EVALUATING EARLY CHANGES IN YOUNG TREE SEEDLINGS UNDER SIMULATED URBAN ENVIRONMENT

***Valentinas Černiauskas , Iveta Varnagirytė-Kabašinskienė**
Lithuanian Research Centre for Agriculture and Forestry, Lithuania
*Corresponding author's e-mail: valentinas.cerniauskas@lammc.lt

Abstract
Urban trees, integral to urban environments, demonstrate intricate responses to atmospheric pollutants like particulate matter (PM), tropospheric ozone (O_3), and carbon dioxide (CO_2). Notably, O_3 induces oxidative stress in leaf tissues, while PM, consisting of fine airborne particles, interacts with urban trees through foliar deposition. This interaction is particularly interesting as tree canopies are highly effective filters, capturing and accumulating PM on their surfaces.
The present study focused on silver birch, small-leaved lime, and Norway maple seedlings responses to elevated O_3 and CO_2 with and without PM. Maple seedlings exhibited the highest stem height increment, followed by lime and birch. Elevated O_3 and CO_2 without PM led to substantial height increments for lime and maple. Elevated O_3 and CO_2 without PM increased the total polyphenols in lime and maple leaves but decreased the content of total flavonoids in birch and lime leaves.
Our findings underscore the adaptability of lime and maple seedlings to elevated O_3 and CO_2, positioning them as promising species for urban environments in the face of changing climates. Birch, while exhibiting biochemical changes, demonstrated less pronounced growth responses. This study's insights into the intricate interactions between urban trees and multiple pollutants, particularly the species-specific responses, are of significant value for urban planning and environmental management.
Keywords: urban trees, particulate matter, ozone, CO_2, seedling height, biochemical effect.

Introduction
Urban trees, integral components of the urban environment, exhibit complex responses to atmospheric pollutants such as particulate matter (PM), tropospheric ozone (O_3) and carbon dioxide (CO_2). Globally, tropospheric O_3 and PM stand out as the foremost air pollutants affecting plants, as highlighted by numerous studies (EEA, 2019; IPCC, 2019; Yeung *et al.*, 2019; Shahid *et al.*, 2019; Zhao *et al.*, 2020; Oksanen & Kontunen-Soppela, 2021). The O_3 infiltrates leaf tissues, instigating oxidative stress and provoking distinct physiological responses. The PM, encompassing fine particles suspended in the air, interfaces with urban trees through foliar deposition. Tree canopies act as effective filters, capturing and accumulating PM on their surfaces (Currie & Bass, 2008; Mondal & Singh, 2022). Plant stomata, especially those on leaves, absorb or sequester gaseous air pollutants such as CO_2 (Nowak & Dwyer, 2007; Nowak *et al.*, 2013; Baraldi *et al.*, 2019). Elevated CO_2 concentrations can induce a stimulation of photosynthesis. Complicating matters, plants in polluted regions face simultaneous exposure to unpredictable combinations of factors, creating multiple stress situations. For example, the PM deposition on leaves disrupted stomatal leaf exchanges, decreasing CO_2 assimilation and water exchange (Singh *et al.*, 2020; Singh, 2021).
The aim of the study was to evaluate stem height growth, total polyphenols, and flavonoid content as early indicators of three deciduous tree species: silver birch (*Betula pendula* Roth), small-leaved lime (*Tilia cordata* Mill.), and Norway maple (*Acer platanoides* L.). It investigated the response of these species to artificially induced exposure to elevated O_3 and CO_2 concentrations with and without PM.

Materials and Methods
One-year-old seedlings of silver birch, small-leaved lime and Norway maple were grown in pots, the substrate of which contained the optimum nutrition and watering. Seedlings were kept in the open field for a year before the start of the simulation experiment. Selected, visually healthy, two-year-old seedlings were placed in the chambers and treated with (*i*) PM + 180 ppb O_3 + 650 ppm CO_2, (*ii*) 180 ppb O_3 + 650 ppm CO_2, (*iii*) PM and (*iv*) control for ten weeks. There were 21 seedlings of each species per treatment.
The height (cm) of seedlings was measured at the beginning of the experiment and ten weeks after the birch, lime, and maple seedlings had grown under simulated conditions in the chambers.
For the analysis of total polyphenols (TPC) and total flavonoid content (TFC), leaf samples were collected in three biological replicates for each tree species from four treatments at the end of the vegetation season. Three composite samples (replicates) were made from each tree species for each treatment. For one composite leaf sample, 4–6 leaves were taken from three seedlings.
Quantifying amounts of TPC and TFC was performed spectrophotometrically using a SpectroStar Nano microplate reader (BMG Labtech, Offenburg, Germany) and 96-well microplates. TPC was determined using the Folin–Ciocalteu reagent according to a modified methodology (Lowry *et al.* 1951). TPC is expressed as micrograms of gallic acid equivalent to one gram of fresh mass (mg/g):

$$\text{Concentration (mg/g)} = (C \times V)/m \quad (1),$$

Here, C is the concentration obtained from the calibration curve (mg/mL), V is the extract volume (ml), and m is the weight of fresh biomass extracted (g).
TFC was estimated by forming a flavonoid-Al (III) complex (Chang *et al.*, 2002). TFC is expressed as micrograms of the quercetin equivalent in one gram of fresh biomass (mg/g):

Concentration (mg/g) = (C × V)/m (2),

Here, C is the concentration obtained from the calibration curve (mg/mL), V is the extract volume (ml), and m is the weight of raw biomass extracted (g).

For statistical analysis, Lilliefors and Kolmogorov–Smirnov tests checked the normality of the variables. The Kruskal–Wallis analysis of variance (ANOVA) test was used to ascertain the significant differences between the treatments. The means are presented with the standard error of the mean (±SE). Statistical analyses were conducted using the Statistica 12.0 software, and a level of significance of $p < 0.05$ was chosen in all cases.

Results and Discussion

In all treatments and the untreated control, the maple, followed by lime and birch seedlings, showed the mean increment in stem height, which was 51-76 cm, 23-27 cm, and 9-11 cm, respectively 'Figure 1'. Elevated O_3 and CO_2 without PM caused a slightly larger height increment for lime and a 1.5 times larger height increment for maple seedlings. PM exposure without elevated O_3 and CO_2 slightly reduced the stem height growth for lime seedlings.

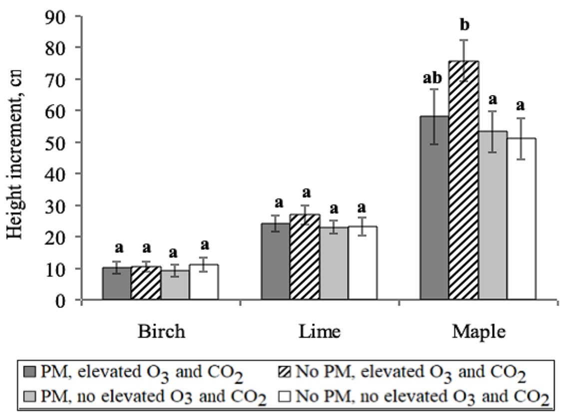

Figure 1. Stem height increment of silver birch, small-leaved lime, and Norway maple seedlings. Experimental treatments include exposure to Particulate Matter (PM), elevated O_3 and CO_2, and the controls. Different letters show statistical significance of the difference between the treatments at $p < 0.05$.

The highest TPC in the control seedlings was found in birch leaves (3.1 mg GA g^{-1}) and maple (2.8 mg GA g^{-1}) 'Figure 2'. The PM treatment and exposure to elevated O_3 and CO_2 did not change the TPC in the lime and maple leaves. The elevated O_3 and CO_2 concentrations without PM caused significantly higher TPC in the leaves of lime (33%) and maple (11%). Compared to the control, the mean TPC of birch leaves was significantly reduced by about 30% when treated with elevated O_3 and CO_2.

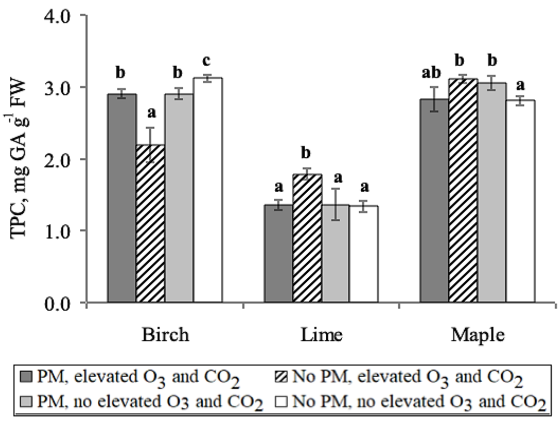

Figure 2. Total polyphenol content (TPC) in leaves of silver birch, small-leaved lime, and Norway maple seedlings. Experimental treatments include exposure to Particulate Matter (PM), elevated O_3 and CO_2, and the controls. Different letters show statistical significance of the difference between the treatments at $p < 0.05$.

Among the seedlings of different tree species, the highest TFC ranging between 0.78 and 1.14 mg QA g^{-1} of fresh weight was found in birch leaves 'Figure 3'. The PM and elevated O_3 and CO_2 treatment resulted in a significant 31-37% decrease in TFC in birch and lime leaves. Compared with untreated controls, mean TFC decreased by 1.4-1.5 in birch seedlings after exposure to elevated O_3 and CO_2, irrespective of PM treatment. The mean TFC slightly decreased in lime seedlings after exposure to elevated O_3 and CO_2 with PM treatment.

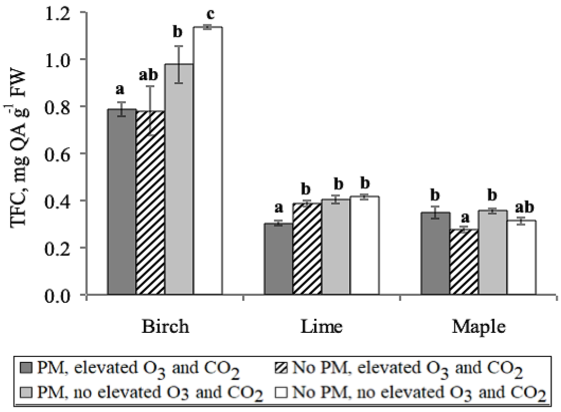

Figure 3. Total flavonoid content (TFC) in leaves of silver birch, small-leaved lime, and Norway maple seedlings. Experimental treatments include exposure to Particulate Matter (PM), elevated O_3 and CO_2, and the controls. Different letters show statistical significance of the difference between the treatments at $p < 0.05$.

Elevated CO_2 enhances plant growth by improving photosynthetic carbon assimilation, though prolonged exposure may lead to photosynthetic down-regulation, especially under nutrient-limiting conditions. Previous studies noted that elevated CO_2 concentrations cause more intensive plant growth followed by higher productivity (Ainsworth & Long, 2005), while O_3 causes phytotoxic effects on vegetation (Agathokleous *et al.*, 2018). The CO_2 and O_3 could negatively affect plant growth (Ashmore, 2005; Karnosky *et al.*, 2005; Wittig *et al.*, 2009).

Summarising, lime seedlings exhibited notable increases in height when subjected to elevated levels of O_3 and CO_2, demonstrating a significant degree of tolerance in terms of biochemical parameters. This adaptability and growth responses make this species well-suited for urban environments. Maple seedlings also experienced heightened growth under elevated O_3 and CO_2 conditions, showcasing adaptability despite variations in specific biochemical responses. The overall growth performance suggests that maple could be a valuable species for urban landscapes. In environments with elevated CO_2 levels due to changing climates, lime displayed increased height increments and exhibited biochemical resilience under elevated O_3 and CO_2 conditions. Similarly, maple demonstrated height increments under elevated O_3 and CO_2. These two species emerge as promising candidates for growing in urban territories under higher CO_2 concentrations.

Conclusions

1. The short-term effect did not thoroughly evaluate how urban tree seedlings would respond to stressful conditions caused by increased PM, O_3 and CO_2 concentrations.
2. Norway maple seedlings, which increased in height under elevated O_3 and CO_2 despite varying biochemical responses, showed potential for adaptation to simulated urban environmental conditions. Among the studied species, small-leaved lime seedlings appeared to have a higher biochemical resistance to simulated conditions than silver birch and could be more adaptable to urban environments.

Acknowledgements
This study is a part of the Valentinas Černiauskas PhD project (2021-2025).

References

Agathokleous, E., Kitao, M., & Kinose, Y. (2018). A review study on ozone phytotoxicity metrics for setting critical levels in Asia. *Asian Journal of Atmospheric Environment (AJAE).* 12, 1–16. DOI: 10.5572/ajae.2018.12.1.001.

Ainsworth, E. A. & Long, S. P. (2005). What have we learned from 15 years of free air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New phytologist*165, 351–371. DOI: 10.1111/j.1469-8137.2004.01224.x.

Ashmore, M. R. (2005). Assessing the future global impacts of ozone on vegetation. *Plant Cell Environ*. 28, 949–964. DOI: 10.1111/j.1365-3040.2005.01341.x.

Baraldi, R., Chieco, C., Neri, L., Facini, O., Rapparini, F., Morrone, L., Rotondi, A., & Carriero, G. (2019). An integrated study on air mitigation potential of urban vegetation: From a multi-trait approach to modeling. *Urban Forestry & Urban Greening*, *41*, 127-138. DOI: 10.1016/j.ufug.2019.03.020.

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of food and drug analysis*, *10*(3), 3. DOI: 10.38212/2224-6614.2748.

Currie, B. A. & Bass, B. (2008). Estimates of air pollution mitigation with green plants and green roofs using the UFORE model. *Urban ecosystems*, *11*, 409-422. DOI:10.1007/s11252-008-0054-y.

EEA. (2019). *Air Quality in Europe - 2019. European Environment Agency (EEA).* Report No 10/2019. 370. Retrieved March 01, 2024, from https://www.eea.europa.eu//publications/air-quality-in- europe-2019.

IPCC. (2019). *Climate change and land*, *Report*. Retrieved March 01, 2024, from https://www.ipcc.ch/site/assets/uploads/2019/08/2c.-Chapter-2_FINAL.pdf.

Karnosky, D. F., Skelly, J. M., Percy, K. E., & Chappelka, A .H. (2007). Perspectives regarding 50 years of research on effects of tropospheric ozone air pollution on US forests. Environmental pollution, 147(3), 489-506. DOI: 10.1016/j.envpol.2006.08.043.

Lowry, O., Rosebrough, N., Farr, A. L., & Randall, R. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, *193*(1), 265-275. DOI: 10.1016/S0021-9258(19)52451-6.

Mondal, S. & Singh, G. (2022). Air pollution tolerance, anticipated performance, and metal accumulation capacity of common plant species for green belt development. *Environmental Science and Pollution Research*, *29*(17), 25507-25518. DOI: 10.1007/s11356-021-17716-8.

Nowak, D. J. & Dwyer, J. F. (2007). Understanding the benefits and costs of urban forest ecosystems. In *Urban and community forestry in the northeast*, pp. 25-46. Dordrecht: Springer Netherlands.

Nowak, D. J., Greenfield, E. J., Hoehn, R. E., & Lapoint, E. (2013). Carbon storage and sequestration by trees in urban and community areas of the United States. *Environmental pollution*, *178*, 229-236. DOI: 10.1016/j.envpol.2013.03.019.

Oksanen, E. & Kontunen-Soppela, S. (2021). Plants have different strategies to defend against air pollutants. *Current Opinion in Environmental Science & Health*, *19*, 100222. DOI: 10.1016/j.coesh.2020.10.010.

Shahid, M., Dumat, N. C., Niazi, N. K., Xiong, T. T., Farooq, A. B. U., & Khalid, S. (2021). Ecotoxicology of heavy metal (loid)-enriched particulate matter: foliar accumulation by plants and health impacts. *Reviews of Environmental Contamination and Toxicology 253*, 65-113. DOI: 10.1007/398_2019_38.

Singh, H. (2021). An integrated approach considering physiological-and biophysical-based indicators for assessing tolerance of roadside plantations of *Alstonia scholaris* towards urban roadside air pollution: an assessment of adaptation of plantations for mitigating roadside air pollution. *Trees* 37(3), 1-15. DOI: 10.1007/s00468-021-02179-8.

Singh, H., Yadav, M., Kumar, N., Kumar, A., & Kumar, M. (2020). Assessing adaptation and mitigation potential of roadside trees under the influence of vehicular emissions: A case study of *Grevillea robusta* and *Mangifera indica* planted in an urban city of India. *Plos one*, 15(1), e0227380. DOI: 10.1371/journal.pone.0227380.

Wittig, V. E., Ainsworth, E. A., Naidu, S. L., Karnosky, D. F., & Long, S. P. (2009). Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. *Global ChangeBbiology*, *15*(2), 396-424. DOI: 10.1111/j.1365-2486.2008.01774.x.

Yeung, L. Y., Murray, L. T., Martinerie, P., Witrant, E., Hu, H., Banerjee, A., Orsi, A., & Chappellaz, J. (2019). Isotopic constraint on the twentieth-century increase in tropospheric ozone. *Nature*, *570*(7760), 224-227. DOI: 10.1038/s41586-019-1277-1.

Zhao, S., Yin, D., Yu, Y., Kang, S., Qin, D., & Dong, L. (2020). PM2.5 and O_3 pollution during 2015–2019 over 367 Chinese cities: Spatiotemporal variations, meteorological and topographical impacts. *Environmental Pollution*, *264*, 114694. DOI: 10.1016/j.envpol.2020.114694.