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Chlorophyll fluorescence characteristics in *Robinia pseudoacacia* L. under conditions of urban forest ecosystems in Dnipro city

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Abstract

The rapid technique for measuring of chlorophyll fluorescence photoinduction is one of modern informative methods for assessing the impact of abiotic factors (temperature, humidity, and environmental light intensity) on physiological traits of photosynthesis responsible for productivity. We explored alterations in activity of different types of chlorophyll fluorescence influenced by above mentioned basic abiotic factors which have been undergoing essential changes in Ukrainian Steppe zone in recent decades. The research object was the black locust, introduced tree species spread widely in the examined region as forest anti-erosion plantings and in the landscaping of industrial towns. To reveal background, stationary, maximum and variable chlorophyll fluorescence, we used a fluorimeter to measure spectrum of light absorption and reflection by leaves. The output was presented as Kautsky curve that depicted the fluorescence response changes over time. We clarified that the background concentration was minimal at low air temperature. With increasing temperature, there was an upward trend in the background fluorescence intensification. The increase in the air humidity caused the opposite effect. With raising temperature, the intensity of maximum fluorescence had the reverse trend compared to the background fluorescence. It decreased when the temperature got higher. Alike the background fluorescence, the stationary fluorescence was minimal at low air temperature, but its rise resulted in the decline in the stationary fluorescence intensity. The research outcomes are methods and mathematical models developed and offered for calculations of different fluorescence types. Statistically significant relationship between the fluorescence, temperature and light intensity was established. The obtained results allow defining the introduction of *Robinia pseudoacacia* L. as successful while its resistance to the impact of abiotic environmental factors can be identified as high. It allowed us to specify black locust as the up-to-date species when forming plantings, unless suitable care, management, and control have been fulfilled.

Key words: adaptation of introduced tree species, climatic factors, Kautsky curve, photosynthetic apparatus in trees, resistance of urban dendroflora, Steppe zone of Ukraine.

Introduction

Research on chlorophyll fluorescence intensity is a modern informative method applicable to ecological monitoring (Banks 2018, Holoborodko et al. 2022). The rapid technique of chlorophyll fluorescence photoinduction is one of the most effective methods intended to identify the impact of abiotic environmental factors on physiological conditions of introduced plant species. Timely diagnostics of plant physiological conditions through chlorophyll fluorescence allow obtaining key data on plant photosynthesis (Guidi et al. 2019, Lin et al. 2022).

The maintenance of active photosynthesis under physiological stress is usually associated with developing plant resilience to unfavourable environmental factors. Recently, the main scientific attention has been focused on exploring the plant response to different stressors. Namely, drought, high temperature (Farooq et al. 2009, Blum 2017, Urban et al. 2017, Zandalinas et al. 2018), and shading (Wan et al. 2020) were identified as the most extensive abiotic factors which cause alterations in plant physiological and biochemical reactions (Johnstone et al. 2014, Martínez-Ferri et al. 2016, Alonso et al. 2017, Baghbani et al. 2019, Lin et al. 2019, Berner et al. 2021, Shupranova et al. 2022).

Belgio et al. (2015) showed that with diminishing illumination of the plant habitat, the relative fluorescence quenching also decreases. It almost disappears when the light intensity varied within 20–30 lux or below. This may indicate a gradual decrease in photosynthetic activity in terms of chlorophyll the plants being shaded. As a result of subsequent structural rearrangements in the photosynthetic apparatus, a channel for a heat dissipation of the excitation energy of chlorophyll molecules opens, and the fluorescence decreases. This mechanism is assumed to play a protective role, defencing the photosynthetic apparatus against the damage caused by strong light and ensuring the optimal rate of electron transport between photosystems (Carvalho et al. 2015). An increase in temperature with a simultaneous increase in excitation light is explained by the protective effect of light against leaf damaging by high temperatures (Krüger et al. 2014).

For the last decades, climatic change has been developing on the local and global scale. The perception of photosynthesis that determines the primary productivity in response to fluctuations in air temperature, humidity, and environmental light intensity, belongs to topical scientific tasks (Kosova et al. 2018). It can be explained by the fact that autotrophic ecosystem component establishes the heterotroph functioning, being a driving environmental factor. Therefore, the analysis of photosynthesis activity in leaves exposed to stressful conditions has been a main focus of the major studies. The invention of next-generation fluorimeters that are easy to use and provide high resolution allowed quick kinetics of chlorophyll induction or fluorescence to be measured (Cheng et al. 2019). Parameters obtained by these means are widely utilised to examine responses of photosystem II to stress factors (Farquhar et al. 2001, Bürling et al. 2013, Chen et al. 2019).

Black locust (*Robinia pseudoacacia* L.) is a deciduous fast-growing woody species with wide range of environmental adaptations and native to North America. It has one of the widest distributions in Europe of any introduced plants (Martin 2019). The future climate is conducive to the northward expansion of black locust with a speed of 21 km per decade (Li et al.

2021). It is one of species most commonly introduced within artificial forests and urban plantations in Steppe zone of Ukraine. The functional categories of the analysed species are as follows: (i) plantations intended for environmental protection, scientific, historical and cultural purposes – 1831.1 ha; (ii) recreational-therapeutic plantations – 7173.5 ha; (iii) anti-erosion protection plantations – 8679.1 ha (Lovynska and Sytnyk 2016).

This work hypothesises that, being introduced in the Steppe zone of Ukraine, the black locust assimilation apparatus responded differently to abiotic factors of the environment in different ranges of their values. Therefore, the purpose of the work was to determine the changes in the chlorophyll fluorescence parameters at different values of air temperature, humidity, and light intensity, as well as to identify the abiotic factors that most significantly affect the chlorophyll fluorescence.

Materials and Methods

This study was carried out in black locust plantations in Steppe zone of Ukraine (Fig. 1).

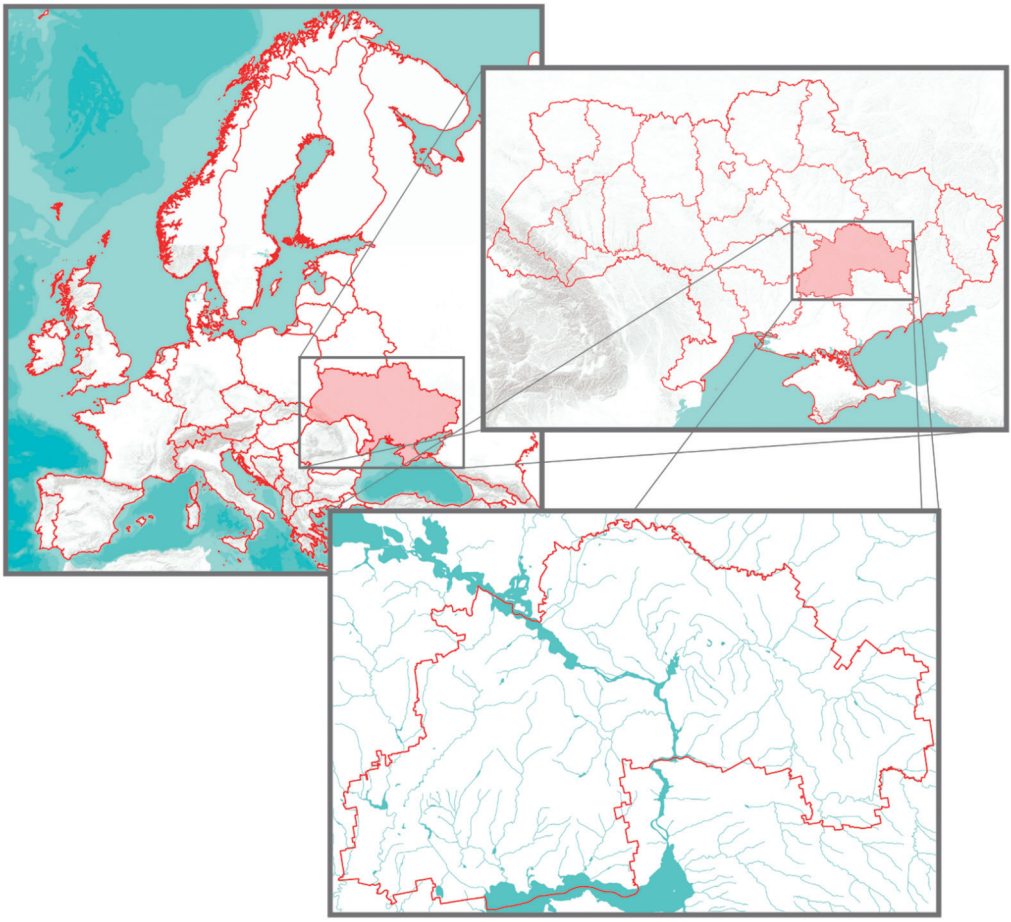

Fig. 1. The study region – Dnipro city, Ukraine.

Geographical parameters are the key regulators of the cultivation of introduced plant species. Their growth and development depend on numerous abiotic factors, but the climatic factors have the greatest limiting effect. Their consistency with ecological demands of the tree species ensures successful development and maintains further functions of tree plantations cultivated in novel geographical areas, essentially different from the relevant native areal.

The abiotic factors of air temperature, humidity, and environmental light intensity have significant effects on the progress of photosynthesis. To detect influence of the abiotic climatic factors, we analysed changes in air temperature and hydrothermic coefficient (HTC) as a ratio of a precipitation quantity (in mm) at a temperature above 10°C to the sum of temperatures for the same time period divided by 10. The considered climatic data were obtained from the Meteorological Site in Dnipro city (synoptic index 34504, altitude: 143 m, geographical coordinates: latitude 48°60´ N, longitude 34°97´ E) for 45 years from 1974 to 2018 (Weather and Climate 2004–2023).

The long-term averaged temperature in the coldest month of January was –3.6 °C, while in the hottest month of July it reached +22.1°C. However, in some years, the air temperature significantly deviated from a typical isotherm. The absolute minimum of –30.0 °C was recorded on January 11, 1950, while the absolute maximum of +40.9 °C was registered on August 8, 2010. Absolute minimum values of air temperature in winter were negative, whereas the corresponding maximum values were positive and equalled 13.6°C in December; 12.6°C in January; 17.5°C in February. The latter were recorded for the previous decade. The frost-free period usually lasts 160 to 220 days. In 1843, the average annual air temperature in Dnipro city was 9°C. It ranged from 6.3°C in 1987 to 10.8 °C in 2007. An annual amplitude between the average temperature in the warmest and coldest months was 25.7°C (Fig. 2).

A hydrothermal coefficient in Dnipro city had a wide range of values between 0.51 and 2.00 (Fig. 3). It meant that

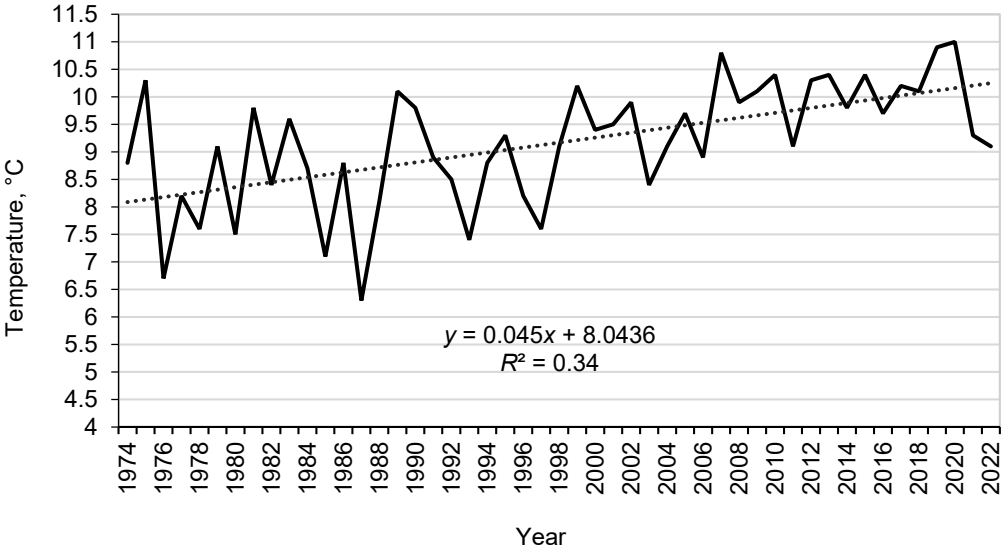

Fig. 2. Trend in average air temperature in Dnipro city.

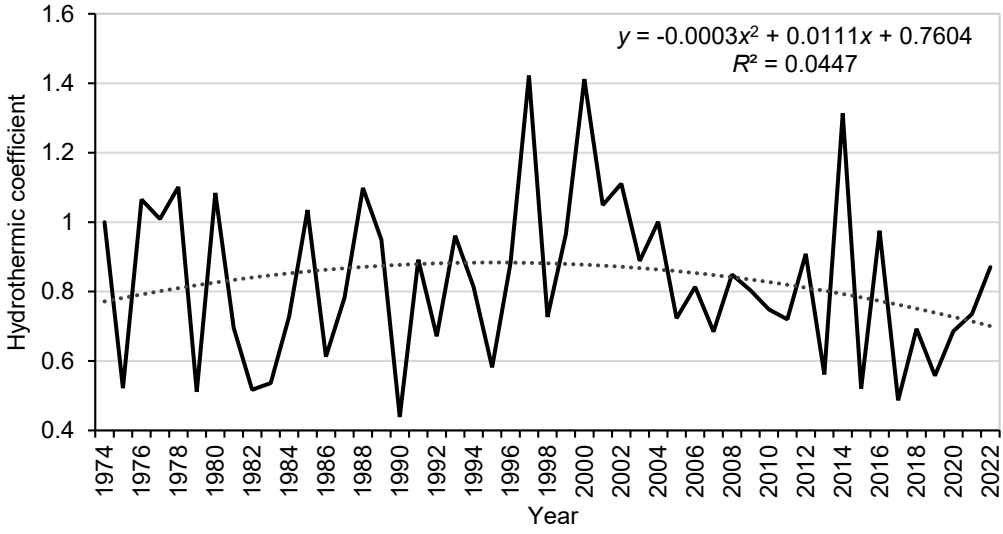

Fig. 3. Hydrothermic coefficient dynamics in Dnipro city.

there were alternating periods of severe droughts (when HTC ≤ 0.5) and excessive precipitations (HTC ≥ 1.5).

The climate of the Dnipropetrovsk region is moderately continental. The most common soil in the Dnipropetrovsk region is ordinary chernozem (or Mollisols in the US soil taxonomy) developed on loess and loess-like sediments. It is a dark-coloured soil containing a high percentage of humic, phosphorus and ammonia compounds. Chernozem is loose and can absorbs and holds water well, and at the same time is easily ventilated. All this creates favourable conditions for plant nutrition and growth. Subsoil water occurs, as a rule, at the depth of more than 5 m and does not influence the soil formation. The thickness of the humus layer varies from 40 to 80 cm. The humus content in the upper horizon of low-humus variety is 3.5–5.5 %, and in the medium-humus, 5.5–6.5 %. The reaction of the soil solution is subacid or near to neutral. The moisture content is subject to considerable seasonal and annual fluctuations (Kunakh et al. 2022).

In the place of the city areas, fine-textured low-humic ordinary chernozem, differently eroded had previously been naturally formed. In a megacity, anthropogenic activity in natural chernozems is often occurs with the formation of transformed soils. In Ukraine, the classification scheme of transformed soils includes the class 'Anthropogenic soils', group of types 'Technological soils' (technozems and lithozems). Soils of urbanized areas have specific differences from natural soils, such as formation of poured mixed upper horison with the presence of various construction and household wastes, shift of acid-alkaline balance towards its alkalization; contamination by heavy metals, oil products, emission components of industrial enterprises; change in physical-mechanical properties (lowered moisture capacity, increased density, rockiness ets.), decreasing buffering capacity (Khokhryakova 2020).

The plant photosystem conditions were explored using the induction in method of chlorophyll fluorescence ob-

served in the plant leaves. For such a reason, the spectra of light reflection and absorption were measured. The output was presented as Kautsky curve, which depicted the time dependence of chlorophyll fluorescence intensity. To study the alterations in native chlorophyll fluorescence of fresh (living) leaves, a portable fluorimeter 'Floratest' (Ukraine) was used (Romanov et al. 2013). Its remote optoelectronic sensor includes a LED that has a maximum radiation intensity at λ (470 + 20 nm). Irradiation indicators in the sensor were: radiation wavelength 470 + 15 nm; irradiated spot size not less than 15 mm^2; luminous intensity within the spot at least 2.4 $W \cdot m^{-2}$. Indicators for signal detection in optoelectronic sensor were: the spectral range of fluorescence intensity measurement 670–800 nm; receiving window size 9 mm^2; photodetector sensitivity at λ = 650 nm was 0.45 $A \cdot W^{-1}$. We carried out this research during the active vegetation season of *R. pseudoacacia* in May–September 2021. The critical parameters of Kautsky curve were measured monthly, namely on 23.05.2021, 20.06.2021, 21.07.2021, 23.08.2021, 20.09.2021 at 12:00 at the height of the sun activity.

Our study was performed in green areas in Dnipro city, and included 7 trees with similar morpho-taxational features. The critical parameters of Kautsky curve were measured on average leaves including 7 pieces of each tree. on the brightly lit side, as well as 7 pieces on the shaded side. The leaves were sampled in dry and sunny weather from the annual vegetative growth in a lower third of a tree crown in different compass points. The leaves were labelled for further comparison.

To clarify the impact of natural factors on fluorescence, we used a number of indicators (Holoborodko et al. 2021):

F_0 – baseline (background) fluorescence;

F_m – maximum fluorescence;

F_{st} – stationary fluorescence;

F_v/F_m – the index of internal or maximum quantum yield of photosystem II (PSII) calculated with the formula (1):

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m}. \quad (1)$$

To address the research goals, we applied mathematical analysis of variance, ANOVA. Like cluster and statistical methods, this approach is an efficient technique utilised to carry out research in miscellaneous fields of natural sciences, e.g. in forestry and agriculture (Vasylieva et al. 2015, 2021). In general, ANOVA allows clarifying the dynamics of parameters and identifying pivot points of significant changes that impact dependent variables in an essential way. In our study, ANOVA enables us to compare the averages of the fluorescence indicators between the groups of observations according to the specific ranges of the factors considered, such as air temperature, humidity, and environmental light intensity.

It involved checking null and alternative hypotheses, such as:

H_0: There is no significant difference between the averages of the compared groups;

H_a: There is significant difference between the averages of the compared groups.

If the impact was found to be insignificant, then the alternative hypothesis was rejected. In contrast, if the revealed influence was significant, then the null hypothesis should be rejected. ANOVA method is based on checking of Fisher criterion to evaluate the ratio of so-called between-group variability to within-group variability.

In more detail, let the entire data be

distributed into M groups consisting of N_m observations X_{mn}, $n = 1…N_m$, $m = 1…M$. Then the total number of observations is calculated by the formula (2):

$$N = \sum_{m=1...M} N_m \quad (2)$$

and, the total mean amounts were calculated by the formula (3):

$$\overline{X} = \frac{\sum_{m=1...M} \sum_{n=1...N} X_{nm}}{N}. \quad (3)$$

Similarly, the mean within the group m is determined by the formula (4):

$$\overline{X}_m = \frac{\sum_{n=1...N_m} X_{mn}}{N_m}, m = 1...M. \quad (4)$$

Fisher ratio is presented by the formula (5):

$$F_{calculated} = \frac{f_2 \sum_{m=1...M} N_m \left(\overline{X}_m - \overline{X}\right)^2}{f_1 \sum_{m=1...M} \sum_{n=1...N_m} \left(X_{mn} - \overline{X}_m\right)^2}, \quad (5)$$

where: $f_1 = M - 1$ is a degree of freedom of the variable between groups explained by an impact of the factor analysed, $f_2 = N - M$ is a degree of freedom of the variable within groups unexplained by the factor considered.

The critical value $F_{critical}$ of Fisher criterion depends on the chosen α level of significance. as well as degrees of freedom f_1 and f_2. Then the inequality $F_{calculated} \leq F_{critical}$ results in rejecting the alternative hypothesis H_α, in other words the changes in the considered factor have no effect on the relevant indicator with the probability of $(1-\alpha)$.

On the contrary, the inequality $F_{calculated} > F_{critical}$ disproves the null hypothesis H_0 and states that the relevant indicator depends on the changes in the factor considered factor with the probability of $(1-\alpha)$.

The calculations carried out covered up to $N = 199$ observations and the significance level of $\alpha = 0.05$.

Results

Several measured indicators of chlorophyll fluorescence intensity, as well as calculated parameters of the variance analysis performed are presented in tables 1, 2, and 3. The sample data size accounted for 60 observations for each of four indicators of fluorescence and each of three environmental parameters. Thus, the total number of the analysed values amounted to 720. For the sake of uniformity every data set was divided into 6 groups or clusters, each of which included 10 observations introducing 6 ranges of factor changes to analyze and compare. In tables 1–3, every cluster by each factor was described by the corresponding group average (AV) and coefficient of variance (CV) equal to ratio of the standard deviation to the group average.

The calculations were performed by means of free software Google Sheets. They are numbered in tables 1–3 and included only clusters relevant to the particular calculation. The clusters excluded from some calculations were marked with dashes (-).

To detect an impact of the abiotic factor conditioned by air temperature, chlorophyll fluorescence was examined within the temperature range of 16 °C to 41 °C. The absolute values of the background fluorescence varied within 304 to 560 relative fluorescence units. The minimum intensity of the background fluorescence was recorded at an air temperature from 18 °C to 19.9 °C. Its maximum was observed at the air temperature in the range of 29–30.9 °C. The applied ANOVA technique allowed the conclusion that temperature has a statistically significant effect on the target indicator. Data presented in Table 1 revealed that the indicator F_0 was essentially different within

Table 1. Chlorophyll fluorescence in *R. pseudoacacia* leaves depending on air temperature.

No	Fluorescence indicators		Range of temperature, °C						$F_{calculated}$	P	$F_{critical}$
			16.0–17.9	18.0–19.9	20.0–26.9	27.0–28.9	29.0–30.9	31.0–41.0			
1	F_0	AV	372.8	310.4	411.8	407.2	449.6	409.6	13.07	0.000	2.39
		CV	0.05	0.04	0.14	0.07	0.16	0.05			
2		AV	-	-	411.8	407.2	449.6	409.6	1.64	0.197	2.87
		CV	-	-	0.14	0.07	0.16	0.05			
3	F_m	AV	1542.4	1534.8	1347.6	1259.6	1361.6	1507.2	2.05	0.086	2.39
		CV	0.23	0.09	0.28	0.18	0.19	0.02			
4	F_{st}	AV	1473.6	1379.2	992.2	918.8	1014.4	1099.2	10.84	0.000	2.39
		CV	0.25	0.09	0.30	0.16	0.16	0.04			
5		AV	1473.6	1379.2	-	-	-	-	0.62	0.442	4.41
		CV	0.25	0.09	-	-	-	-			
6		AV	-	-	992.2	918.8	1014.4	1099.2	1.58	0.211	2.87
		CV	-	-	0.30	0.16	0.16	0.04			
7	F_v/F_m	AV	0.77	0.78	0.74	0.67	0.66	0.65	13.42	0.000	2.39
		CV	0.09	0.04	0.10	0.05	0.06	0.03			
8		AV	0.77	0.78	0.74	-	-	-	1.03	0.369	3.35
		CV	0.09	0.04	0.10	-	-	-			
9		AV	-	-	-	0.67	0.66	0.65	1.18	0.323	3.35
		CV	-	-	-	0.05	0.06	0.03			

the whole temperature range of 16 °C to 41 °C (Table 1, calculation 1). This was true because the calculated Fisher criterion was larger than the corresponding critical coefficient. However, fluctuations in the intensity of the background fluorescence turned out to be non-essential at an air temperature of 20 °C to 41 °C (Table 1, calculation 2).

The intensity of the maximal chlorophyll fluorescence developed a reverse trend compared to the background one. It decreases when the air temperature increases. The minimal average level of this physiological indicator of fluorescence was recorded within a temperature range of 27 °C to 28.9 °C. At the same time, this study detected no any significant impact of air temperature on maximal fluorescence F_m (Table 1, calculation 3). The stationary fluorescence showed a maximum intensity at low air temperature of 16°C to 17.9°C. Its growth resulted in a decrease in the stationary fluorescence values. Such tendency was confirmed by the calculated Fisher criterion. At the same time, indicator F_{st} was statistically uniform within temperature range of 16 °C to 19.9 °C, as well as 20 °C to 41 °C (Table 1, calculations 5 and 6). The index of the maximum quantum yield of PSII (F_v/F_m) varied from 0.59 to 0.86. According to the calculated Fisher criterion, this coefficient showed a clear downward trend triggered by an increase in air temperature. The significant decrease in the discussed index occurred

at the temperature of around 27 °C. Namely, indicator F_v/F_m was relatively stable within the temperature range of 16 °C to 26.9°C, as well as 27°C to 41 °C (Table 1, calculations 8 and 9).

Background, maximum and stationary chlorophyll fluorescence intensity were measured under the condition of relative air humidity from 31 % to 50 %. The background fluorescence associated with an ecological factor of relative air humidity reached its maximum, when the latter showed low values 31 % to 33.9 %. A further increase in humidity caused a reduction in the background fluorescence intensity. According to data accumulated in Table 2, the background fluorescence F_0 was relatively uniform within the range of humidity between 34.0 % and 43.9 % (Table 2, calculation 2).

The highest values of the maximum chlorophyll fluorescence in *R. pseudoacacia* leaves were observed at the air humidity of 31 % to 33.9 %, as well as of 44 % to 50 %. Meanwhile, stationary fluorescence reached its maximum when air humidity ranged from 39 % to 41.9 %. Its intensity dropped with the boundary values of air humidity. The research conducted conclude no essential reliable changes in maximum F_m or stationary F_{st} fluorescence associated with an increase in relative air humidity (Table 2, calculations 3 and 4). The average minimal index (0.62) of the maximum quantum yield of PSII was far from optimal at the air humidity of 31 % to 33.9 % (Table 2, calculation 5). With increasing environmental humidity, the active centres of PSII ensured stable functioning that resulted in growing of the ratio F_v/F_m. However, it was statistically stable within the rest of observed air humidity range (Table 2, calculation 6).

The study analysed the impact of the environment factors on *R. pseudoacacia* growth in phytocoenoses of Dnipro city with respect to different types of chlorophyll fluorescence. The research findings clarified that the maximum background fluorescence occurred under the condition when the light intensity was within the ranges of 150 to 299 lx and 700 to 999 lx.

Table 2. Chlorophyll fluorescence in *R. pseudoacacia* leaves depending on air humidity.

No	Fluorescence indicators		Range of air humidity, %						$F_{calculated}$	P	$F_{critical}$
			31.0–33.9	34.0–36.9	37.0–38.9	39.0–41.9	42.0–43.9	44.0–50.0			
1	F_0	AV	598.4	386.8	360.0	370.4	363.2	527.8	24.17	0.000	2.39
		CV	0.06	0.02	0.16	0.05	0.10	0.27			
2		AV	-	386.8	360.0	370.4	363.2	-	1.093	0.365	2.87
		CV	-	0.02	0.16	0.05	0.10	-			
3	F_m	AV	1532.8	1489.6	1369.6	1469.2	1412.8	1533.2	0.93	0.467	2.39
		CV	0.06	0.04	0.21	0.11	0.19	0.19			
4	F_{st}	AV	1080.0	1126.2	1131.2	1225.6	1084.8	1081.4	0.75	0.588	2.39
		CV	0.10	0.03	0.22	0.15	0.31	0.17			
5	F_v/F_m	AV	0.62	0.72	0.71	0.71	0.71	0.66	4.00	0.004	2.39
		CV	0.04	0.03	0.14	0.08	0.09	0.07			
6		AV	-	0.72	0.71	0.71	0.71	0.66	1.21	0.320	2.58
		CV	-	0.03	0.14	0.08	0.09	0.07			

Meanwhile, the corresponding minimum value associated with the environmental light intensity was 300 to 499 lx. The calculated Fisher criterion obtained using ANOVA technique was less than the critical one. It enabled us to reject the hypothesis that there is a significant relationship between the background fluorescence intensity F_0 and environmental light intensity (Table 3, calculation 1).

Both the maximum and stationary chlorophyll fluorescence depended on light intensity. In particular, the maximal fluorescence reached its peak when an environmental light intensity was within 300–499 lx, and dropped down when the light intensity was within 700–999 lx. Indicator F_m was statistically uniform when the light intensity varied between 150 and 699 lx, as well as from 1000 to 7450 lx (Table 3, calculations 3 and 4). The average values of the stationary chlorophyll fluorescence F_{st} were constant when the light intensity varied between 150 and 699 lx, as well as between 700 and 7450 lx (Table 3, calculations 6 and 7).

The coefficient of maximum quantum yield of PSII had a near-optimal value, except when light intensity was within 700–999 lx. However, the indicator F_v/F_m was

Table 3. Chlorophyll fluorescence in leaves of *R. pseudoacacia* depending on light intensity.

No	Fluorescence indicators		Range of light intensity, lx						$F_{calculated}$	P	$F_{critical}$
			150–299	300–499	500–699	700–999	1000–1699	1700–7450			
1	F_0	AV	454.4	363.2	369.2	441.0	364.8	367.6	9.29	0.000	2.39
		CV	0.06	0.04	0.05	0.17	0.18	0.06			
2		AV	1614.4	1636.8	1557.4	1110.4	1448.0	1435.8	6.39	0.000	2.39
		CV	0.04	0.03	0.06	0.17	0.31	0.22			
3	F_m	AV	1614.4	1636.8	1557.4	-	-	-	3.20	0.057	3.35
		CV	0.04	0.03	0.06	-	-	-			
4		AV	-	-	-	-	1448.0	1435.8	0.005	0.945	4.41
		CV	-	-	-	-	0.31	0.22			
5		AV	1190.4	1209.6	1141.6	895.6	1038.4	1105.4	4.21	0.003	2.39
		CV	0.03	0.03	0.15	0.13	0.25	0.25			
6	F_{st}	AV	1190.4	1209.6	1141.6	-	-	-	1.22	0.310	3.35
		CV	0.03	0.03	0.15	-	-	-			
7		AV	-	-	-	895.6	1038.4	1105.4	2.13	0.139	3.35
		CV	-	-	-	0.13	0.25	0.25			
8		AV	0.70	0.71	0.70	0.50	0.72	0.74	22.01	0.000	2.39
		CV	0.02	0.05	0.07	0.17	0.13	0.06			
9	F_v/F_m	AV	0.70	0.71	0.70	-	-	-	0.13	0.878	3.35
		CV	0.02	0.05	0.07	-	-	-			
10		AV	-	-	-	-	0.72	0.74	0.72	0.407	4.41
		CV	-	-	-	-	0.13	0.06			

relatively stable in the light intensity range of 150–699 lx, as well as 1000–7450 lx, showing a strong dependence on the light intensity of the environment (Table 3, calculations 9 and 10).

The next stage of the fulfilled research suggested establishing a mathematical relationship between different kinds of fluorescence and the analysed environmental factors by means of econometric non-linear regressions.

According to the data given, some of the modelled equations have statistically insignificant coefficients of determination. The one-factor models linked F_0 and F_{st} to temperature and also F_m and F_{st} to light intensity, had statistically reliable coefficients of their regressions. The two-factor models created linking F_{st} to temperature, and light intensity had statistically reliable coefficients (*a*, *b, c*) of their regressions. Statistically significant coefficients *a* and c were identified for F_m, while statistically significant coefficients *a* and *b* were detected for F_0. The most reliable three-factor regression (as a mathematical relationship based on temperature, humidity, and light intensity) was calculated for F_{st}. Overall, due to statistical analysis with regard to the mathematical models considered, this research revealed a fairly strong dependence of the fluorescence on the temperature and light intensity, but a similar relationship between the fluorescence and the humidity was not confirmed.

Discussion

PSII is considered to be the most sensitive to overheating. The thermal exposure was shown to cause disintegration of the components within PSII complex and a significant decrease in the efficiency of excitation energy transfer from light-harvesting pigments to PSII reaction centres. The background fluorescence F_0 (under normal conditions it corresponds to Chl_a when PSII reaction centres are fully open) was proposed to use as a test for the thermal stability of F_0 (Kalaji et al. 2012, 2016). Several authors (Crafts-Brandner and Salvucci 2002) found a significant increase in the F_0 level after heating of chrysanthemum leaves at temperatures above 38 °C for 30–120 min. At the same time, the researchers registered a sharp decrease in the F_v/F_m ratio, which characterizes the photochemical activity of PSII.

The value of background fluorescence (F_0) depends on the excitation energy lost during its transfer across a pigment matrix and also on the quantity of chlorophyll molecules without functional links to reaction centres (Sayed 2003, Zhang et al. 2020). This fact can be attributable to structural changes in a pigment complex caused by a combined impact of air temperature, humidity, and environmental light intensity. A decrease in the quantity of antenna chlorophylls reduces the initial level of fluorescence, and *vice versa* (Demmig-Adams et al. 2012). The non-photochemical decrease in the fluorescence intensity of PSII-associated chlorophyll is explained by the establishment of the proton gradient on the thylakoid membrane and redistributing the excitation energy in favour of PSI. The formation of ΔpH on the thylakoid membrane leads to the protonation of a special PsbS protein and the conversion of the carotenoid pigment violaxanthin into zeaxanthin. The mechanism for optimising the joint action of two photosystems includes reversible phosphorylation of a special light-harvesting chlorophyll-protein complex and its relocation between membrane areas with different content of PSI and PSII complexes (Mathur et al. 2011).

The parameter F_{st} describes the highest level of fluorescence; in other words, it is the maximum value on the induction curve. It tends to be the most variable due to its high adaptability (Zhao et al. 2018). The minimal indicators of F_{st} in the structure of a plant pigment complex were revealed in *R. pseudoacacia* trees to be affected by three factors: air temperature, humidity, and environmental light intensity. This combination is also responsible for a decrease in both light harvesting and antenna chlorophylls. The value of the stationary fluorescence F_{st} decreased coincidently with a downward trend for air temperature, humidity, and environmental light intensity (Sepúlveda and Johnstone 2019, Shupranova et al. 2019) This parameter featured a dynamic balance between processes that explain rising and decreasing (Ruban 2016).

The ratio F_v/F_m has been largely used as a sensitive indicator of plant photosynthetic performance (Guidi et al. 2019). Some scientists suggest that the decrease in this index indicates reducing PSII efficiency, namely photoinhibition. Being sensitive to inhibition of a light-dependent stage of photosynthesis, the parameter F_v/F_m provides an effective means to monitor the stressful effects on plants (Sonti et al. 2020**)**. This parameter is informative with regard to basic processes which boost changes in photosynthetic performance, in particular non-photochemical quenching (Uhrin and Supuka 2016, Uhrin et al. 2018). The values of F_v/F_m identify the maximum quantum yield of the potential PSII and are utilised as a sensitive productivity indicator of the plant photosystem. Its optimal value is close to 0.83 in major plants (Suchocka et al. 2021).

A sharp decrease in the ratio F_v/F_m, which characterises the maximum photochemical activity of PSII, also revealed the suppression of the functional activity in the photosynthetic apparatus after heating a leaf to over 38 °C. As for the background fluorescence level F_0 when PSII reaction centres open, it remains practically unchanged up to temperatures of 42 °C, which could indicate no significant disturbances in the aggregation of the light harvesting antenna and reaction centres (Janka et al. 2013). Perhaps, F_v/F_m is the parameter most frequently used for measuring of chlorophyll fluorescence and assessing plant responses to some stresses. However, some scientists concluded that F_v/F_m represents the plant resistance to moderate drought (Tezara et al. 1999, Oukarroum et al. 2007, Wan et al. 2020).

Our study disclosed that the discussed index varies between 0.65 and 0.66 regardless of the abiotic environmental factors considered. This means that its impact on plants growing in urban technogenic areas resulted in photoinhibition in all three cases associated with light intensity, temperature, and humidity. In this regard, a significant decrease in F_v/F_m can be interpreted as a factor of photo-damage, while an insignificant decline might be explained by photoprotection (Golan et al. 2015). In turn, some researchers associated a relatively moderate decrease in F_v/F_m with a reduced risk of photooxidative stress or so-called positive photoinhibition caused by activity of PSII-associated proteins (Giardi et al. 1996).

Conclusion

The absolute values of the background fluorescence fluctuated between 304 and 560 relative units. The minimum intensity of the background fluorescence was found at an air temperature of 18 °C to 19.9 °C.

The stationary fluorescence amounted to maximum when the air temperature dropped to 16–17.9 °C. The maximum level of background fluorescence was associated with low air humidity of 31 % to 34 %. The subsequent increase in humidity resulted in a decrease in background fluorescence intensity. The highest values of the maximum chlorophyll fluorescence produced by *R. pseudoacacia* were detected at humidity values of 31 % to 33.9 % and 44 % to 50 %. Stationary fluorescence amounted to its maximum intensity at an air humidity of 39 % to 41.9 %. Background fluorescence was the most active at a light intensity within 700–999 lx. The stationary fluorescence reached its maximum at a light intensity of 300–499 lx, but it was minimal when the light intensity increased to 700–999 lx. The coefficient of the maximum quantum yield of PSII was stable and relatively close to its optimum except for an environmental light intensity of 700–999 lx.

Our research resulted in confirming the hypothesis about different levels of influence of the studied abiotic factors on the photosynthetic processes observed in *R. pseudoacacia* under conditions of its introduction. Chlorophyll fluorescence appeared to be most sensitive to changes in light intensity levels. The marginal values of all types of fluorescence were determined for a wide range of values of the studied abiotic factors. According to data analysis, the values of chlorophyll fluorescence intensity varied significantly depending on abiotic factors. The research findings identified the values an air temperature, air humidity and light intensity at which background, maximum and stationary fluorescence reach their minimums and maximums.

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