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PHOTOSYNTHETIC PIGMENTS AND PHYTOCHEMICAL ACTIVITY OF PHOTOSYNTHETIC APPARATUS OF MAIZE (*Zea mays* L.) LEAVES UNDER THE EFFECT OF THIAMETHOXAM

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Abstract

In the last decade, neonicotinoid insecticides have been actively used to protect plants from pests. Moreover, their effect on the plants, in particular on the state of photosynthetic pigments, has been studied paucity. In the present work, it was shown for the first time that treatment of maize (*Zea mays* L.) leaves with a thiamethoxam (TMX) insecticide leads to a decrease in the functional activity of photosystem II and a decrease in the energization of thylakoid membranes. In addition, the effect of thiamethoxam depends on the genotype of maize. The aim of the work was to study the effect of thiamethoxam pesticide on photosynthetic pigments and the photochemical activity of the photosynthetic apparatus of maize leaves of two genotypes. The experiments were carried out in 2018–2019. The object of the study was samples of maize leaves of the inbred line zppl 225 and hybrid zp 341 with high rates of germination, grain quality and yield (Institut za kukuruz “Zemun Polje”, Belgrade, Serbia). The seeds were germinated until the roots appeared (length not less than 5 mm), after which they were planted in the soil (vermiculite: chernozem mixture, 1: 1) and grown under 16-hour daylight at a constant temperature of 25 °C. When the third true leaf appeared (more than 4 cm in length), the plants were sprayed with a TMX solution at a concentration of 0.2 mg/l. Plants grown under similar conditions without TMX spraying were used as controls. The measurements were carried out when the fifth true leaf reached a size of 12–14 cm. The content of photosynthetic pigments (chlorophyll a, b and carotenoids) was determined spectrophotometrically in 100 % acetone extract and calculated using the Holm-Wetstein formula. A change in the conformation of carotenoid molecules was recorded by Raman spectroscopy. Light-induced kinetics of prompt fluorescence (PF), delayed fluorescence (DF) and modulated reflection at $\lambda = 820$ nm (MR) were recorded simultaneously using a multifunctional plant efficiency analyzer M-PEA-2 (Hansatech Instruments, Great Britain). PF induction curves (OJIP curves) were analyzed using a standard JIP-test. It was found that in the phase of the fifth true leaf, in the inbred line zppl 225, the chlorophyll content in the presence of TMX decreased from 0.74 to 0.61 mg/g: the amount of chlorophyll a decreased by 17 %, chlorophyll b by 24 %. In contrast, no changes in pigment composition were detected in the leaves of the zp 341 hybrid when exposed to TMX. The OJIP curves of the control and TMX-treated leaves had a typical curve with characteristic O-J, J-I, and I-P phases, which reflected the processes of sequential reduction of carriers in the electron transport chain of photosynthesis (ETC) between two photosystems. The effect of the pesticide on the leaves of two maize genotypes was manifested in a decrease in the functional state of photosystem II, determined by the fluorescence parameter (PI_{ABS}), which was obtained based on the analysis of OJIP curves using the JIP-test. Comparison of PI_{ABS} in the control and under the influence of TMX revealed statistically sig-

nificant ($p < 0.05$) differences: in the leaves of zppl 225 and zp 341 samples treated with TMX, the PI_{ABS} parameter decreased by 29 and 24%, respectively. Changes in the fast phase of delayed fluorescence, associated with a decrease in the energyzation of the thylakoid membrane upon exposure to TMX, were detected in the leaves of maize. An analysis of the maximum oxidation and reduction rates of P700 (MR kinetics) indicates a decrease in the acceptor pool on the acceptor side of PSI in zppl 225 leaves when exposed to TMX. It was found that the reaction centers (RCs) of PSI zp 341 showed resistance to TMX (no change in the redox transformations of P700). TMX caused changes in the conformation of carotenoid molecules, but did not change their content in the leaf. The proposed combination of methods for prompt fluorescence, delayed fluorescence, modulated reflection at $\lambda = 820$ nm and Raman spectroscopy can be the basis for the formation of an effective technology for the diagnosis of early defects of photosynthetic pigments when pesticides enter an intact plant.

Keywords: *Zea mays* L., pesticides, Raman spectroscopy, thiamethoxam, chlorophyll, carotenoids, chlorophyll fluorescence

In the last decade, neuroactive nicotine-based insecticides (imidacloprid, acetamipride, dinotefuran, thiamethoxam) have been widely used to protect plants from pests. Neonicotinoids act as acetylcholine agonists by binding to nicotinic acetylcholine receptors (nAChRs) on the postsynaptic membrane, which causes blocking of synaptic transmission, inhibition of excitation and death of the insect [1]. However, there is no consensus on the effect of neonicotinoids on the state of the plant itself [2]. On the one hand, neonicotinoids are able to improve the morphological and physiological characteristics of some plants even under stressful conditions, which contributes to their growth and yield increase [3], and on the other hand, there is evidence of phytotoxicity of neonicotinoids [4, 5].

It is known that maize production is complicated by the influence of various abiotic and biotic factors, as well as the ability of phytophages to damage the plant at the very early stages of development. It is prospective to investigate changes in the state of pigment composition of maize plants under the action of neonicotinoids in both laboratory and field conditions

The photosynthetic apparatus (PSA) of higher plants, consisting of pigment-protein complexes, includes two types of pigments, chlorophylls and carotenoids. After absorption of light quanta, the molecules of the antenna pigments transfer energy to the reaction centers (RCs) of two photosystems, in an excited state, give electrons to acceptors, which then reduce $NADP^+$ to NADPH, synthesize ATP and organic substances. The state of PSA is one of the sensitive indicators of stress in plants [6]. In its study, fluorescence methods are effective, which are highly sensitive and allow one to detect violations of the state of pigments long before the appearance of morphological changes [7, 8]. In studying the state of carotenoid molecules, the Raman spectroscopy method is widely used, which makes it possible to determine changes in the conformation of the carotenoid molecule of photosystem II (PSII) antenna via evaluating the contribution of C=C bonds valence vibrations (I_{1520}/I_{1006}), transitions from the planar to bent configuration of the molecule (I_{960}/I_{1006}), as well as changes in the length of the polyene chain (I_{1520}/I_{1160}) [9].

In the present work, it was shown for the first time that treating maize leaves with a TMX insecticide leads to a decrease in the functional activity of photosystem II and a decrease in the energyzation of thylakoid membranes. In addition, the effect of thiamethoxam is shown to depends on the genotype of maize. So, in the inbred line zppl 225, a decrease is shown in the content of chlorophyll and the acceptor pool on the acceptor side of PSI. TMX causes opposite changes in the conformation of carotenoid molecules in the antenna, but does not change their content.

The aim of the work was to investigate the effect of the TMX pesticide

on the photosynthetic pigments and the photochemical activity of the leaf photosynthetic apparatus in the two maize genotypes.

Materials and methods. Experiments were carried out in 2018-2019. Samples of maize (*Zea mays* L.) leaves of the inbred line zppl 225 and hybrid zp 341 with high germination rates, grain quality and yield (Institut za kukuruz “Zemun Polje”, Belgrade, Serbia) were used [10]. Seeds were germinated until roots appeared (length not less than 5 mm), thereafter the germinated seeds were sown in soil (vermiculite mixed with chernozem, 1:1) and grown under 16-hour daylight at a constant temperature of 25 °C. When 10 days after germination the 3rd true leaf appeared (longer than 4 cm), plants were sprayed with a 0.2 mg/l thiamethoxam [5-methyl-3-(2-chlorothiazol-5-ylmethyl)-1,3,5-oxadiazinane-4-ylidene-N-nitroamine]. Maize leaves grown under similar conditions without spraying with thiamethoxam were a control. The measurements were carried out when the 5th real sheet reached a size of 12-14 cm.

The content of photosynthetic pigments (chlorophyll a, b, and carotenoids) was measured spectrophotometrically in a 100% acetone extract (a Hitachi-557, Hitachi, Japan) with calculation according to Holm-Wetstein formulas [11]. A change in the conformation of carotenoid molecules was recorded by Raman spectroscopy (a DFS 24 Raman spectrometer, LOMO, Russia) equipped with a 473 nm laser (Ciel, Eurolase, Germany) and a MORC 1/3648 recording system (LOMO, Russia) based on a linear CCD matrix TCD1304DG (Toshiba, Japan) with an LPO2-473RS-50 filter (Shemrock, USA). In the experiment, a fragment of the leaf blade was fixed on a stage and a Raman signal was recorded for 5 s; the laser power on the sample was 3 mW.

Light-induced kinetics of prompt fluorescence (PF), delayed fluorescence (DF) and modulated reflection at $\lambda = 820$ nm (MR) were recorded simultaneously (a M-PEA-2 multifunctional plant analyzer, Hansatech Instruments, Great Britain). The intensity of the actinic light and the duration of illumination were $3000 \mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and 60 s, respectively. Measurements were performed on the adaxial surface of intact leaves placed into a measuring unit with a leafclip. Before measurement, the plants were adapted to darkness for 15 min so that the reaction centers (RC) of the photosystems went into an “open” state with oxidized Q_A . Three signals were recorded during alternating light and dark intervals, the duration of which is described in detail by Strasser et al. [12].

PF induction curves (OJIP curves) were analyzed by a standard JIP test, estimating the energy fluxes through different parts of the photosynthesis electron transport chain [13] at 20 μs (F_O), 270 μs ($F_{270\mu\text{s}}$), 2 ms (F_J), 30 ms (F_I) and maximum fluorescence (F_M). These characteristics were used to calculate the maximal quantum yield of PSII photochemistry as a ratio of variable fluorescence (F_V) to F_M as $F_V/F_M = 1 - F_O/F_M$, probability that an electron moves further than Q_A^- $\psi_{E_0} = 1 - (F_J - F_O)/(F_M - F_O)$, light energy (ABS) absorption flux per RC as

$$\text{ABS/RC} = 4 \times (F_{270\mu\text{s}} - F_O) \times (F_M - F_O)^{-3} \times (F_J - F_O)^{-1} \times F_M,$$

and an indicator of plant PSII performance per the absorbed energy (PSII performance index):

$$\text{PI}_{\text{ABS}} = (\text{ABS/RC})^{-1} \times F_V/F_M \times (1 - F_V/F_M)^{-1} \times \psi_{E_0} \times (1 - \psi_{E_0})^{-1} [13].$$

Kinetics of modulated reflection/absorption of light at $\lambda = 820$ nm (MR) characterize the redox transformations of the PSI pigment (P700) and plastocyanin [12]. In our study, the MR signal was normalized to MR_0 and calculated as $\text{MR}/\text{MR}_0 - 1$, where MR_0 is the signal intensity at 0.7 ms.

Data statistical processing was carried out using a nonparametric Krus-

kal-Wallis test for the set of independent variables (Kruskal-Wallis test) (Statistica v.10 software, StatSoft, Inc., USA). Values $p < 0.05$ were deemed statistically significant. The presented table shows the mean (M) values and standard error of the means (\pm SEM).

Results. The pigment composition of the leaves did not differ much between the two maize genotypes (zpl 225 and zp 341), but after the thiamethoxam (TMX) application, obvious changes were found which depended on the genotype. Thus, TMX decreased the content of chlorophyll from 0.74 mg/g to 0.61 mg/g (chlorophyll a decreased by 17%, chlorophyll b by 24%; $p < 0.05$) in leaves of the inbred line zpl 225. In contrast, in leaves of the zp 341 hybrid exposed to TMX there were no changes in the pigment composition.

OJIP curves in both control and TMX-treated leaves have a typical pattern with characteristic phases O-J, J-I, and I-P, which reflects the sequential reduction of carriers in photosynthetic ETC between two photosystems [13] (Fig. 1, A, D). The initial level O (origin) corresponds to the fluorescence intensity at "open" PSII RCs (F_0), when all acceptors in PSII are oxidized and can accept an electron. The increase in fluorescence in the O-J phase is due to the photoinduced reduction of Q_A^- . The next J-I-P phases reflect a further increase in fluorescence associated with the Q_A^- accumulation due to the reduction of Q_B electron acceptors, the quinone pool, and primary acceptors in photosystem I (PSI) [14].

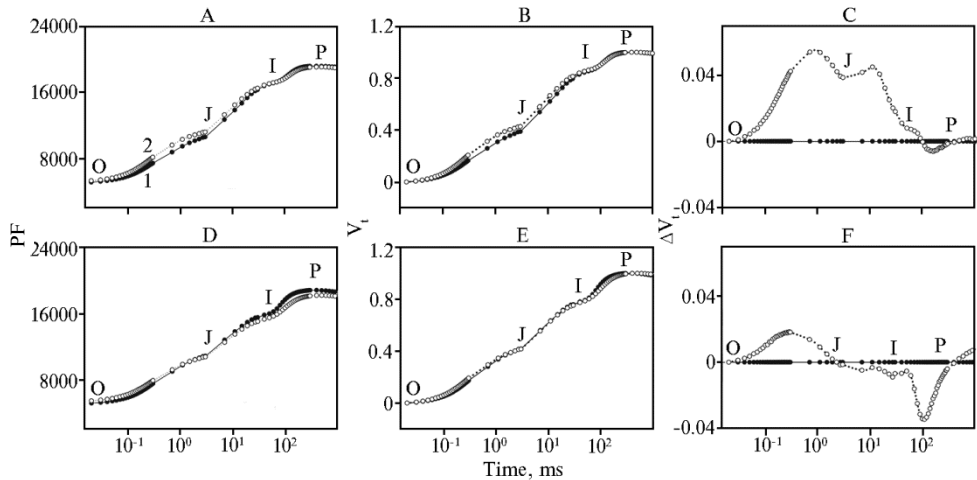


Fig. 1. OJIP curves of maize (*Zea mays* L.) leaves (A, D), the derived curves of relative variable fluorescence $V_t = (F_t - F_0)/(F_M - F_0)$ after normalization to O and P levels (B, E) and the difference in the values of the functions $\Delta V_t = V_{t(\text{TMX})} - V_{t(\text{control})}$ (C, E) for the inbred line zpl 225 (A, B, C) and the hybrid zp 341 (D, E, F) in control (1) and upon thiamethoxam treatment (2). O, J, I, and P are 20 μ s, 2 ms, 30 ms, and 300 ms peaks of the induction curve.

TMX exposure caused slight changes in the OJIP of both the inbred line zpl 225 and the hybrid zp 341 (see Fig. 1, A, D). For a more detailed analysis, the induction curves were normalized to O and P levels to derive a relative fluorescence variable (V_t) functions (see Fig. 1, B, E) and the difference in the values of the V_t functions between the control and samples processed with TMX (ΔV_t) (see Fig. 1, C, F). The effect of the pesticide on the leaves of two maize genotypes appeared as an increase in ΔV_t in the O-J phase, which indicates the accumulation of reduced Q_A as a result of an increase in the proportion of Q_B non-reducing centers of PSII, i.e. centers that are not capable of electron transfer along the ETC [15]. Changes in O-J phase were more apparent in leaves of the zpl 225 inbred line than in the zp 341 hybrid. In addition, the effect of

TMX in zppl 225 leaves was accompanied by an increase in the J-I phase amplitude, i.e. ΔV_t increased in the O-J and J-I phases, which indicates the accumulation of reduced Q_A forms and plastoquinone molecules that are not capable of electron transfer for dark photosynthesis reactions [8].

JIP-test parameters derived from analysis of OJIP induction curves for leaves of two maize (*Zea mays* L.) genotypes upon thiamethoxam (TMX) treatment ($M \pm SEM$)

Fluorescence parameter	zppl 225	zppl 225 + TMX	zp 341	zp 341 + TMX
F_V/F_M	0.740 \pm 0.004 (100 %)	0.740 \pm 0.004 (99 %)	0.740 \pm 0.010 (100 %)	0.710 \pm 0.010 (97 %)
ψ_{E_0}	0.69 \pm 0.01 (100 %)	0.65 \pm 0.01 (93 %)*	0.67 \pm 0.01 (100 %)	0.66 \pm 0.02 (100 %)
ABS/RC	2.62 \pm 0.09 (100 %)	2.93 \pm 0.13 (112 %)	2.57 \pm 0.12 (100 %)	2.95 \pm 0.18 (115 %)
PI_{ABS}	2.50 \pm 0.10 (100 %)	1.77 \pm 0.15 (71 %)*	2.24 \pm 0.23(100 %)	1.70 \pm 0.09 (76 %)*

Note. F_V/F_M — the maximal quantum yield of PSII photochemistry, ψ_{E_0} — probability that an electron moves further than Q_A^- , ABS/RC — absorption flux per reaction center (RC), PI_{ABS} — an indicator of plant PSII performance per the absorbed energy. The values in parentheses are a percentage of control.
* Difference from control are statistically significant ($p < 0.05$).

PF induction curves were analyzed by the JIP-test [13] (Table). As known, the most adequate parameter of the JIP-test is the F_V/F_M ratio which correlates with the maximal quantum yield of the PSII primary photochemical reaction and is used as an indicator of photosynthesis efficiency [6]. The F_V/F_M value in the control at 5th leaf phase of zppl 225 and zp 341 was 0.74 \pm 0.01. The obtained photosynthetic activity values derived from F_V/F_M were comparable with the F_V/F_M values reported for C4 plants [16]. There were no statistically significant differences in the F_V/F_M between control and TMX-treated leaves for zppl 225 and zp 341 ($p > 0.05$).

Unlike the F_V/F_M parameter, the PI_{ABS} performance index changed significantly when leaves were exposed to TMX. It is known that PI_{ABS} correlates with plant viability and reflects the current PSA functioning under stress [17]. Comparison of PI_{ABS} in the control and under the influence of TMX revealed statistically significant ($p < 0.05$) differences, i.e. in the zppl 225 and zp 341 leaves treated with TMX the PI_{ABS} decreased by 29% and 24%, respectively.

PI_{ABS} is an indicator that integrates three independent parameters, the fraction of active RCs (ABS/RC), the efficiency of exciton-captured electron transfer in the electron transfer chain beyond Q_A (ψ_{E_0}), and the probability that the exciton will be trapped in the RC (F_V/F_M) [13]. The decrease in PI_{ABS} in zppl 225 leaves exposed to TMX was due to a significant decrease in the electron transport at acceptor side of PSII (ψ_{E_0}), which is confirmed by the changes in the PF induction curves shown above ($p < 0.05$). In contrast, a decrease in PI_{ABS} in zp 341 leaves could be associated with a slight decrease in the fraction of active RCs (increase in ABS/RC) and PSII photochemistry (F_V/F_M).

We evaluated the redox transformations of the reaction centers of PSI (P700) molecules upon TMX treatment by modulated reflection at $\lambda = 820$ nm (MR) [18] (Fig. 2, A, C). It is known that the kinetics of the light-induced decrease of the MR signal in the first 15-20 ms reflects the P700 oxidation (fast phase) and reaches a minimum at ~ 20 ms (MR_{min}). MR_{min} is a transitory steady state with equal rate of P700 oxidation and re-reduction. Subsequently, the reduction rate prevails over the oxidation rate due to electron donation from PSII, leading to a decrease in the absorption at $\lambda = 820$ nm and an increase in the MR signal (slow phase) to a maximum at ~ 200 ms (MR_{max}) [12].

TMX causes changes in the redox P700 transformations in leaves of both the inbred line zppl 225 and hybrid zp 341 (see Fig. 2, A, C). Note that zppl 225 leaves showed changes in chlorophyll in the fast and slow phases of MR associated with a decrease in amplitude at ~ 20 ms (see Fig. 2, A). An analysis of

the maximum P700 oxidation and reduction rates (MR kinetics) indicates a decrease in the oxidized and an increase in the reduced PSI (P700) acceptors in zpl 225 leaves under the influence of TMX. This is probably due to a decrease in the pool of acceptors, such as P700, on the acceptor side of the PSI. On the contrary, PSI RCs of zp 341 hybrid leaves showed resistance to TMX (no change in MR kinetics) (see Fig. 2, B).

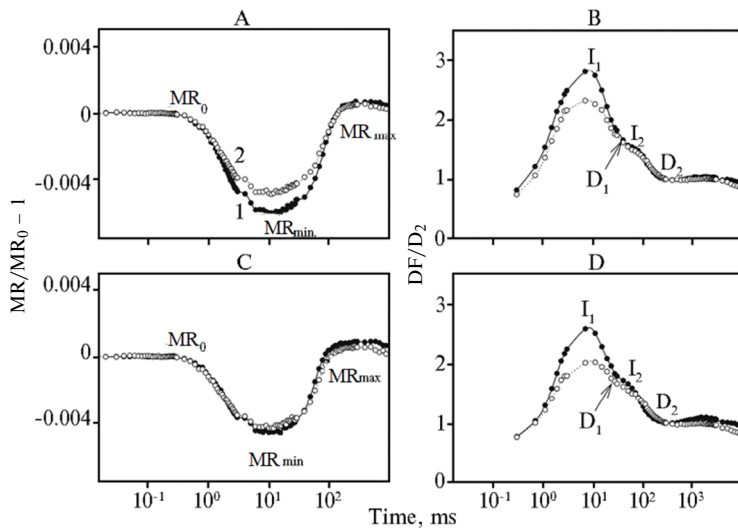


Fig. 2. Light-induced kinetics of modulated reflection at $\lambda = 820$ nm (MR) (A, C) and delayed fluorescence (DF) normalized to the minimum signal level D2 at 200 ms (B, D) in leaves of maize (*Zea mays* L.) inbred line zpl 225 (A, B) and hybrid zp 341 (C, D) in control (1) and upon thiamethoxam treatment (2). MR kinetics are normalized and expressed as $MR/MR_0 - 1$, where MR_0 is the signal intensity at 0.7 ms. I_1 , I_2 , D_1 , D_2 are maximums and minimums on the DF induction curve.

We estimated the degree of thylakoid membrane energization based on peaks I_1 and I_2 of delayed fluorescence measured in the microsecond time range (0-0.09 ms). It is known that DF is proportional to the rate of recombination reactions in PSII, which are affected by the energization of the thylakoid membrane [6]. We considered the fast phase of the DF associated with the membrane potential formation. It is known that the fast phase of the DF has two peaks, I_1 (~ 7 ms) and I_2 (~ 100 ms), after which it drops to a D_2 minimum (~ 200 ms) [19]. Figure 2 (B, D) shows the induction curves of the DF of zpl 225 and zp 341 leaves treated with TMX, normalized to the minimum fluorescence D_2 . Under the TMX influence I_1 amplitude decreased in zpl 225 and zp 341 leaves by 23 and 21%, respectively, compared to control. A decrease in the I_1 peak in the microsecond time range could be caused by a decrease in the Q_A re-oxidation rate (disturbance of electron transport on the acceptor side of PSII) and/or a decrease in the Z^+ reduction from 4MnCa of oxygen-evolving complex (OEC) [19].

The state of OEC and its ability to donate an electron for $P680^+$ through Z^+ were monitored by the appearance of an additional peak on the PF induction curve. For this, we calculated the relative fluorescence between the O and J peaks as $W_{OJ} = (F_t - F_O)/(F_J - F_O)$ (see Fig. 3, A, C) and the difference in the values of the functions between the control and the samples treated with TMX as $\Delta W_{OJ} = W_{OJ(TMX)} - W_{OJ(control)}$ (see Fig. 3, B, D). In TMX-treated leaves of zpl 225 and zp 341 an additional peak appeared between O and J at ~ 300 μ s (peak K) (see Fig. 3, B, D) which is characteristic of high-temperature stress due to the limitation of electron transfer from OEC [13].

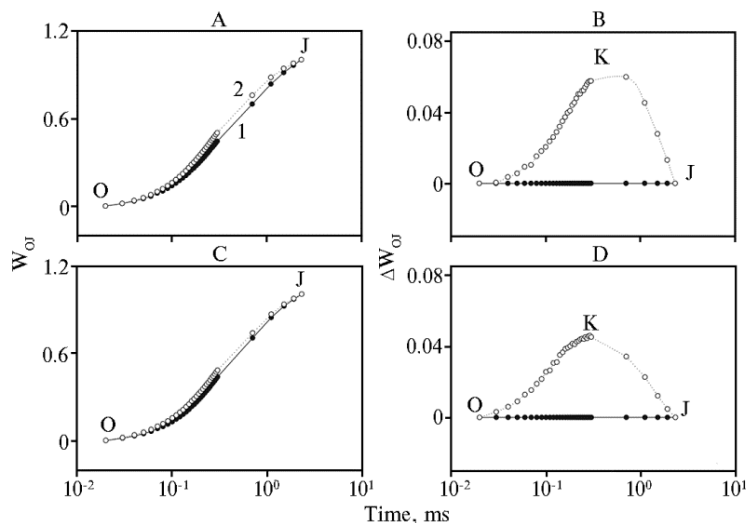


Fig. 3. The relative variable fluorescence $W_{OJ} = (F_t - F_0)/(F_J - F_0)$ between the O and J levels (A, C) and the difference in the function values $\Delta W_{OJ} = W_{OJ(TMX)} - W_{OJ(control)}$ (B, D) in leaves of maize (*Zea mays* L.) inbred line zppl 225 (A, B) and hybrid zp 341 (C, D) in control (1) and upon thiamethoxam treatment (2). O, K, J are the peaks on the induction curve at 20 μ s, 300 μ s, and 2 ms, respectively.

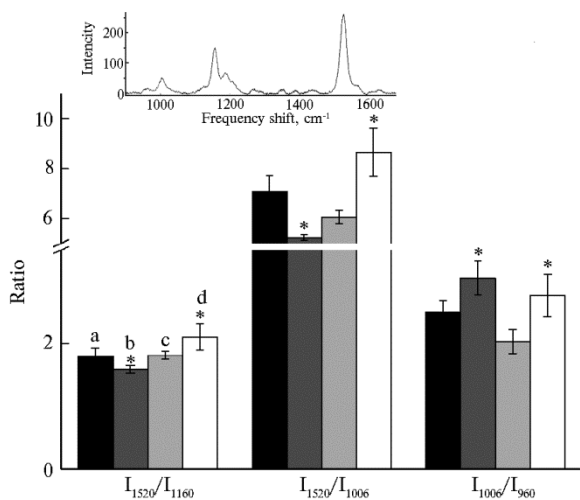


Fig. 4. Changes in Raman spectra of carotenoids in leaves of maize (*Zea mays* L.) of two genotypes upon thiamethoxam treatment (TMX): a – inbred line zppl 225, b – zppl 225 + TMX, c – hybrid zp 341, d – hybrid zp 341+ TMX. Asterisks indicate statistically significant differences ($p < 0.05$). The inset shows the Raman spectrum of leaf carotenoid for the inbred maize line zppl 225.

1200 cm^{-1} in the Raman spectra are indicative of a 15-*cis* conformation of carotenoids (data not shown).

We found an increase in the ratio I_{960}/I_{1006} bands in maize leaves when TMX was applied (see Fig. 4). This may indicate a change in the conformation of carotenoid molecules in the PSA antenna. Under the influence of TMX, the conformation of the polyene chain of the carotenoid molecule changed in the leaves of the zppl 225 inbred line, with a 26% decrease in the contribution of the $-\text{C}=\text{C}-$ valence vibrations (I_{1520}/I_{1006} ratio). In the leaves of the zp 341 hybrid

In the Raman scattering spectra of maize leaves, we revealed the bands characteristic of carotenoids (960 cm^{-1} , 1006 cm^{-1} , 1156 cm^{-1} , 1190 cm^{-1} , 1200 cm^{-1} , and 1520 cm^{-1}) which are due to electron valence vibrations in the polyene chain of the molecule (Fig. 4). The maximum changes in the amplitude of the Raman spectrum bands were observed for I_{1520}/I_{1006} (reflects contribution of $-\text{C}=\text{C}-$ bond valence vibrations of the carotenoid polyene chain), I_{1520}/I_{1160} (reflects changes in the contribution of $-\text{C}=\text{C}-$ bonds vs. $-\text{C}-\text{C}-$ bonds of the polyene chain) and I_{1006}/I_{960} (reflects the position of carotenoid polyene chain relative to the pyrrole rings) [20]. Peaks at 1190 and

TMX caused a 16% and 43% increase in the ratios I_{1520}/I_{1160} and I_{1520}/I_{1006} , respectively, which indicates a change in the conformation of carotenoids in the leaves of the zp 341 hybrid, but not their content.

In this work, we investigated the effect of TMX pesticide on the photosynthetic pigments and the photochemical activity of the photosynthetic apparatus of leaves of two maize genotypes using modern spectral and fluorescence methods.

The obtained results show that the zppl 225 inbred line is subject to greater changes when spraying leaves with TMX than the zp 341 hybrid. The zppl 225 inbred line shows lower chlorophyll content, which probably indicates that the synthesis of these pigments decreases under the influence of TMX. Note that in the zppl 225 leaves a decrease in the ratio of chlorophylls (a + b) carotenoids are revealed, which, as a rule, correlates with an increase in the content of carotenoids under stress [21]. On the contrary, no changes in the pigment composition are revealed in the leaves of the zp 341 hybrid. Earlier on the example of cotton, neonicotinoids were shown not to affect the chlorophyll content, while in okra leaves, when exposed to acetamipride, a gradual increase in the total chlorophyll content was reported [22]. At the same time, TMX can act as a bioactivator of rice plants, increasing their physiological and metabolic activity [23].

In our work, the TMX effect on PSII, PSI, and electron carriers between photosystems in leaves of zppl 225 and zp 341 was evaluated based on a set of parameters obtained after analysis of the kinetic curves of PF, DF and MR. An analysis of the kinetic curves of PF by the JIP-test, which describes the energy fluxes through different parts of the electron transport chain of photosynthesis [13], revealed that TMX had an insignificant ($p > 0.05$) effect on PSII photochemistry (F_V/F_M) in leaves of both studied genotypes. Changes were detected on the acceptor side of PSII associated with a decrease in the probability of electron transport after the acceptor Q_A^- (ψ_{E_0}) in the leaves of the inbred line zppl 225. Lower probability of electron transport on the acceptor side of PSII (ψ_{E_0}) caused a decrease in the functionality of PSII (PI_{ABS}). Other neonicotinodine pesticides, such as acetamipride, also do not affect F_V/F_M and lead to a significant decrease in the functional activity of PSII due to a decrease in the pool size and electron transport on the acceptor side of PSII in the cyanobacteria *Synechocystis* sp. [24].

Thus, spraying maize plants with pesticide thiamethoxam (TMX) causes a decrease in the chlorophyll content in leaves of the zppl 225 inbred line compared to the hybrid zp 341. In addition, a decrease in the acceptor pool on the acceptor side of photosystem I (PSI) is found in leaves of zppl 225. It was established that TMX affects the functional activity of photosystem II (PSII), decreasing the PI_{ABS} parameter, and reduces the potential on the thylakoid membrane (I_1) in both zppl 225 and zp 341. TMX causes opposite changes in the conformation of carotenoid molecules in the antenna, but does not change their content, which, apparently, is associated with a more efficient dissipation of excess energy through the system of carotenoids in leaves of zp 341 compared to zppl 225. The proposed combination of methods makes it possible to efficiently assess the functional state of photosynthetic apparatus in leaves under field and lab conditions both during biomonitoring and when comparing different lines of crops.

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