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THE EFFECTS OF EUROPEAN MISTLETOE (*VISCUM ALBUM* L.) ON URBAN TREE HEALTH

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Background. Urban forests play a crucial role in making the urban environment and provide for sustainable development of urban areas. At the same time, urban habitats are stressful for trees therefore the latter become more susceptive to a range of disturbances, in particular to biotic agents such as the European mistletoe. The paper aims to evaluate the detrimental impacts of this hemiparasitic plant on urban trees.

Materials and Methods. The study was conducted in an urban watershed of central and northwestern parts of the city of Kharkiv, Ukraine. Urban tree health survey took place in different stand types along 4 transects of the total length of 6.54 km. Totally, 956 individual trees were surveyed during the vegetation season of 2019. The health status of individual trees was assessed applying the 7-grade scale after O. D. Maslov, modified by the Ukrainian Forestry Research Institute. The mistletoe presence on individual trees was quantified using a semi-logarithmic index. The statistical analysis of the data collected involved the comparison of two empirical distributions, the normality test of the initial data, and the determination of the relationship between two independent samples.

Results and Discussion. The analysis of the collected data revealed that the studied trees divided into two groups – “non-infested” and “mistletoe-infested” – grew in quite similar conditions and showed quite similar distribution by stand types, classes of soil conditions and age classes. We found differences in the mean health status between non-infested (transient from “weakened” to “dry-top”), and mistletoe-infested trees (“dry-top”): the tree health index of 2.43±0.03 for the former vs. 2.98±0.08 for the latter. Empirical distributions of the number of trees by the tree health classes in both groups were different too (the Kolmogorov–Smirnov criterion $\lambda = 3.14$ at $P > 0.999$). We found a significant positive correlation between both mistletoe abundance and the age of individual infested trees (Spearman's R = 0.36 at P >0.999), as well as the health index of individual infested trees (Spearman's R = 0.23 at P >0.95).

Conclusions. The study has proven a negative effect of mistletoe's infestation on the tree crown foliage assessed with the tree health index. Such an effect increases with the age of infested trees. The findings of this study suggest that in urban forests the mistletoe acts as a trigger towards acceleration of tree stand health deterioration.

***Keywords**:* urban forest, the European mistletoe, tree health status, trigger, Kharkiv

INTRODUCTION

Urban tree stands, a.k.a. urban forests, play a pivotal role in making the urban environment and securing sustainable development of urban areas (Cavender & Donnelly, 2019). Urban terrestrial vegetation is a major natural asset for such ecosystem services as microclimatic conditioning, atmospheric pollution and anthropogenic noise buffering, water cycle regulation, etc. (Chiabai *et al.*, 2018; Muresan *et al.*, 2022). At the same time, in urban settings trees tend to grow in a more stressful environment (Cavender & Donnelly, 2019), therefore being more susceptible to influences of various biotic agents, such as the European mistletoe (*Viscum album* L.).

This species of the *Loranthaceae* family (or the *Santalaceae* family, by another classification) is a dioecious semi-parasite dwarf with yellowish evergreen leathery leaves, that lives in crowns of many tree species (Ivchenko *et al.*, 2014). According to the data from different sources, in temperate climatic zones the European mistletoe ramets grow up to 100–120 cm in diameter, with a maximum of 4 m, in tropic climatic zones. The European mistletoe's lifespan may reach 40 years (Ivchenko *et al.*, 2014; Lyu *et al.*, 2000).

The role of the European mistletoe in plant communities is controversial (Ivchenko *et al.*, 2014). On the one hand, it is considered to be a major biotic stressor to the host trees alongside herbivores (insects, nematodes) and pathogenic microorganisms that cause tree stands die-off. On the other hand, damaging effects of the majority of mistletoe species are often overestimated as mistletoes perform important ecological functions by increasing both trophic and structural diversity of plant communities and thus supporting a variety of interactions within biotic communities (Krasylenko *et al.*, 2020). The mistletoe taxa are members of a powerful interaction network between their host, herbivore, pollinator and seed dispersing species. At present, the role of parasitic and hemi-parasitic plants as ecosystem engineers is being more and more acknowledged (Griebel *et al.*, 2017).

In addition, it is assumed that specific symbiotic mutual relationships are established between the European mistletoe and its host trees (Ivchenko *et al.*, 2014), which is related to the ability of the semi-parasite plant to produce certain organic compounds that a host tree can utilize for its own metabolism.

The majority of known studies are focused on an integrated assessment and forecasting of the effects caused by different mistletoe species on natural ecosystems (e.g. forests), as well as agricultural ones (e.g. fruit orchards), while urban forests have not been considered until quite recently (Orlov *et al.*, 2023; Zhytova *et al.*, 2024). So far, the harmfulness of the European mistletoe to urban tree stands has been assessed insufficiently. Hence, the aim of this study is to assess the influence of the European mistletoe on the urban tree health.

MATERIALS AND METHODS

The study area comprised uphill terrain of the central and north-western parts of the city of Kharkiv, in local watershed of the Lopan' and Kharkiv rivers. Four survey routes (transects) were selected in a semi-random way: the start points were chosen randomly, then each route was directed along the nearby streets and, where possible, across city blocks in order to represent both major land use and urban forest types. The total length of transects was 6.54 km. Each route was divided into smaller sections depending on the street network configuration and differences in urban forest typology (**Table 1**).

Table 1. **General information on the survey transects**

Transect ID	Section ID	Description	Length, km	Tree stand type
M1	M1-1	From Chornoglazivs'ka (f. Marshal Bazhanov) St. to Darwin St.	1.57	street
	M1-2	From Darwin St. to Mystetstv St.		- " -
	M1-3	Mystetstv St.		- " -
	M1-4	Politekhnichna St.		- " -
	M1-5	Maksymylianivs'ka St.		- " -
M2	M2-1	From Pushkins'kyi entrance to Yaroslav Mudryi St.	1.66	backyard
	M2-2	Yaroslav Mudryi St.		street
	M2-3	Alchevs'ky St.		- " -
	M2-4	Student Residences "Gygant"		backyard
	M2-5	'Molodizhnyi' Park (on the place of the Old Cemetery)		park
	M2-6	from Alchevs'ky St. to Myronosyts'ka St.		street
M3	M3-1	from Vartovykh Neba (f. Tobol's'ka) St. to Otakar Jaroš St.	1.91	backyard
	M3-2	from Otakar Jaroš St. to Yevropeys'ka (f. Novhorods'ka) St.		sidewalk
	M3-3	from Yevropeys'ka (f. Novhorods'ka) St. to Kosmichna St.		street
	M3-4	from Kosmichna St to Yenin (f. Bakulin) St.		backyard
M4	M4-1	Akademik Lyapunov St.	1.40	street
	M4-2	Kolomens'ka St.		- " -
	M4-3	Yenin (f. Bakulin) St. – Aviatsiyna St. – Fanins'kyi Ln.		- " -
	M4-4	Kryms'ka St.		- " -

The field survey was conducted in late May of 2019. Along each route, all trees were counted within a 50-m fixed-width band on both sides. For each tree we noted its species name, the transect and section ID, type of tree stands, index of soil conditions, age class, crown and trunk health conditions, and the mistletoe's abundance index. Tree species were identified and their scientific names used as by (Prokudin, 1987), then checked with modern data base of the plants of the world (Plants of the World Online).

Soil conditions at each tree's location were assessed using the following scale: 1 – natural or close-to-natural, 2 – slightly modified, 3 – significantly modified by human activities, 4 – severely modified (e.g., as a result of construction/development activities), 5 – soil is buried under artificial surfaces (e.g., pavements, construction wastes, etc.) (Vergeles, 2011).

Tree age classes were defined as follows: 1 – young trees (up to 20 years old), 2 – early generative (21–40 years old), 3 – middle-aged (41–60 years old), 4 – maturing (61–80 years old), 5 – mature and senile (80+ years old).

Tree health was assessed using the following scale initially developed by O. D. Maslov in 1973 and further modified by the Ukrainian Forestry Research Institute in 1987: 1 – healthy, 2 – weakened (less than 15 % of crown is damaged, no or slight trunk damage), 3 – dry-topped (crown damage between 1/6 and 1/3 of the total volume, some trunk damage), 4 – dry-crowned (crown damage between 1/3 and 2/3 of the total volume, multiple trunk damage), 5 – dying-up (more than 2/3 of the crown damaged, multiple trunk damage, open roots, traces of xylophage insect attacks), 6 – dead tree (died during current season), 7 – long-dead tree (died in previous years) (Vergeles, 2011).

The abundance of European mistletoe on individual infested trees was assessed with an original semi-logarithmic index as follows: 1 – from 1 to 5 mistletoe dwarves on a single tree, 2 – 6-10, 3 – 11-20, 4 – 21-40, 5 – 41-80, etc. (Rybalka *et al.*, 2016).

The statistical analysis of the collected data was carried out in several stages. The first step was to compare the two empirical distributions (characteristics of non-infested and mistletoe-infested trees) using a specialized Kolmogorov–Smirnov test. Next, we assessed the normality of the distribution of the original data. For this purpose, we calculated the Kolmogorov-Smirnov criterion (applied if the sample size exceeds 50 variants), and determined the ratio of the range of variability in each empirical variation series to its standard deviation (C) and calculated the skewness and kurtosis indicators. Correlation analysis was performed to identify the functional relationship between the variation series. The significance of the empirical correlation coefficient was evaluated using Student's *t*-test. The mathematical processing of the collected data was carried out according to standard approaches (Mac Berthouex & Brown, 2002) using MS Excel® (comparison of distributions; calculation of the C value and indicators of skewness and kurtosis; checking the significance of correlation coefficients) and Statistica® (calculation of the Kolmogorov–Smirnov criterion; correlation analysis of data).

RESULTS AND DISCUSSION

The size of the studied sample was 956 trees. The total number of non-infested trees was 845, of which 6 trees had regrown a secondary crown after pruning (the sample does not include trees that crowned 5 years ago or later). Non-infested trees (control) were represented by 36 species. The most numerous among them were: Norway maple (*Acer platanoides* L. – 26.27 % of the total subsample of non-infested trees), horse chestnut (*Aesculus hyppocastanum* L. – 13.25 %), European or Caucasian lime-tree (*Tilia x europaea* L. – 10.77 %), large-leaved lime-tree (*Tilia platyphyllos* Scop. – 6.63 %), and other species, such as silver birch (*Betula pendula* Roth), common oak (*Quercus robur* L.), black locust (*Robinia pseudoacacia* L.), common ash (*Fraxinus excelsior* L.), yellow box elder (*Acer negundo* L.), balsam poplar (*Populus balsamifera* L. = *P. tacamahacca* Mill.), etc., whose share in the subsample did not exceed 5 % each. Four individual trees from this subsample were assessed as "long-dead"; they should be removed from tree stands according to the national Urban Forestry Maintenance Regulations (Order…, 2006).

The European mistletoe dwarves were found on 111 trees in total. The host trees belonged to 14 species: Norway maple (*Acer platanoides* L. – 31.53 % of the total subsample of the infested trees), balsam poplar (*Populus balsamifera* L. – 5.32 %), black locust (*Robinia pseudoacacia* L. – 11.71 %), silver maple (*Acer saccharinum* L. – 9.01 %), eastern cottonwood (*Populus deltoides* W.Bartram ex Marshall – 8.11 %), mountain-ash (*Sorbus aucuparia* L. – 6.31 %), green ash (*Fraxinus pennsylvanica* Marshall = *F. lanceolata* Borkh. – 6.31 %) and other species (European or Caucasian lime-tree (*Tilia* x *europaea* L.), broad-leaved lime-tree (*Tilia platyphyllos* Scop.), common ash (*Fraxinus excelsior* L.), golden Lombardy, or black, poplar (*Populus nigra* L.), Mongolian poplar (*P. suaveolens* Fisch. ex Poit. & A.Vilm.), white poplar (*P. alba* L.), and field maple (*Acer campestre* L.), whose share in the subsample did not exceed 5 % each). Thus, every ninth tree in urban tree stands along the route appeared to be mistletoe-infested.

The analysis of the collected data revealed that both non-infested and mistletoe-infested trees grew in fairly similar conditions and showed similar patterns of distribution by plantation type, soil class, and age class:

- distribution of mistletoe-free trees by stand type: street tree stands – 334 specimens (39.53 % of the total number of mistletoe-free trees), backyard stands – 382 (45.21 %, respectively), parks – 129 (15.27 %); distribution of mistletoe-infested trees by plantation type: street stands – 39 specimens (35.14 % of the total number of infested trees), backyard stands – 58 (52.25 %, respectively), parks – 14 (12.61 %);
- distribution of non-infested trees according to indices of soil condition: 1 – 98 specimens (11.60 % of the total number of trees without mistletoe), 2 – 418 (49.47 %, respectively), 3 – 329 (38.93 %); distribution of mistletoe-infested trees according to indices of soil condition: 1 – 19 specimens (17.12 % of the total sample), 2 – 61 (54.95 %, respectively), 3 – 31 (27.93 %);
- distribution of non-infested trees by age class: 2 – 46 specimens (5.44 % of the total number of mistletoe-free trees), 3 – 458 (54.20 %, respectively), 4 – 330 (39.05 %), 5 – 11 (1.30 %); distribution of mistletoe-infested trees by age class: 2 – 3 specimens (2.70 % of the total sample), 3 – 57 (51.35 %, respectively), 4 – 50 (45.05 %), 5 – 1 (0.90 %).

It is worth noting that no mistletoe-infested juvenile trees were found in the total sample. This can be explained by the fact that it is difficult for mistletoe seeds to gain a foothold on a young tree with branches in the crown that are just forming and have a small diameter. This hemi-parasitic plant species emerged on the host trees starting from the second age class, while the maximum number of mistletoe-infested trees was recorded in the third and fourth age classes (i.e. in the middle-aged and maturing stands), which is important in terms of controlling the mistletoe spread.

According to the sanitary condition, trees not infected with mistletoe were assessed as transitional from "weakened" to "dry-top" (mean value of the tree health index is 2.43±0.03), and infested trees – as "dry-top" (mean value of the tree health index is 2.98±0.08). The distributions of non-infested and mistletoe-infested trees by classes of sanitary condition according to the 7-point scale of O. D. Maslov also differ (**Fig. 1**). The „zero" hypothesis assumes that the differences between the two examined empirical distributions are not systematic, but purely random.

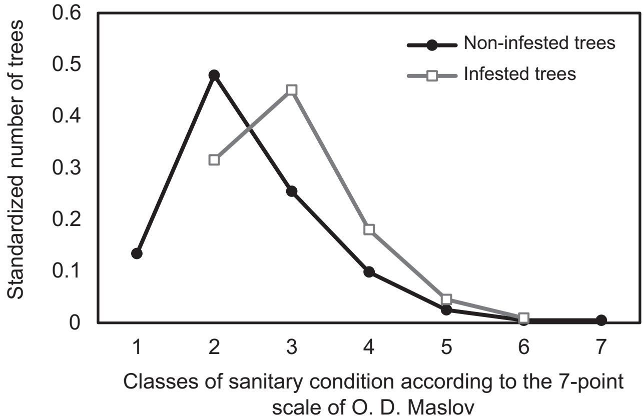

Fig. 1. The distributions of non-infested and mistletoe-infested trees by classes of sanitary condition according to the 7-point scale of O. D. Maslov

To test the "zero" hypothesis, we applied a specialized Kolmogorov–Smirnov λ test. Since the examined samples significantly differed in size, with the number of mistletoe-infested trees being much smaller than the number of non-infested trees, we standardized the levels of the empirical distribution for non-infested trees in the range from 1 to 111. The standardized series looked as follows: 15; 53; 28; 11; 3; 1; 1.

The result of calculating the Kolmogorov–Smirnov criterion when comparing the two empirical distributions (after standardization) is as follows: λ = 3.14, which exceeds the critical value of 1.95 at P = 0.999. Thus, the "zero" hypothesis is rejected, which means the groups differ significantly in the considered attribute.

It is also interesting to note that when the distribution for infested trees was shifted one class forward (**Fig. 2**), the Kolmogorov–Smirnov criterion λ = 1.91, i.e., at P >0.95 (critical value 1.36) and P >0.99 (critical value 1.63), the results did not change – the difference between the two empirical distributions was significant, while at P = 0.999 (critical value 1.95) there was no difference between the frequencies for the same test. If we exclude healthy trees (health class „1") in **Fig. 2**, the difference between the empirical distributions at P = 0.95, P = 0.99, and P = 0.999 disappears: λ = 0.91. Thus, mistletoe causes deterioration in tree sanitary conditions by one grade. Similar patterns are also observed if we standardize the empirical distribution for mistletoe-infested trees in the range from 1 to 845, but those are less pronounced.

Thus, the negative impact of mistletoe on the host tree can be traced to the level of the entire organism of the woody plant. The analysis of the collected data shows that mistletoe was not found at all on healthy trees with a sanitary condition index of «1». Infestation by a hemi-parasite plant occurs when a tree becomes weakened and transits to the 2nd class of sanitary condition (however, not all weakened trees in the sample were infested by the mistletoe). The maximum number of host trees in the sample had a sanitary condition assessed as "dry-top", and the numbers of trees with subsequent condition indices decrease significantly. This indicates that a weakened tree can still provide mistletoe with resources for development, but at the same time it degrades much faster towards the third class of sanitary condition ("dry-top"), and then both the host tree and mistletoe are destined to die. If the root system of the host tree is intact, pruning can be applied to control the abundance and further spread of the mistletoe; otherwise, the whole tree should be removed.

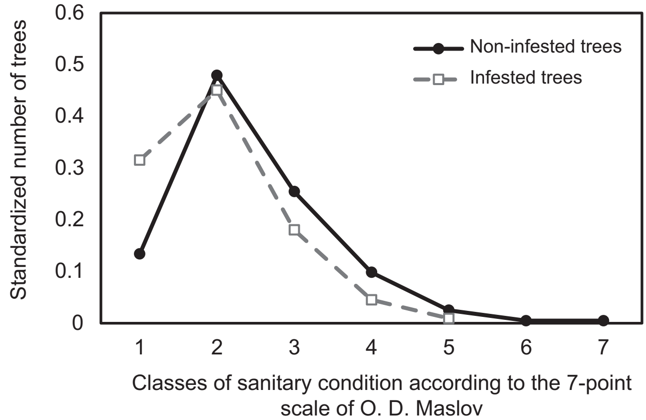

Fig. 2. The transformed distributions of non-infested and mistletoe-infested trees by classes of sanitary condition according to the 7-point scale of O. D. Maslov (the distribution of infested trees shifted one grade forward)

Let us consider the statistical complex of data on mistletoe-infested trees separately. Based on the results of three tests (the Kolmogorov–Smirnov criterion, C-test, skewness and kurtosis), the working hypothesis about the normality of the distribution of the observed data in the studied sample was rejected. In order to identify the functional relationship between the variation series, the nonparametric Spearman's rank correlation coefficient R was calculated. The results of the correlation analysis are shown in **Table 2**.

Table 2. **Results of calculating Spearman's rank correlation coefficient R**

Indexes*	1	2	3	4	5	6	7
1	1.00	**-0.20****	**-0.27**	**-0.42**	**-0.28**	-0.08	0.11
2		1.00	**-0.23**	**0.21**	**0.29**	0.14	0.13
3			1.00	**-0.39**	0.00	**-0.27**	-0.10
4				1.00	0.12	**0.20**	-0.02
5					1.00	0.03	**0.36**
6						1.00	**0.23**
7							1.00

Note: * – symbol «1» denotes the transect ID, «2» – section ID, «3» – type of tree stand, «4» – soil condition index, «5» – tree age class, «6» – tree health index, «7» – mistletoe abundance index; ** – significant correlation is shown in bold

The correlation analysis revealed significant positive correlation between mistletoe abundance indices and tree age ($R = 0.36$, $k = 109$, $t = 4.03 > t_{st} = 3.38$ at $P > 0.999$), as well as their sanitary condition ($R = 0.23$, $k = 109$, $t = 2.47 > t_{st} = 1.98$ at $P > 0.95$). Thus, the hemiparasite plant has a negative effect on the host tree foliage, and this effect increases with age. The relationship between the indices of abundance and age classes was most clearly observed in silver maple ($R = 0.65$; $k = 8$, $t = 2.42 > t_{st} = 2.31$ at $P > 0.95$), while between the indices of mistletoe abundance and tree health condition – in black locust (however, in this case it was statistically insignificant). In contrast, for the statistical set of data on non-infested trees, the relationships were different: the sanitary condition of trees was significantly correlated with the tree stand type (tree health conditions were better in parks, R = -0.22, t = 6.55), and soil condition (the more transformed and damaged were soils, the worse was tree health, R = 0.22, t = 6.55, k = 843, t_{st} = 3.30 at P >0.999).

In general, urban tree stands (by obtained sample of 956 trees) were assessed as transitional from "weakened" to "dry-top" (average value of the sanitary condition index 2.50 ± 0.03). According to the previous results of tree health assessment in 1998, the average value of the sanitary condition index for urban green spaces in Kharkiv was then 2.37 ± 0.02 (Vergeles & Vyshnevetsky, 2001), i.e., over the past twenty years, a slight deterioration in the quality of arboreal vegetation in the city can be noted.

The study has proven that in the urban environment where stressors combine and reinforce each other, the European mistletoe can be regarded as a trigger that turns the trajectory of urban green spaces onto the path of accelerated degradation.

CONCLUSION

1. The sanitary condition of trees not infested with the European mistletoe was assessed as transitional from "weakened" to "dry-top" (mean value of the tree health index is 2.43±0.03), whilst the sanitary condition of mistletoe-infested trees was assessed as "dry-top" (mean value of the tree health index is 2.98±0.08).
2. The distributions of non-infested and mistletoe-infested trees by classes of sanitary condition according to the 7-point O. D. Maslov scale differ: the Kolmogorov–Smirnov criterion λ = 3.14 exceeding the critical value of 1.95 at P = 0.999. At the same time, mistletoe causes deterioration in the sanitary condition of trees in urban forest by about one grade.
3. The correlation analysis revealed a significant positive relationship between mistletoe abundance indices and tree age (R = 0.36, t = 4.03 > t_{st} = 3.38 at P >0.999), as well as tree health condition (R = 0.23, t = 2.47 > t_{st} = 1.98 at P >0.95).
4. According to the sanitary condition, urban tree stands in the city of Kharkiv were assessed as transitional from "weakened" to "dry-top", with the tree health index increasing from 2.37±0.02 to 2.50±0.03 over the past two decades.
5. In the urban settings where some environmental stressful factors reinforce others, the European mistletoe is acting as a trigger that turns the trajectory of urban green spaces on the path of accelerated degradation.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: this article does not contain any studies with animal subjects performed by any of the authors.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [I.R.; Y.V.]; methodology, [I.R.; Y.V.]; formal analysis, [I.R.]; investigation, [Y.V.]; writing – original draft preparation, [I.R.; Y.V.]; writing – review and editing, [I.R.; Y.V.]; visualization, [I.R.].

All authors have read and agreed to the published version of the manuscript.

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ВПЛИВ ОМЕЛИ БІЛОЇ (*VISCUM ALBUM* L.) НА ЗДОРОВ'Я МІСЬКИХ НАСАДЖЕНЬ

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Вступ. Зелені насадження відіграють ключову роль у формуванні довкілля урбанізованих територій і в забезпеченні їхнього сталого розвитку. Водночас в умовах міста дерева розвиваються у більш стресових умовах, а отже, є більш уразливими до впливу біотичних чинників, зокрема, омели білої. Шкодочинність цієї рослини-напівпаразита для міських зелених насаджень до цього часу є мало вивченою.

Матеріали та методи. Дослідження проведене на вододілах у центральній та північно-західній частинах м. Харкова з використанням методу маршрутних обстежень. Загальна довжина маршрутів становила 6,54 км, розмір дослідженої вибірки – 956 дерев. Поточний санітарний стан дерев визначали з використанням семибальної шкали, запропонованої О. Д. Масловим у 1973 р. й у подальшому модифікованої Українським науково-дослідним інститутом лісового господарства та агролісомеліорації імені Г. М. Висоцького. Чисельність кущів омели на індивідуальних деревах відображали за допомогою напівлогарифмічного індексу.

Статистичний аналіз зібраних даних передбачав порівняння двох емпіричних розподілів, перевірку вихідних даних на нормальність, визначення взаємозв'язку між двома незалежними вибірками. Розрахунки здійснено з використанням програм Statistica та MS Excel.

Результати. Аналіз зібраних даних дає можливість встановити, що досліджувані дерева, розділені на дві групи – "неінфіковані" та "інфіковані омелою", – зростають у досить подібних умовах і є приблизно однаково розподілені за типами насаджень, класами ґрунтових умов і класами віку. Водночас санітарний стан не інфікованих омелою дерев визначено як перехідний від послабленого до суховерхівкового (середнє значення індексу санітарного стану 2,43±0,03), а санітарний стан дерев-живителів омели – як суховерхівковий (середнє значення індексу санітарного стану 2,98±0,08). Розподіли неінфікованих та інфікованих омелою дерев за класами санітарного стану за семибальною шкалою А. Д. Маслова також відрізняються: критерій Колмогорова–Смірнова λ = 3,14, що перевищує 1,95 за P = 0,999. На основі кореляційного аналізу виявлено тісний додатний зв'язок між індексами чисельності омели та віком уражених дерев, а також їхнім санітарним станом.

Висновки. Проведене дослідження довело, що омела біла негативно впливає на облиствіння крони дерев-живителів (інтегральним показником стану якого є санітарний стан), і цей вплив посилюється з віком. Автори розглядають роль омели білої в умовах міста як своєрідний тригер, що прискорює деградацію міських зелених насаджень.

Ключові слова: міські насадження, омела біла, санітарний стан, тригер, Харків

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