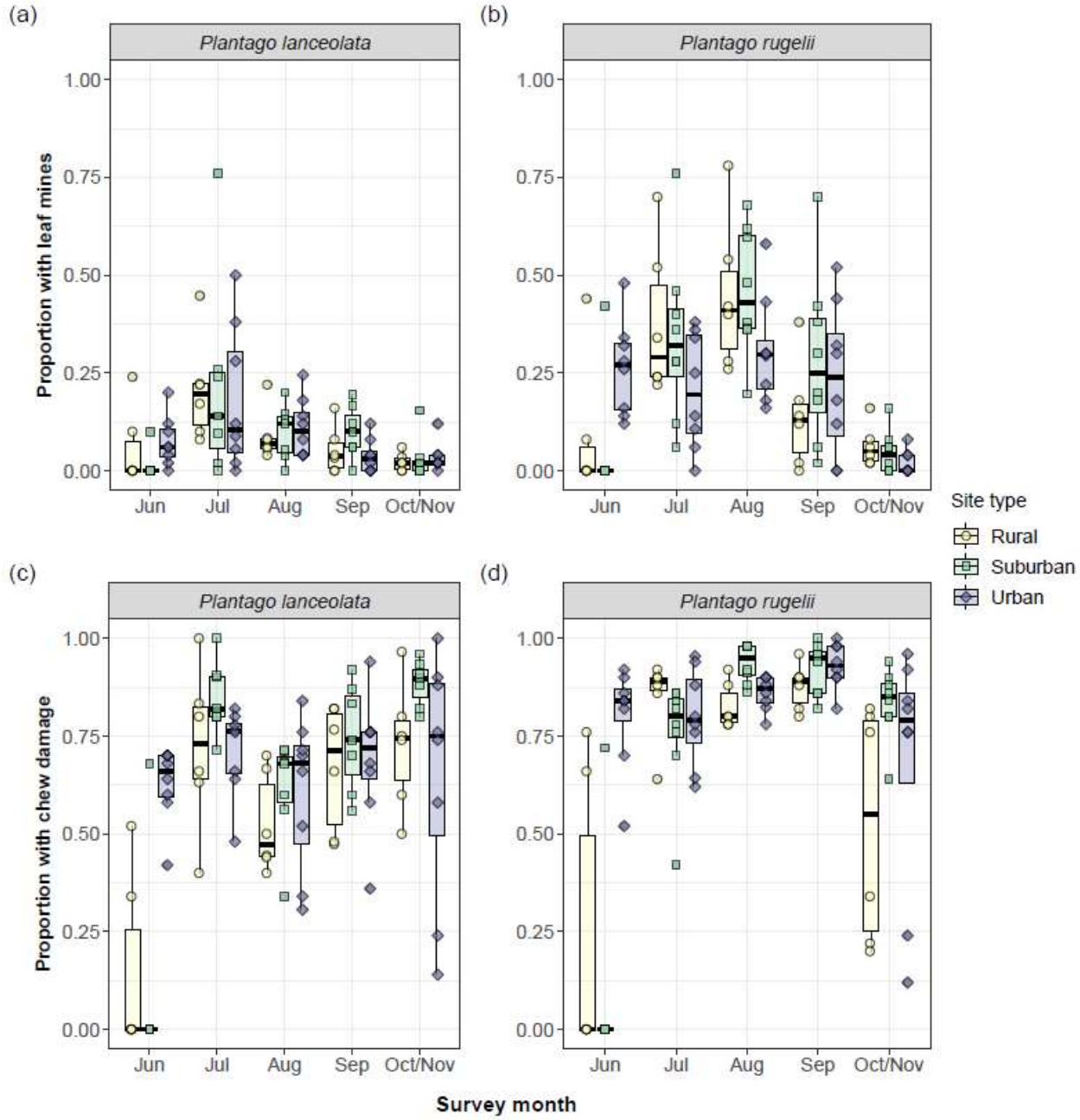

(a)
Plantago lanceolata
(b)
Plantago rugelii
(c)
Plantago lanceolata
(d)
Plantago rugelii
Proportion with leaf mines
Proportion with chew damage
1.00
0.75
0.50
0.25
0.00
Jun
Jul
Aug
Sep
Oct/Nov
Survey month
Site type
Rural
Suburban
Urban

Figure 5

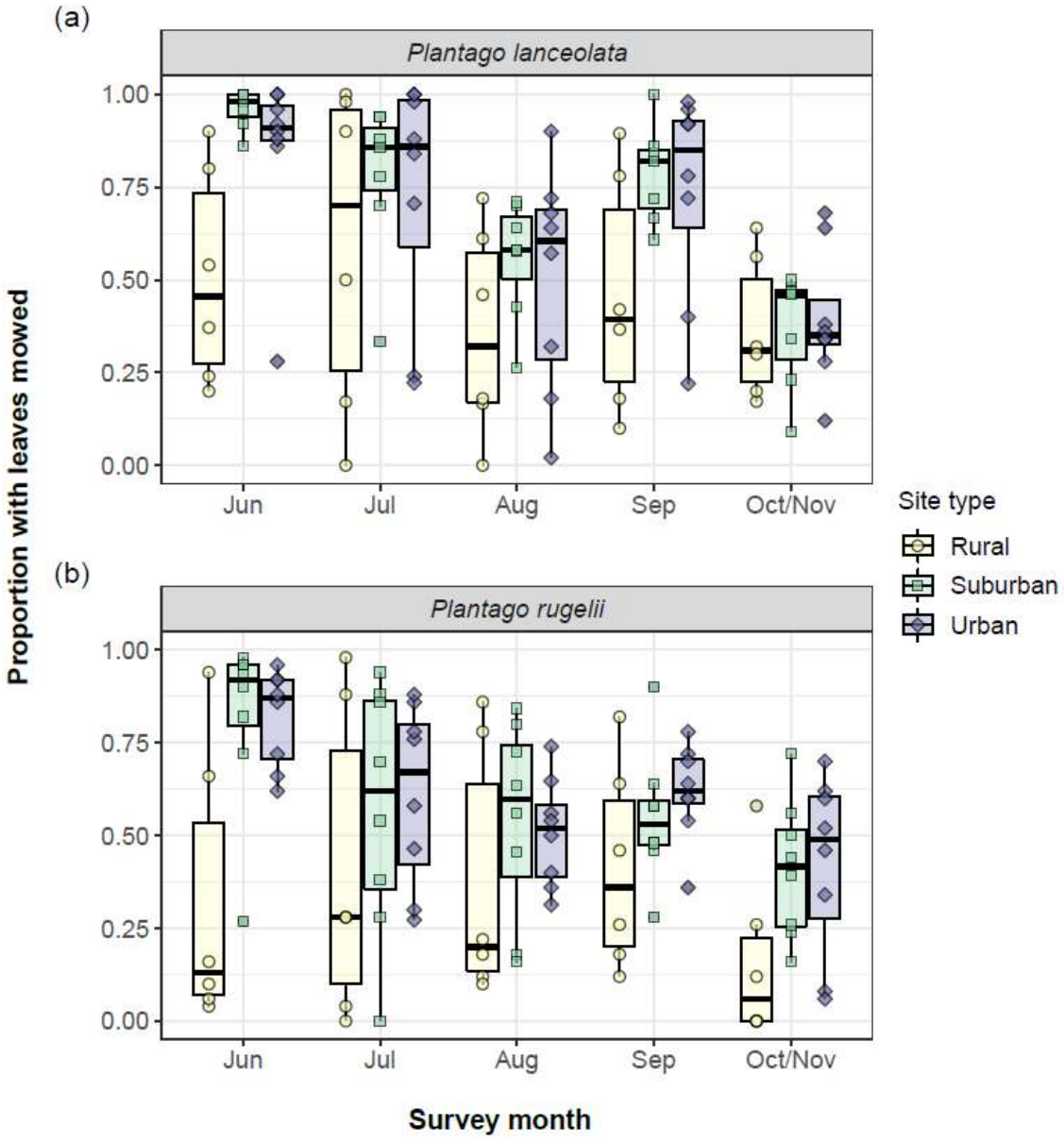
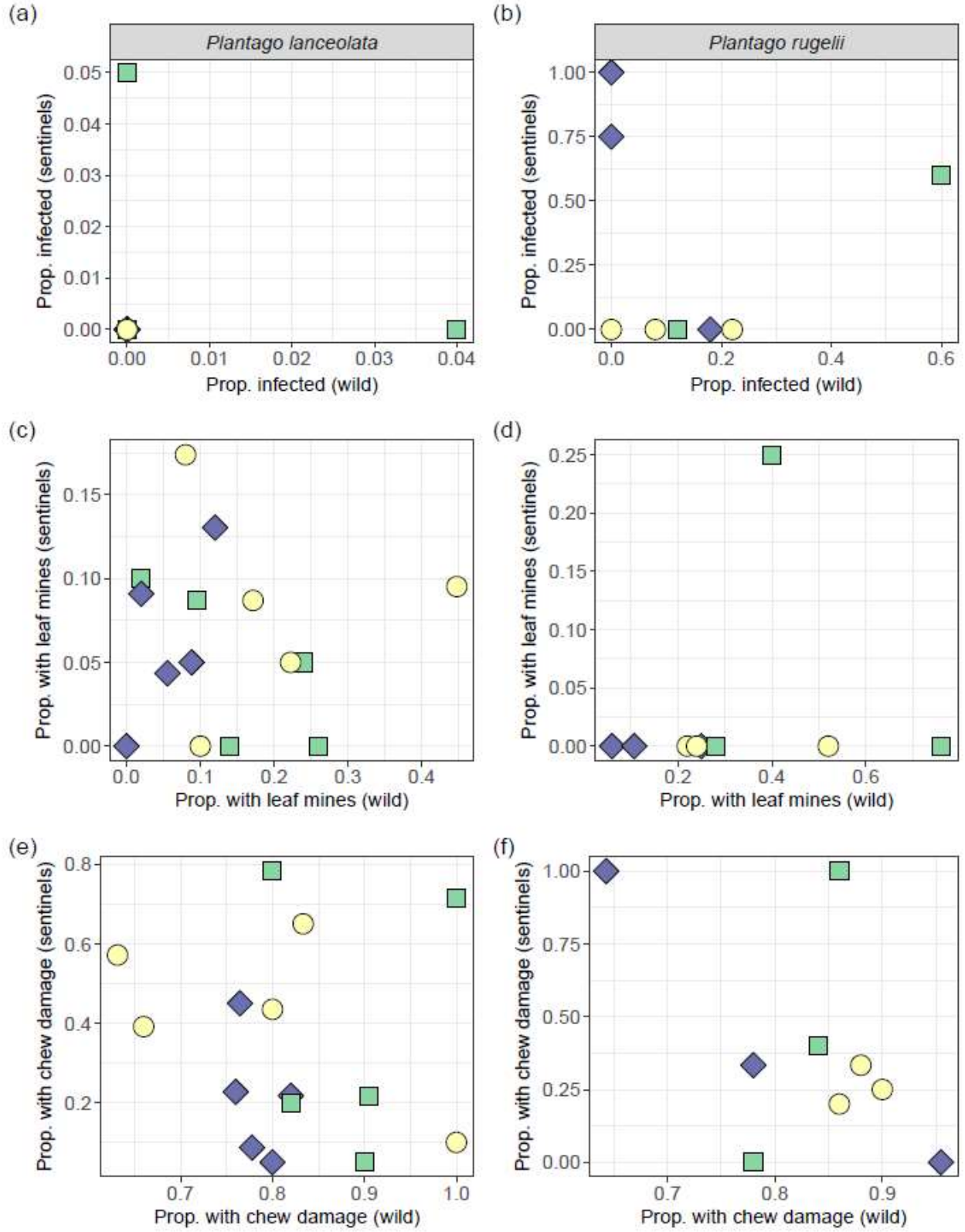

(a)
Plantago lanceolata
1.00
0.75
0.50
0.25
0.00
Jun
Jul
Aug
Sep
Oct/Nov
(b)
Plantago rugelii
Proportion with leaves mowed
Survey month
Site type
Rural
Suburban
Urban

Figure 6


(a)
Plantago lanceolata
Prop. infected (sentinels)
Prop. infected (wild)
(b)
Plantago rugelii
Prop. infected (sentinels)
Prop. infected (wild)
(c)
Prop. with leaf mines (sentinels)
Prop. with leaf mines (wild)
(d)
Prop. with leaf mines (sentinels)
Prop. with leaf mines (wild)
(e)
Prop. with chew damage (sentinels)
Prop. with chew damage (wild)
(f)
Prop. with chew damage (sentinels)
Prop. with chew damage (wild)