

Community Structure, Diversity, and Population Dynamics of Black Poplar Leaf Miners and Galling Aphids in the Urban Environment

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ABSTRACT

Trees in the urban environment, such as the black poplar (*Populus nigra* L.), are strongly affected by environmental changes, which can even lead to their decline. Since the development of large cities on the river banks, black poplar is losing its habitat, but is also threatened by factors such as pollution, climate change, and altered habitat characteristics. These changes affect the insects feeding on black poplar, especially the endophagous species, which are in close contact with the host through their entire development, such as the leaf miners (LMs) and galling aphids (GAs). That is why we examined the community structure, diversity, and population dynamics of black poplar LMs and GAs. The study was conducted in Belgrade, Serbia, on five urban sites differing in vegetation characteristics. Sampling was conducted once per month in the period May-September, in 2016 and 2017. Thirteen species of LMs and seven GAs were identified on the studied trees. The seasonal and site differences affected the LM and GA communities, showing their different adaptation strategies for coexistence on the same host, while the effect of the study year was modest. Greater GAs species richness and LMs diversity were recorded on sites with higher participation of canopy and green area cover. The results highlight the importance of urban green structure for sustaining specialized herbivorous insects and suggest that LMs and GAs on black poplar can serve as sensitive indicators of ecological quality in urban environments.

Keywords: abundance; green areas; canopy cover; *Populus nigra* L.; seasonal dynamics; species richness

INTRODUCTION

With increasing urbanization, many tree species are disappearing from the cities or are being suppressed to the city edges (Helen et al. 2019, Korányi and Markó 2022, Łukaszkiwicz et al. 2024, Xie et al. 2024). One such species is the black poplar (*Populus nigra* L.), a key tree species of floodplain and riverbank ecosystems in the basins of major European rivers (Lefèvre et al. 2001, de Rigo et al. 2016, Łukaszkiwicz et al. 2024). Black poplars are ecologically significant as they stabilize and remedy the soil in sensitive habitats, sustain great biodiversity, and improve air quality due to their rapid growth and large leaf area (Gilman and Watson 1994, Lefèvre et al. 2001, Šiler et al. 2014, de Rigo

et al. 2016, Levei et al. 2021, Łukaszkiwicz et al. 2024). However, several of its traits are considered a drawback for urban environments: its brittle branches break easily and can pose safety risks, its strong roots can damage infrastructure, and its abundant pollen and fluffy seeds can cause discomfort to people (Gilman and Watson 1994, Costello et al. 1997, Minić et al. 2020, Costache et al. 2021, Shams et al. 2021, Łukaszkiwicz et al. 2024).

Besides the direct effect of urbanization on the decline of black poplar from the urban environment, the trees in the cities can be affected in many ways (Rickman and Connor 2003, Dobrosavljević et al. 2020, 2023, Korányi and Markó 2022, Percival 2023, Franceschi et al. 2023, Gao et al. 2023). Elevated temperatures and air pollution

in the urban environment can cause significant stress, which is further increased by altered soil properties and groundwater regimes (Percival 2023, Franceschi et al. 2023, Gao et al. 2023). These, and other environmental disturbances, negatively affect organisms trophically connected to the black poplar trees (Southwood 1961, Schowalter 1995, Dobrosavljević et al. 2023). Among these organisms, insects are the most numerous and significant (Southwood 1961, Lefèvre et al. 2001, Vanden Broeck 2003). The majority of them are herbivorous species, which are highly sensitive to environmental change (Raupp et al. 2010, Valdés-Correcher et al. 2022, Dobrosavljević et al. 2023). They are affected both directly and indirectly, through the host plants (Schowalter 2006, Jones and Leather 2012, Sallé et al. 2021). The most sensitive are the species that cannot migrate far from their hosts, and those that develop in close contact with their hosts, such as the leaf miners and gall-inducing insects (Moir et al. 2014, Dobrosavljević et al. 2020, 2023, Sallé et al. 2021, Valdés-Correcher et al. 2022). They respond to environmental changes by altering their population spatial distribution and seasonal dynamics (Sunil et al. 2023, Boggs 2024, Ojija et al. 2025). LMs and GAs are diverse insect groups with numerous species that are taxonomically well studied in the study area and globally distributed across a wide range of habitats (Hařaj and Osiadacz 2013, Dobrosavljević et al. 2017, Ellis 2020, Dobrosavljević and Marković 2024).

As many European capitals are located near big rivers, the black poplar is becoming increasingly endangered (Lefèvre et al. 2001, de Rigo et al. 2016). Its status is further threatened by the practice of conversion of wetlands to agricultural land, which is now largely prohibited across Europe (Ramsar Convention Secretariat 2016). To determine how the insect community inhabiting black poplar behaves in an urban setting, we investigated the community structure, diversity, and population dynamics of LMs and GAs. The study aimed to determine the effect of (1) interannual variation, (2) seasonal dynamics, and (3) site characteristics on the LMs and GAs abundance, species richness, and diversity in the urban environment.

MATERIALS AND METHODS

Study Design and Sampling

The study was conducted in 2016 and 2017 on five sites in Belgrade: Ada Ciganlija (44°47'09"N, 20°24'41"E), Dorćol (44°49'49"N, 20°27'29"E), New Belgrade block 69 (44°48'11"N, 20°25'14"E), New Belgrade Studenjak (44°49'32"N, 20°24'00"E), and Rakovica (44°46'13"N, 20°26'23"E). On each site, five trees measuring at least 30 cm in diameter and at least 10 m in height were selected. Once per month in the period May-September, five 50-100 cm long lateral branches with leaves were collected from each tree to a height of about 4 m (5 branches · 5 trees · 5 sites = 125 branches per session or 25 branches per site).

All samples were brought to the Entomological laboratory of the Faculty of Forestry, University of Belgrade, where the total number of leaves was counted. Leaves with mines and galls were separated and individually analyzed.

The presence of live larvae or pupae within mines and galls was recorded. However, not all mines and galls contained live individuals due to factors such as prior emergence, mortality, or parasitism.

Species identification was primarily based on the characteristics of mines and galls. If immature stages (eggs, larvae, pupae, larval cases, and cocoons) were present in the analyzed material, they were examined and used for morphological identification. To increase the reliability of the identification and to confirm the species' identity, adults were reared from a part of the collected larvae and pupae (Hering 1957, Blackman and Eastop 1994, Hařaj and Osiadacz 2013, Doorenweerd et al. 2014, Haarder and Liston 2018, Laštůvka et al. 2018, Langmaid 2019, Lepiforum 2019, Liston et al. 2019, Ellis 2020, Babichev and Kirichenko 2020).

Site Characteristics

Belgrade, the capital of Serbia, was selected as the study area because it represents a large urban centre situated at the confluence of two major rivers, the Sava and the Danube. This geographic setting, combined with diverse levels of urbanization and green space distribution, makes Belgrade an ideal environment for studying how urban conditions and proximity to riverbanks influence insect communities associated with black poplar. The trees at the sampling sites were all distributed in groups of up to 10 trees. All of the studied sites were located in urban areas, New Belgrade and Dorćol in heavily urbanized areas, while Ada Ciganlija and Rakovica in areas with slightly less intense urbanization. Sites New Belgrade block 69, Dorćol, and Ada Ciganlija were located in the immediate vicinity of the Sava and Danube rivers. At the same time, sites New Belgrade, Studenjak, and Rakovica were more than 500 meters from the large river banks. At the sampling sites, 200 m diameter buffer zones were selected. In each buffer zone, the green spaces (grasslands, parks, dirt trails, etc.), spaces with no greenery (impervious areas, bodies of water, railroad tracks, etc.), as well as the total area covered by tree canopies were measured. The aerial images used for the analysis were obtained from Google Earth Pro 7.3.6.10201, from the images made in 2018 (Google Earth Pro (Desktop), 2025). ImageJ 1.53e (Schneider et al. 2012) was used for all the area measurements and creation of the buffer zone circles.

Characterisation of the Leaf Miner (LM) and Gall-ing Aphid (GA) Community Analyzed

LM and GA communities were quantified by the following parameters at the level of a single tree (Dobrosavljević et al. 2020, 2023):

- The abundance of individual species (A_i) is calculated as the number of mines per 100 leaves:

$$A_i = \frac{N_i}{l_i} \cdot 100 \quad (1)$$

where n_i is the number of mines of one particular species found in one sample, and l_i is the number of leaves per sample.

- The total abundance of (A_b) is calculated as the sum of all individual species' abundance.



Figure 1. Buffer zones (200 m) around the sampled trees in each of the studied sites: **(a)** Ada Ciganlija; **(b)** Dorćol; **(c)** New Belgrade Block 69; **(d)** Rakovica; **(e)** New Belgrade Studenjak.

- Species richness (S) (number of species identified).
- Shannon's index of diversity (H'), calculated as:

$$H' = -\sum_{i=1}^S (p_i \cdot \ln(p_i)) \quad (2)$$

where S is the Species richness and p_i is the proportion of individuals belonging to the i -th species in the dataset (Shannon 1948).

Dominant species were defined as the subset of the most abundant species that cumulatively accounted for 90% of the total LMs or GAs abundance in a given year. The 90% threshold was selected to capture the core community while excluding rare species (Magurran 2004).

Statistical Analysis

As the Kolmogorov–Smirnov test indicated that the data distribution does not fit any of the standardized distributions, nonparametric methods were used for the analysis. The differences in the LM and GA abundance, species richness, and diversity between the months in the vegetative season and locations were determined by the Kruskal–Wallis test, while the differences between the studied years were determined by the Mann–Whitney U test. Only dominant species were included in the individual species analysis. When the Kruskal–Wallis test highlighted significance, the Mann–Whitney U test was used as a post hoc test to conduct pairwise analysis. Linear regression was used to determine how the analyzed parameters relate to the green spaces and canopy cover of the investigated sites. Data were analyzed at a significance level of $p < 0.05$. The diversity index was calculated in PAST 5.2.2. (Hammer et al. 2001). All of the statistical analyses were conducted in Statistica 12.0 (StatSoft 2014).

RESULTS

Thirteen species of LMs and seven species of GAs were identified on the leaves of the studied trees (Table 1). The most abundant LM species were *Phyllocnistis unipunctella* (Stephens, 1834), *Ph. extrematrix* Martynova, 1955, and *Stigmella trimaculella* (Haworth, 1828), while the most abundant GAs were *Pemphigus spyrothecae* Passerini, 1860 and *Thecabius lysimachiae* Börner, 1916.

Dominant LM species in 2016 were by their participation in the total abundance *Ph. unipunctella* (27.5%), *Ph.*

extrematrix (27.1%), *S. trimaculella* (19.9%), and *A. populi* (13.1%), while in 2017 the community was dominated by *Ph. unipunctella* (40.2%), *L. sinuella* (20.2%), *P. populifoliella* (10.8%), *S. trimaculella* (7.9%), *Ph. extrematrix* (5.4%), and *F. hortulana* (4.1%). Dominant GA species in 2016 were, by their participation in the total, *P. spyrothecae* (50.7%), *T. lysimachiae* (19.9%), *T. affinis* (13.4%), and *P. populinigrae* (5.7%), while in 2017 the community was dominated by *T. lysimachiae* (46.7%), *P. spyrothecae* (21%), *T. affinis* (12.7%), and *P. populinigrae* (7.9%).

LM abundance differed significantly between the investigated years for all the dominant species except *Ph. extrematrix* (Table 1). Only half of the dominant GA species were affected by the interannual variation (*T. affinis* and *T. lysimachiae*) (Table 1). The dominant LM species were affected by the differences between the months in the vegetative season, both in 2016 and 2017 (Figure 2a,b, Table A1), while the GAs species showed a different pattern, as only one species was significantly affected by the seasonality, only in 2016 (Figure 2c,d, Table A1). Site characteristics significantly affected all the dominant LM and GA species in both of the analyzed years (Table 2, Table A1).

The total LM and GA abundance did not differ significantly between the investigated years ($Z = -1.258$, $p = 0.208$; $Z = 0.839$, $p = 0.401$). LM abundance was highest in August, while GA abundance remained relatively consistent throughout the season, with the highest values noted in May (Figure 3a,c). Month of the vegetative season as well as site characteristics affected both LMs ($H = 108.542$, $p < 0.01$; $H = 22.764$, $p < 0.01$) and GAs abundance ($H = 241.001$, $p < 0.01$; $H = 12.140$, $p = 0.016$) (Figure 3, Table A2, Table A3).

LM species richness and diversity did not differ significantly between the two years ($Z = -1.097$, $p = 0.265$; $Z = 0.868$, $p = 0.385$). GA species richness differed significantly between the two years ($Z = 2.002$, $p = 0.045$), while the diversity did not ($Z = 1.551$, $p = 0.060$). Further down, the characteristics of the community that did not vary between years were analyzed using pooled data, whereas those that showed yearly variation were analyzed separately for each year.

The total LM species richness was affected by both the study site and seasonality ($H = 13.923$, $p = 0.008$; $H = 76.930$, $p < 0.001$) (Figure 4, Table A4), while the diversity was affected only by seasonality ($H = 20.745$, p

Table 1. Abundance of LMs and GAs species in 2016 and 2017 (\pm sd), and the influence of the interannual variation on the dominant species assessed by the Mann–Whitney U test.

Taxonomic information	2016	2017	Z	P
LEAF MINERS (LMs)				
Diptera				
Agromyzidae				
<i>Agromyza albitarsis</i> Meigen, 1830	0.180 \pm 0.702	0.050 \pm 0.366		
<i>Aulagromyza populi</i> (Kaltenbach, 1864)	4.753 \pm 10.618	1.149 \pm 3.244	5.242	0.000
<i>A. populicola</i> (Walker, 1853)	0.205 \pm 0.638	0.009 \pm 0.104		
Hymenoptera				
Tenthredinidae				
<i>Fenusella hortulana</i> (Klug, 1818)	0.344 \pm 1.179	1.243 \pm 3.045	-2.172	0.030
<i>Heterarthrus ochropoda</i> (Klug, 1818)	0.694 \pm 1.759	1.276 \pm 2.799		
<i>Zeugophora flavicollis</i> (Marsha, 1802)	0.659 \pm 2.077	0.688 \pm 1.255		
Lepidoptera				
Nepticulidae				
<i>Stigmella trimaculella</i> (Haworth, 1828)	5.934 \pm 7.602	2.567 \pm 3.293	3.471	0.001
Gracillariidae				
<i>Caloptilia stigmatella</i> (Fabricius, 1781)	0.137 \pm 0.533	0.162 \pm 0.644		
<i>Phyllocnistis extrematrix</i> Martynova, 1955	9.958 \pm 20.341	1.275 \pm 2.753	1.062	0.288
<i>Ph. unipunctella</i> (Stephens, 1834)	10.238 \pm 19.901	17.156 \pm 26.696	-2.433	0.015
<i>Phyllonorycter pastorella</i> (Zeller, 1846)	0.086 \pm 0.438	0.388 \pm 1.187		
<i>P. populifoliella</i> (Treitschke, 1833)	0.867 \pm 1.643	3.405 \pm 6.341	-3.307	0.001
Lyonetiidae				
<i>Leucoptera sinuella</i> Reutti, 1853	0.693 \pm 2.096	7.211 \pm 17.638	-3.517	0.000
GALLING APHIDS (GAs)				
Hemiptera				
Aphididae				
<i>Pemphigus bursarius</i> (Linnaeus, 1758)	0.226 \pm 0.934	0.415 \pm 1.338		
<i>P. populi</i> Couchet, 1881	0.307 \pm 1.039	0.658 \pm 1.830		
<i>P. populinigrae</i> (Schrank, 1801)	0.331 \pm 0.932	0.978 \pm 2.420	-1.152	0.249
<i>P. protospirae</i> Lichtenstein, 1885	0.220 \pm 1.200	0.367 \pm 1.638		
<i>P. spyrothecae</i> Passerini, 1860	3.149 \pm 8.051	2.519 \pm 7.258	0.527	0.599
<i>Thecabius affinis</i> (Kaltenbach, 1843)	0.786 \pm 2.447	0.311 \pm 1.994	2.260	0.020
<i>T. lysimachiae</i> Börner, 1916	1.469 \pm 3.493	1.149 \pm 3.425	2.184	0.030

NOTE: Statistically significant differences in abundance of LMs and GAs species between 2016 and 2017 are highlighted in bold

= 0.004) (Figure 5a,b, Table A5). GA species richness was affected by the locality both in 2016 and 2017 ($H = 28.381$, $p < 0.001$; $H = 75.649$, $p < 0.001$) (Figure 6b,d, Table A4), while it was not affected by seasonality in neither in 2016, nor in 2017 ($H = 1.783$, $p = 0.776$; $H = 5.467$, $p = 0.243$) (Figure 6a,c, Table A4). The total GA diversity was only affected by the study site ($H = 86.775$, $p < 0.001$) (Figure 5c,d, Table A5).

Green areas in the 200 m radius buffer zone comprised, on average, $53.4 \pm 14.26\%$ of the total area, while the tree canopies covered, on average, $29.4 \pm 13.31\%$ of the total area. The site with the greatest green area participation

was Rakovica (79%), while Dorćol had the lowest (35%), mainly because the water area comprised a large part of the buffer zone (31%). Tree canopy cover was greatest at NBG Studenjak (45%), and lowest at NBG block 69 (9%) (Figure 1, Table 3).

Although the number of investigated sites was not great enough for a detailed insight into the effect of their characteristics on the studied insect communities, we determined that greater green area and canopy cover positively affected GA abundance and species richness, LM diversity, while the green area on the studied sites affected GA species richness and LM diversity (Table 4).

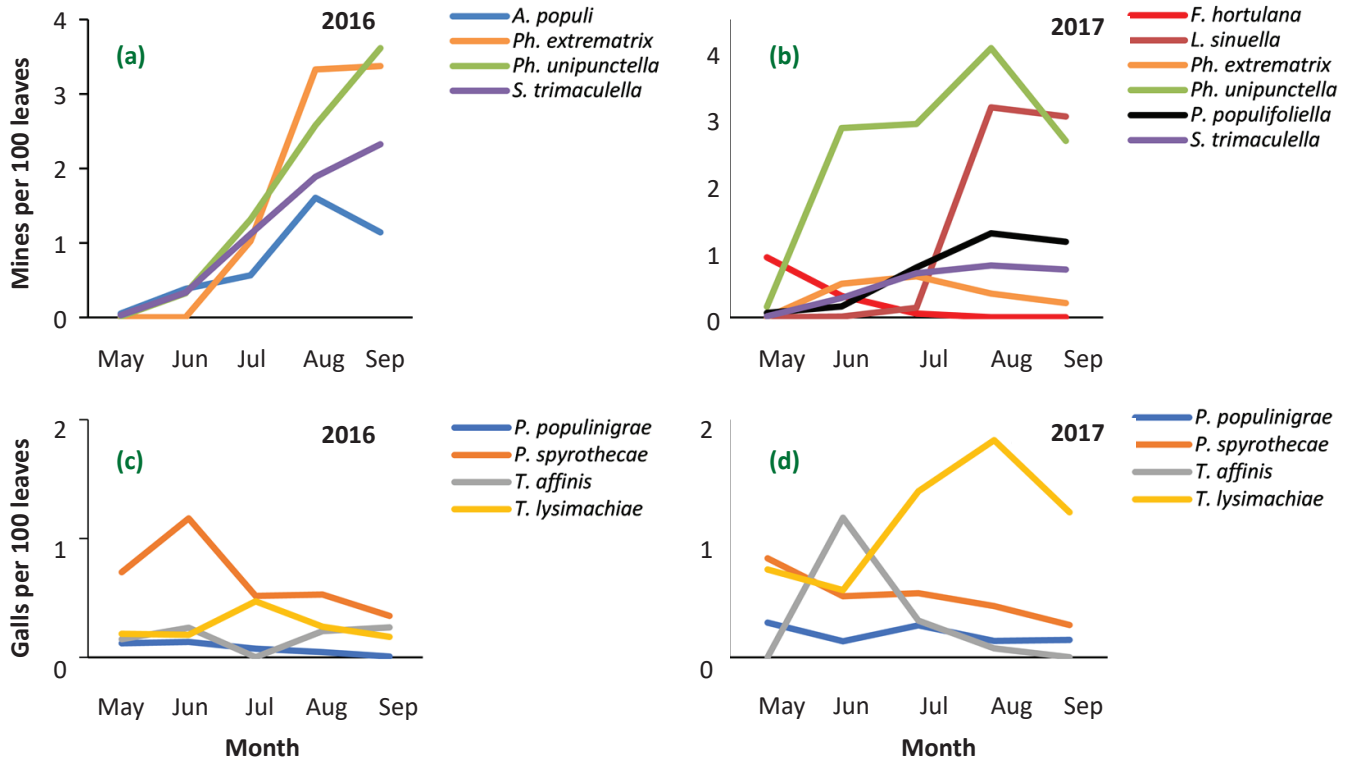


Figure 2. Seasonal variation of the dominant (a, b) leaf miner (LM) and (c, d) galling aphid (GA) species' abundance.

Table 2. Site and interannual variation of the dominant leaf miner (LM) and galling aphid (GA) species' abundance.

Year	Species	Site				
		Ada	Dorc.	Bl. 69	Stud.	Rak.
2016	<i>A. populi</i>	0.050	0.384	0.564	1.606	1.141
	<i>Ph. extrematrix</i>	n/a	n/a	1.022	3.328	3.375
	<i>Ph. unipunctella</i>	0.012	0.321	1.312	2.578	3.614
	<i>S. trimaculella</i>	0.027	0.331	1.120	1.886	2.321
	<i>P. populinigrae</i>	0.117	0.129	0.071	0.042	0.007
	<i>P. spyrothecae</i>	0.716	1.169	0.517	0.528	0.348
	<i>T. affinis</i>	0.152	0.248	n/a	0.219	0.250
	<i>T. lysimachiae</i>	0.198	0.187	0.472	0.257	0.172
2017	<i>F. hortulana</i>	0.905	0.324	0.057	n/a	n/a
	<i>L. sinuella</i>	n/a	0.009	0.145	3.170	3.033
	<i>Ph. extrematrix</i>	n/a	0.507	0.620	0.357	0.215
	<i>Ph. unipunctella</i>	0.157	2.859	2.921	4.065	2.664
	<i>P. populifoliella</i>	0.067	0.163	0.752	1.269	1.142
	<i>S. trimaculella</i>	0.017	0.290	0.666	0.785	0.723
	<i>P. populinigrae</i>	0.292	0.135	0.268	0.138	0.145
	<i>P. spyrothecae</i>	0.832	0.514	0.538	0.431	0.271
	<i>T. affinis</i>	0.000	1.175	0.310	0.074	n/a
	<i>T. lysimachiae</i>	0.740	0.564	1.398	1.828	1.219

NOTE: Abbreviation n/a stands for not applicable; abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorc. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica

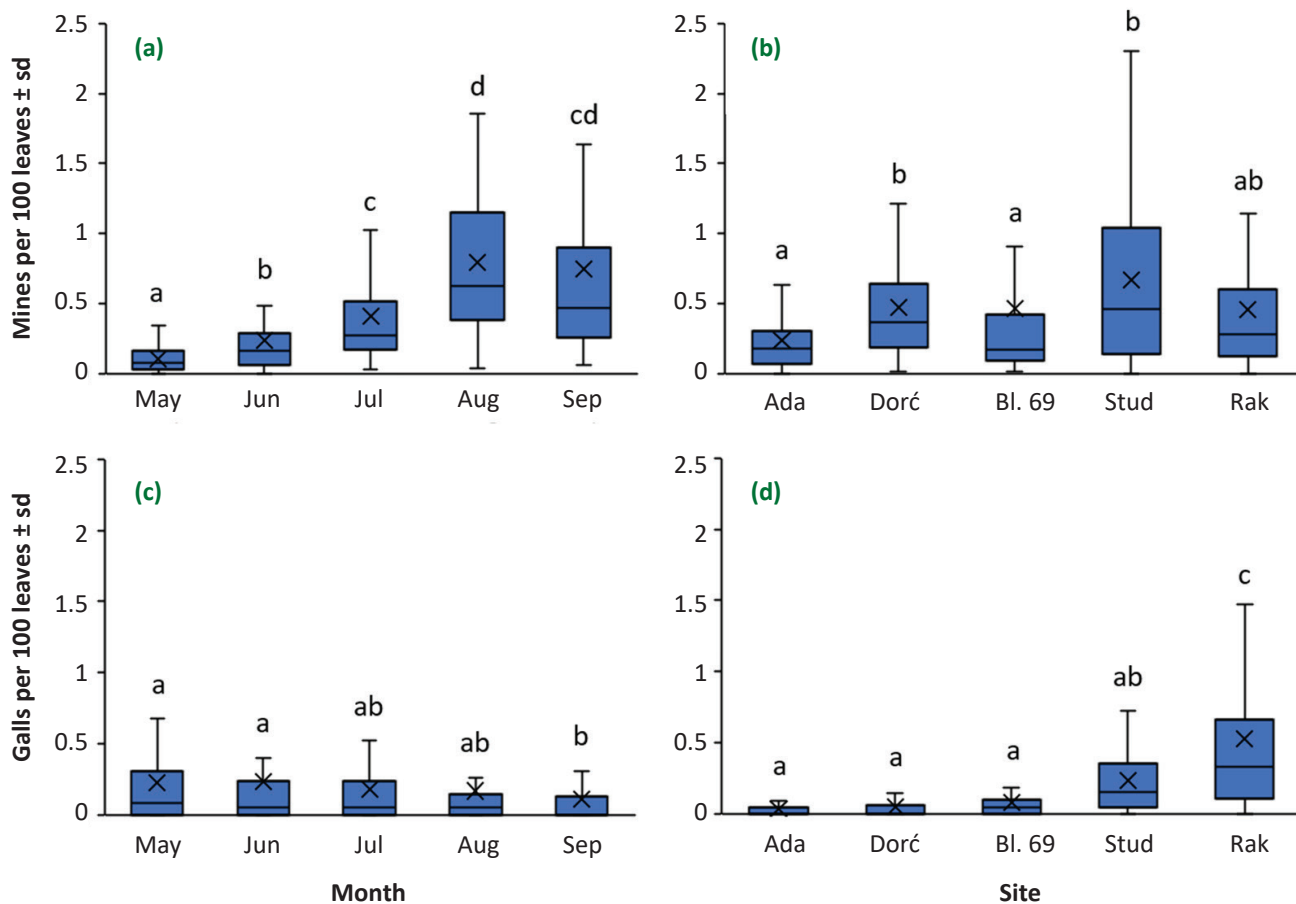


Figure 3. Seasonal variation of (a) leaf miner (LM) and (c) galling aphid (GA) abundance, and site variation of (b) LM and (d) GA abundance (x – mean, line – median, variation – ± standard deviation). Abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorć. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica. Different lowercase letters next to data points indicate a statistically significant difference ($p < 0.05$) in abundance between different months and sites for mines and galls.

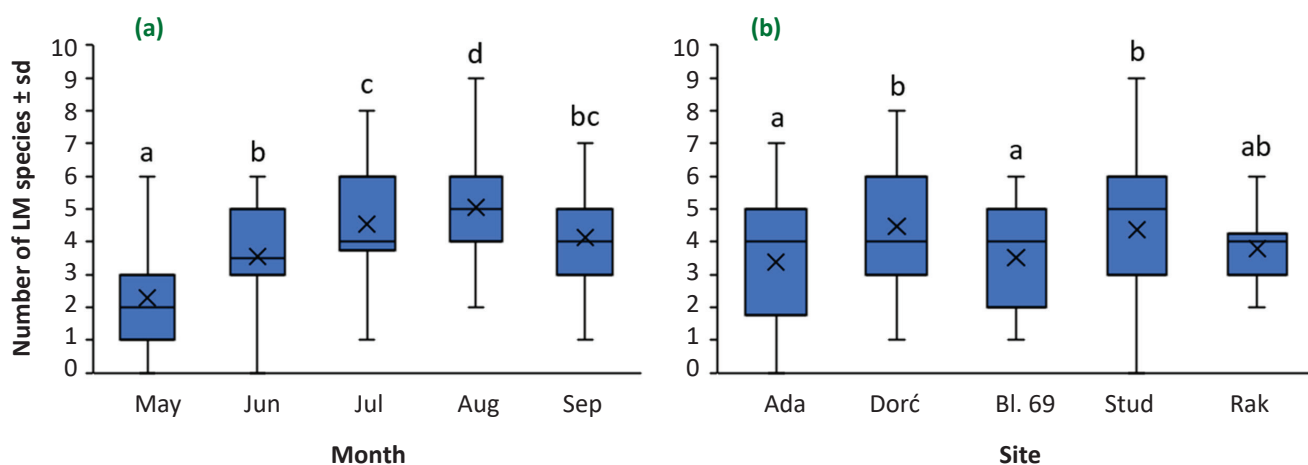


Figure 4. (a) Seasonal and (b) site variation of leaf miner (LM) species richness (x – mean, line – median, variation – ± standard deviation). Abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorć. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica. Different lowercase letters next to data points indicate a statistically significant difference ($p < 0.05$) in number of LM species between different months and sites.

Table 3. Analyzed site characteristics and characteristics of the leaf miner (LM) and galling aphid (GA) community.

Analyzed characteristic	Study site				
	Ada Ciganlija	Dorćol	NBG Block 69	NBG Studenjak	Rakovica
Green area (%)	53	35	50	50	79
Tree canopy cover (%)	36	19	9	45	38
LM abundance	0.237	0.471	0.468	0.673	0.457
GA abundance	0.031	0.044	0.079	0.235	0.525
LM species richness	3.380	4.480	3.520	4.380	3.780
GA species richness	0.580	0.480	0.920	1.340	3.200
LM diversity	1.346	1.512	1.432	1.519	1.445
GA diversity	0.186	0.146	0.257	0.318	1.280

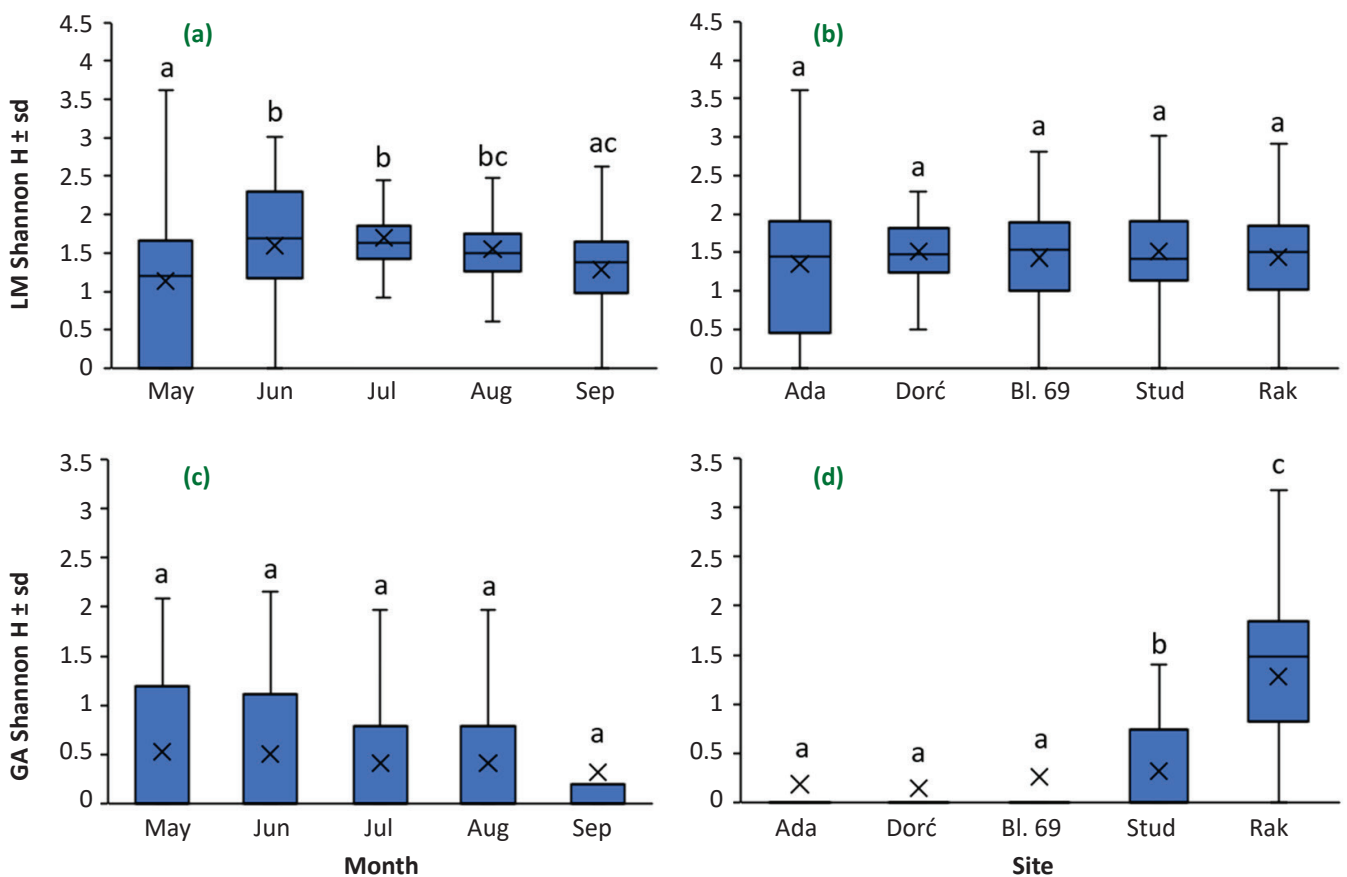


Figure 5. Seasonal variation of (a) (leaf miner) LM and (c) (galling aphid) GA diversity, and site variation of (b) LM and (d) GA diversity (x – mean, line – median, variation – \pm standard deviation). Abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorć. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica. Different lowercase letters next to data points indicate a statistically significant difference ($p < 0.05$) in Shannon between different months and sites for leaf miner and gallid aphids.

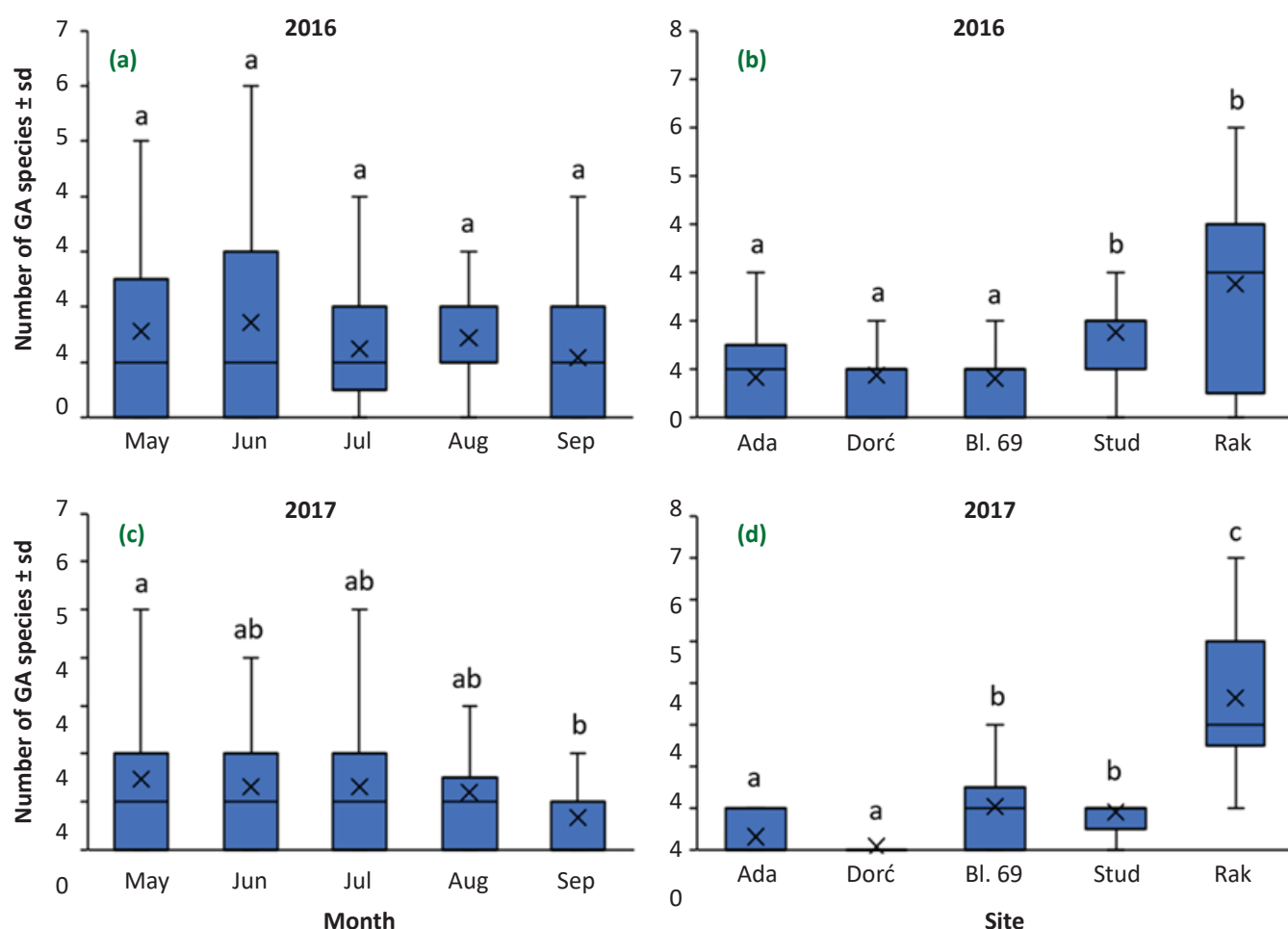


Figure 6. Seasonal (a, c), site (b, d), and interannual variation of galling aphid (GA) species richness (x – mean, line – median, variation – ± standard deviation). Abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorć. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica. Different lowercase letters next to data points indicate a statistically significant difference ($p < 0.05$) in the number of GA between different months and sites.

Table 4. Regression results of the influence of site characteristics on the leaf miners (LM) and gallid aphids (GA) community characteristics.

Site characteristics	Insect community characteristics	Regression results			
		R	R ²	F	p
Green area (%)	LM abundance	0.025	0.001	0.153	0.696
	GA abundance	0.457	0.208	65.329	0.000
	LM species richness	0.060	0.004	0.888	0.347
	GA species richness	0.141	0.020	5.004	0.026
	LM diversity	0.158	0.025	6.331	0.012
	GA diversity	0.081	0.007	1.637	0.202
Canopy cover (%)	LM abundance	0.043	0.002	0.461	0.498
	GA abundance	0.268	0.072	19.148	0.000
	LM species richness	0.001	0.000	0.000	0.983
	GA species richness	0.038	0.001	0.354	0.552
	LM diversity	0.179	0.032	8.178	0.005
	GA diversity	0.021	0.000	0.113	0.737

NOTE: Statistically significant differences are highlighted in bold

DISCUSSION

The results of this study highlight that different group of black poplar endophagous insects - leaf miners (LMs) and galling aphids (GAs) - respond differently to changes in environmental and temporal factors in the urban environment. Among the analyzed drivers, interannual variation had the lowest influence on the overall community patterns, whereas microclimatic and habitat-related factors played a more prominent role, consistent with previous studies on urban insect assemblages (Korányi and Markó 2022, Dobrosavljević et al. 2023).

Interannual differences significantly affected GA species richness, but their impact on community-level abundance remained limited. However, the differences between the studied years had a significant effect on the abundance of most of the dominant LM species, such as *S. trimaculella* and *Ph. unipunctella*. These species were abundant in both years, but the environmental differences between the two studied years affected them in opposite ways. While the abundance of *S. trimaculella* declined, *Ph. unipunctella* increased its abundance significantly, which can be a consequence of utilizing the increased available resources, such as leaf availability and improved leaf quality, according to Balaji et al. 2024. This highlights the importance of individual species biology for adaptation to even subtle differences in environmental conditions, and stressors such as temperature or humidity, as well as diversity of surrounding plants, and other factors (Connor and Taverner 1997, Rickman and Connor 2003, Fernandes et al. 2004, Rodriguez-Castañeda et al. 2017, Barantal et al. 2019, Dobrosavljević et al. 2023, Chen et al. 2023). These differences show that even though the individual species were affected by the interannual variation, the overall community abundance remained stable, suggesting a compensatory response among dominant species that maintained overall community stability. On the other side, the interannual effect was not that significant for the GA community, as only half of the dominant species were affected. *P. spyrothecae*, the most abundant GA species in this study, likely maintained its population levels due to biological traits like the protective features of the gall, as noted in previous studies (Blackman and Eastop 1994, Alton 1999, Hařaj and Osiadacz 2013, Kot and Kmieć 2020). The effect of interannual variation on the LM and GA communities is likely to become more pronounced under climate change, as growing variability in temperature, humidity, and precipitation can directly influence insect development, survival, and phenology, as well as indirectly alter host plant quality and defenses (Bale et al., 2002, Parmesan, 2006, Pincebourde et al. 2012, Robinet and Roques, 2010). Additionally, the increasing frequency of extreme climatic events may further amplify year-to-year fluctuations in insect populations, particularly in urban environments where microclimatic conditions are already highly heterogeneous (Oke 1982, Meineke et al. 2013, Korányi and Markó 2022). The importance of the interannual variation, due to climate change, as many environmental factors, such as the temperature, humidity, and precipitation patterns, will change. That is why long-term monitoring of the insect-plant interactions, especially in the urban environment, is necessary.

The LM and GA communities reacted differently to seasonal variation. The LMs' abundance, species richness, and diversity peaked in the middle of the studied period (July and August), similar to previous studies conducted in both rural and urban environments (Southwood et al. 2004, Dobrosavljević et al. 2020, Marković et al. 2021b). This trend also occurs in other insect species such as coccinellids and LM communities on other host plants (Southwood et al. 2004, Marković et al. 2018). This is a consequence of the better leaf quality as food for the LMs and interguild competition in the earlier part of the year (Connor and Taverner 1997, Southwood et al. 2004). Another reason why the LM community peaked in the middle of the studied period is that most of the dominant LM species are multivoltine, and their second generation, which feeds during July and August, is more abundant than the first generation (Hering 1951, Ellis 2020). The only dominant species that did not peak during this period was *Fenusa hortulana*, as it develops only one generation in early spring (Hering 1951, Ellis 2020, Dobrosavljević et al. 2023). In contrast, the GA community characteristics peaked much earlier in the vegetative season, in the period May-June. One of the reasons for this lies in the behaviour of the most abundant dominant species, *P. spyrothecae*, which tends to form galls on the petioles of young, developing leaves (Blackman and Eastop 1994, Alton 1999, Hařaj and Osiadacz 2013, Kot and Kmieć 2020). Their contrasting seasonal dynamics suggest temporal partitioning between the two endophagous herbivore guilds, which may facilitate their coexistence on the same host trees by reducing potential overlap in resource use. Besides the vegetative season, the studied guilds, especially the GA community, which was less affected by the seasonal variation, were affected by habitat characteristics. We determined that the analyzed habitat characteristics (percentage of green area and canopy cover on the 200 m buffer zones) had a significant effect on some of the investigated insect community characteristics, although not with a strong correlation coefficient (GA abundance and species richness, and LM diversity), similar to other studies (Knuff et al. 2020, Fenoglio et al. 2023, Matilda Collins et al. 2024, Abrego and Medianero 2025, Sinclair et al. 2025). This highlights the importance of urban greenery, especially of the urban trees, for diversity conservation. Even though the investigated factors influenced the LM and GA communities, there are also many other factors such as landscape, microclimate, host trees characteristics, predator and parasitoid community characteristics, etc. which can also affect insect communities in the urban environment (Rickman and Connor 2003, Baraniak et al. 2004, Fenoglio et al. 2009, Bairstow et al. 2010, Peralta et al. 2011, Jones and Leather 2012, Cebeci et al. 2018, Milanović et al. 2020, Dobrosavljević et al. 2020, 2023, 2025, Marković et al. 2021a, Valdés-Correcher et al. 2022, Matilda Collins et al. 2024, Horák et al. 2024). That is why more detailed studies, which can give insights into the influence of other individual environmental factors on the herbivorous insect behaviour in urban environments, need to be conducted. All this suggests that habitat characteristics and seasonal variation have a stronger effect on the investigated insect guilds than interannual variation, similar to studies on plant-insect interactions in

similar climate conditions (Southwood et al. 2004, Korányi and Markó 2022).

Although the LMs and GAs behave similarly (both feed endophagously on the same host plant), they are differently affected by the environmental differences. This is even more pronounced on the level of individual species, where, i.e. the abundance of the miner *P. unipunctella* varies significantly between years and sites, reflecting its sensitivity to microhabitat conditions, while the dominant aphid species *P. spyrothecae* shows a more stable dynamics due to the differences in their biology (Hering 1951, Alton 1999, Ellis 2020, Kot and Kmiec 2020). Although LMs and GAs comprise a significant component of black poplar insect diversity, the influence of environmental changes caused by urbanization and climate change on other insects needs to be addressed, as black poplar is a host for more than 100 insect species (Southwood 1961). Conversely, insects can cause significant damage to trees, especially when they are young and sensitive (Marković et al. 2024), which is why the significance of these insect species as pests should be reviewed. Even after identifying significant pests, their management in the urban environment is challenging, as chemical insecticides, which could be used in their natural habitat, can pose risks for the already fragile environment, and even for people in the cities (Md Meftaul et al. 2020). This highlights the importance of constant monitoring of insect populations in the cities, and their integrated pest management (IPM) approaches (Dreistadt and Flint 1995, Dhang 2014, Tabassum et al. 2024, Milanović et al. 2025). Integrating such strategies into urban forestry planning can help balance tree protection, biodiversity conservation, and ecosystem health, thereby supporting the black poplar and its insect communities.

CONCLUSIONS

This study showed that leaf miners (LMs) and galling aphids (GAs) on black poplar in urban habitats react differently to temporal and environmental changes. The interannual variation had a modest influence, affecting only GA species richness and a few dominant LM species. Seasonal changes and habitat characteristics influenced

their abundance and diversity much more significantly. While GAs abundance remained relatively consistent throughout the season, with the highest values noted in May, LMs' notably peaked mid-summer, reflecting the differences in their life cycles and survival strategies. Such differences may facilitate their coexistence on the same host trees by reducing overlap in resource use, rather than implying direct competitive interactions. Habitat characteristics, particularly the proportion of green areas and canopy cover, were important drivers of insect community structure, highlighting the role of urban greenery in supporting biodiversity. At the species level, responses were highly variable, emphasizing the importance of species-specific traits in shaping population dynamics under urban conditions. Given the ecological importance and ongoing decline of black poplar in cities, these findings underline the need for its conservation and for the implementation of sustainable, ecologically based pest management strategies. Overall, the results contribute to a better understanding of plant–insect interactions in urban ecosystems and provide a basis for integrating biodiversity conservation with urban forestry planning.

Author Contributions

JD, ČM and MM conceived and designed the research, JD, ČM and MM carried out the field measurements, JD performed laboratory analysis, JD processed the data and performed the statistical analysis, JD and ČM secured the research funding, supervised the research and helped to draft the manuscript, JD and ČM wrote the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Appendix A

Table A1. Influence of the seasonality and study site on dominant leaf miner (LM) and galling aphid (GA) species' abundance assessed by the Kruskal-Wallis test.

Factor	Seasonality				Site				
	Year	2016		2017		2016		2017	
Species	H	p	H	p	H	p	H	p	
LM	<i>F. hortulana</i>	n/a	n/a	58.203	0.000	n/a	n/a	11.076	0.026
	<i>L. sinuella</i>	n/a	n/a	72.809	0.000	n/a	n/a	12.752	0.013
	<i>A. populi</i>	32.465	0.000	n/a	n/a	14.438	0.006	n/a	n/a
	<i>Ph. extrematrix</i>	59.999	0.000	33.877	0.000	16.557	0.002	11.619	0.020
	<i>Ph. unipunctella</i>	48.582	0.000	24.765	0.000	16.790	0.002	51.956	0.000
	<i>P. populifoliella</i>	n/a	n/a	24.163	0.000	n/a	n/a	45.979	0.000
	<i>S. trimaculella</i>	78.868	0.000	43.109	0.000	12.309	0.015	12.273	0.015
GA	<i>P. populinigrae</i>	6.146	0.189	2.873	0.579	16.723	0.002	66.288	0.000
	<i>P. spyrothecae</i>	3.673	0.452	6.934	0.139	49.833	0.000	45.673	0.000
	<i>T. affinis</i>	12.513	0.014	8.422	0.077	17.084	0.002	14.766	0.005
	<i>T. lysimachiae</i>	7.289	0.121	8.536	0.074	25.006	0.000	35.573	0.000

Table A2. Influence of the study site on LM (leaf miner) and GA (galling aphid) abundance assessed by Mann–Whitney U test.

M-W U TEST		LM		GA	
f1	f2	Z	p	Z	p
Ada Ciganlija	NBG Block 69	-0.842	0.400	-1.230	0.219
Ada Ciganlija	Dorćol	-3.243	0.001	0.277	0.782
Ada Ciganlija	Rakovica	-1.263	0.207	-8.965	0.000
Ada Ciganlija	NBG Studenjak	-2.956	0.003	-2.662	0.008
NBG Block 69	Dorćol	-2.268	0.023	1.459	0.145
NBG Block 69	Rakovica	-0.424	0.672	-7.661	0.000
NBG Block 69	NBG Studenjak	-2.026	0.043	-1.469	0.142
Dorćol	Rakovica	1.907	0.056	-9.019	0.000
Dorćol	NBG Studenjak	0.226	0.821	-2.872	0.004
Rakovica	NBG Studenjak	-1.661	0.097	6.004	0.000

Table A3. Influence of the seasonality on LM (leaf miner) and GA (galling aphid) abundance assessed by Mann–Whitney U test.

M-W U TEST		LM		GA	
f1	f2	Z	p	Z	p
May	June	-3.145	0.002	0.087	0.930
May	July	-5.995	0.000	0.781	0.435
May	August	-7.553	0.000	0.761	0.447
May	September	-5.369	0.000	2.081	0.037
June	July	-3.064	0.002	0.677	0.498
June	August	-4.809	0.000	0.664	0.507
June	September	-2.671	0.008	2.020	0.043
July	August	-1.966	0.049	-0.006	0.995
July	September	0.010	0.992	1.292	0.196
August	September	1.868	0.062	1.343	0.179

Table A4. The influence of study site and seasonality on LM (leaf miner) and GA (galling aphid) species richness, assessed by Mann–Whitney U test.

M-W U TEST		LM		GA			
f1	f2	Z	p	2016		2017	
		Z	p	Z	p	Z	p
Ada Ciganlija	NBG Block 69	-0.345	0.730	0.058	0.954	-2.639	0.004
Ada Ciganlija	Dorćol	-2.609	0.009	-0.233	0.816	1.446	0.148
Ada Ciganlija	Rakovica	-0.838	0.402	-3.337	0.001	-5.821	0.000
Ada Ciganlija	NBG Studenjak	-2.468	0.014	-3.085	0.002	-2.969	0.003
NBG Block 69	Dorćol	-2.554	0.011	-0.330	0.742	3.745	0.000
NBG Block 69	Rakovica	-0.641	0.521	-3.347	0.001	-4.928	0.000
NBG Block 69	NBG Studenjak	-2.389	0.017	-3.347	0.001	0.029	0.977
Dorćol	Rakovica	1.923	0.054	-3.240	0.001	-5.995	0.000
Dorćol	NBG Studenjak	0.283	0.777	-3.056	0.002	-4.191	0.000
Rakovica	NBG Studenjak	-1.830	0.067	1.940	0.052	5.268	0.000
May	June	-4.226	0.000	-0.223	0.823	0.175	0.861
May	July	-6.549	0.000	0.223	0.823	0.689	0.491
May	August	-7.228	0.000	-0.136	0.892	0.407	0.684
May	September	-5.388	0.000	0.844	0.399	1.979	0.048
June	July	-3.054	0.002	0.475	0.635	0.475	0.635
June	August	-4.505	0.000	0.087	0.930	0.223	0.823
June	September	-1.834	0.067	1.048	0.295	1.717	0.086
July	August	-1.703	0.089	-0.398	0.691	-0.310	0.756
July	September	1.086	0.278	0.699	0.485	1.154	0.248
August	September	2.637	0.008	1.096	0.273	1.610	0.107

Table A5. The influence of study site and seasonality on LM (leaf miner) and GA (galling aphid) diversity, assessed by Mann–Whitney U test.

M-W U TEST		LM		GA	
f1	f2	Z	p	Z	p
Ada Ciganlija	NBG Block 69	-0.548	0.584	-0.562	0.574
Ada Ciganlija	Dorćol	-0.658	0.510	0.190	0.850
Ada Ciganlija	Rakovica	-0.438	0.662	-5.925	0.000
Ada Ciganlija	NBG Studenjak	-0.565	0.572	-1.741	0.082
NBG Block 69	Dorćol	-0.093	0.926	0.782	0.434
NBG Block 69	Rakovica	0.176	0.860	-5.808	0.000
NBG Block 69	NBG Studenjak	-0.014	0.989	-0.989	0.323
Dorćol	Rakovica	0.252	0.801	-6.173	0.000
Dorćol	NBG Studenjak	0.196	0.844	-1.989	0.047
Rakovica	NBG Studenjak	-0.179	0.858	5.622	0.000
May	June	-2.699	0.007	0.014	0.989
May	July	-3.595	0.000	0.455	0.649
May	August	-2.809	0.005	0.489	0.625
May	September	-1.110	0.267	1.127	0.260
June	July	-0.172	0.863	0.555	0.579
June	August	0.924	0.356	0.669	0.504
June	September	2.272	0.023	1.293	0.196
July	August	1.565	0.118	0.107	0.915
July	September	3.261	0.001	0.700	0.484
August	September	1.958	0.050	0.527	0.598

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