Dependence of Guaiacol Peroxidase Activity and Lipid Peroxidation Rate in Drooping Birch (*Betula pendula* Roth) and Tillet (*Tilia cordata* Mill) Leaf on Motor Traffic Pollution Intensity

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Abstract

Hormesis and paradoxical effects are frequently found for different plant parameters. These phenomena were also observed for lipid peroxidation (LP) rate at environmental pollution. However, the role of antioxidant enzymes, particularly guaiacol peroxidases (GPX), in a nonmonotonic variation in the LP rate remains insufficiently explored. Therefore, dependence of GPX activity and LP rate in *Betula pendula* and *Tilia cordata* leaf on motor traffic pollution intensity was studied. Regression analysis revealed dependences of LP rate and GPX activity on traffic intensity. In *B pendula*, GPX activity enhanced significantly (up to 2.8 times relatively control) under increased traffic that induced biphasic paradoxical effect for LP rate. In the first phase, LP level increased in comparison with the control, and in the second phase, it was normalized by enhanced GPX activity. In *T cordata*, dependences of GPX activity and LP rate on traffic pollution were paradoxical effects. However, there was no connection between change of GPX activity and LP rate under middle- and high-level pollution: LP level reduced relatively the control or normalized even if GPX activity was lower than the control. This indicates that in *T cordata*, other regulatory mechanisms instead of GPX were activated which could control LP rate under middle- and high-level pollution.

Keywords

Betula pendula, *Tilia cordata*, guaiacol peroxidases, lipid peroxidation, plant leaf, motor traffic pollution

Introduction

Various environmental stress factors, including environmental pollution, increase production of reactive oxygen species (ROS) in plant tissues (Polesskaya 2007). This leads to an increase in the lipid peroxidation (LP) rate in cell membranes because some ROS, especially hydroxyl radical (•OH), can initiate LP. Antioxidant enzymes play an important role in the regulation of ROS and LP levels. Peroxidase enzymes reduce the content of hydrogen peroxide as a main source of •OH because cells have no enzymatic mechanism to eliminate •OH. They catalyze oxidation–reduction reactions, such as the following (Raven 2003; Naji and Devaraj 2009):

$$2AH + H_2O_2 \rightarrow 2A + 2H_2O.$$
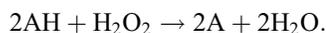
Guaiacol peroxidases are heme-containing proteins that preferably oxidize aromatic electron donors such as guaiacol and pyragallol at the expense of H_2O_2 (Sharma et al. 2012). Guaiacol peroxidase is associated with many important biosynthetic processes and defense against abiotic and biotic stresses. The GPXs are widely accepted as a stress "enzyme" (Sharma et al. 2012). Various stressful conditions of the environment, including different pollutants such as heavy metals (Maheshwari and Dubey 2009; Mishra et al. 2011; Srivastava and Dubey 2011), herbicides (Ivanov et al. 2013), ozone (Li et al. 2013), and polycyclic aromatic hydrocarbon (Song et al. 2012), have been shown to induce the activity of GPX.

It is known that nonmonotonic dose–response dependences that include hormesis (Cedergreen et al. 2007; Calabrese 2008) and paradoxical effects (Schatz 1999; Batyan et al. 2009; Smith et al. 2012) are frequently found for different plant parameters (Calabrese and Blain 2009; Erofeeva 2012; Erofeeva 2014a;

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Erofeeva 2014b). Hormesis is a biphasic dose–response phenomenon characterized by low-dose stimulation and high-dose inhibition (Calabrese 2008; Calabrese 2013). It is known that the manifestation of paradoxical effects consists of the following: as the dose or concentration of the toxic agent is reduced, its toxicity increases, and vice versa, such that with an increase in the dose, its effect is reduced (Schatz et al. 1964; Batyan et al. 2009). In fact, all nonmonotonic dose–response dependences except hormesis are paradoxical effects.

Hormesis and paradoxical effects for LP were found upon exposure to different pollutants (Sinha et al. 2007; Zhang et al. 2007; Qiu et al. 2008; Gupta and Singha 2009; Xu et al. 2010; Jia et al. 2013; Erofeeva 2014a; Mitton et al. 2014) and motor traffic pollution (Erofeeva et al. 2011) on plants. However, the role of GPX activity in a nonmonotonic variation of the LP rate remains insufficiently studied.

Road transport is a major source of air and soil pollution in the most megacities of Russia, which is caused by the rapid growth in the number of cars (Belkina 2008). Drooping birch (*Betula pendula* Roth) and tillet (*Tilia cordata* Mill) often grow in roadside forest strips of cities in Russia. These species are bioindicators, and many of its parameters are used for bioindication (Schebek et al. 1984; Kryazheva et al. 1996; Kosiba 2008; Sameska-Cymerman et al. 2009). Therefore, these species of woody plant were selected for this study. The aim of this study was to study the dependence of GPX activity and LP rate in *B pendula* and *T cordata* leaf on motor traffic pollution intensity.

Materials and Methods

Study Area and Sampling Sites

Our research was carried out in 2009 (for *B pendula*) and 2014 (for *T cordata*). We studied parameters in middle-aged generative trees of *B pendula* and *T cordata*. The trees grew in 9 (*T cordata*) or in 13 (*B pendula*) sampling sites of tree stands planted along roadsides in the upland part of the city of Nizhni Novgorod (Russia). Motor traffic is a major source of pollution in this part of the city. All sites were characterized by similar soil conditions (light gray forest soils with anthropogenically mixed upper horizons) and a normal moistening regime. A conditionally clean area near the village of Kiselikha, 20 km north of Nizhni Novgorod, was chosen as the control site for *B pendula*. Forest Park Shchelkovsky Farm which is situated in the upland part of Nizhni Novgorod was chosen as the control site for *T cordata*. The control sites were located far from highways and other pollution sources. About 30 to 35 leaves were collected from 10 trees of every site on the side of the crown facing the road (n = 10), at a height of 1 to 3 m.

Estimation of Motor Traffic Pollution

Motor traffic pollution was estimated by the traffic intensity (vehicles/h). Site location was chosen so that traffic intensity varied within a wide range, with the minimum and maximum values differing by a factor of several tens. The traffic intensity was the median of vehicles/h counted 3 times on a weekday, in the morning (from 8 until 10), in the afternoon (from 12 until 15), and in the evening (from 17 until 19; Ruzskiy et al. 2008). We previously demonstrated that the traffic intensity correlated with the content of the main pollutants (oxides of sulfur, nitrogen, carbon, benzine, kerosene, benzopyrene, and formaldehyde) in the air along highways in Nizhni Novgorod ($r = .8$-$.9$; $P < .05$).

Lipid Peroxidation

Lipid peroxidation rate was estimated in pooled samples of 30 to 35 leaves from each of 10 trees on every site (10 trees/site; n = 10). The level of LP was determined in terms of malondialdehyde (MDA) concentration according to the method of Heath and Packer (1968) with the modifications of Kamyshnikov (2002). Approximately 0.2 g of fresh leaves was homogenized in 2 mL of 3 mmol/L EDTA. Then, 2 mL of 20% trichloroacetic acid and 2 mL of 0.75% 2-thiobarbituric acid were added to 2 mL of the homogenate. The mixture was then incubated at 96°C for 30 minutes and then transferred into an ice bath to stop the reaction. The tubes were centrifuged at 10 000g for 15 minutes, and the absorbance of the resulting supernatant was measured at 532 nm using spectrophotometer SF 2000 (OKB Spectrum, Russia). Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using the extinction coefficient of 155 mmol/L^{-1} cm^{-1}. The concentration of MDA was expressed as nmol g^{-1} FW (fresh weight of leaf).

Guaiacol Peroxidase Activity

Guaiacol peroxidase activity was estimated in pooled samples of 30 to 35 leaves from each of 10 trees on every site (10 trees/site; n = 10). The GPX activity was determined according to the method of Ridge and Osborne (1971) with the modifications of Shevyakova et al. (2002). Approximately 1 g of fresh leaves was homogenized in an ice-cold 17 mL of 0.066 mol/L K, Na phosphate buffer (pH = 7.4), with the addition of polyvinylpyrrolidone. The homogenate was centrifuged at 10 000g at 4°C for 20 minutes. The activity of GPX in the supernatant was determined spectrophotometrically by measuring the increase in absorbance at 470 nm. The reaction mixture contained 80 mmol/L guaiacol and 10 mmol/L H_2O_2 in 0.066 mol/L phosphate buffer, pH = 7.4. The enzymatic reaction was started by adding 0.1 mL of the extract to 3 mL of reaction mixture. Enzyme activity was calculated as the increase in absorbance (ΔE) min^{-1} g^{-1} fresh weight.

Statistical Analysis

Statistical analyses were executed using the programs Statistica 8.0. and Primer of Biostatistics 4.03. Parametric criteria were
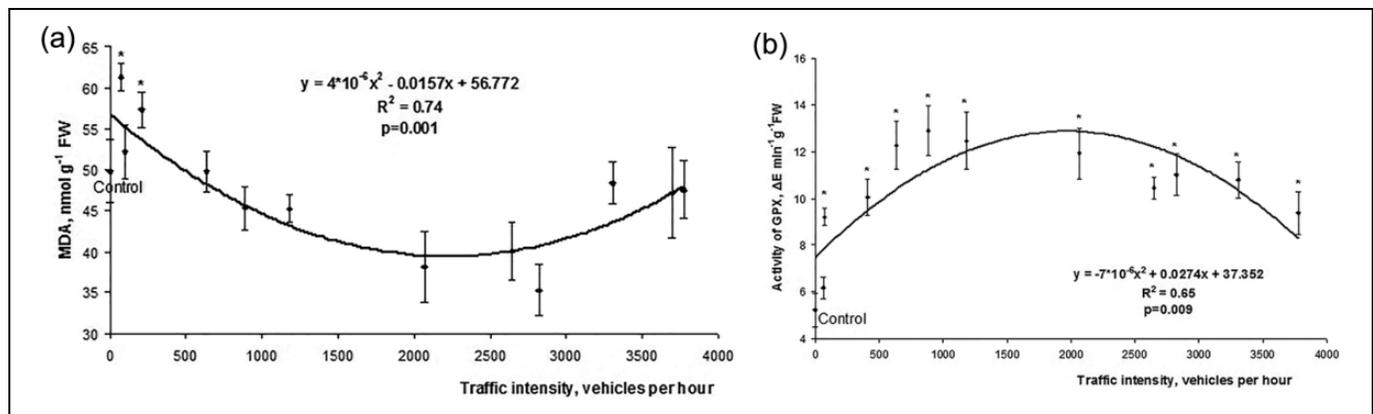
Figure 1. Dependence of lipid peroxidation rate (a) and guaiacol peroxidase activity (b) on motor traffic intensity in the *Betula pendula* leaf. Values represent mean ± standard error (SE; n = 10). * indicates significant differences between treatments and control at *P* < .05. FW, fresh weight of leaf.

used, since the Shapiro-Wilk criterion showed that the sampling distribution in all treatments and controls did not differ from the Gaussian distribution. One-way analysis of variance and parametric Newman-Keuls tests were used for multiple comparisons of studied quantitative parameters. Regression analysis was used to evaluate the dependence of the studied parameters on traffic intensity. One or two points of some dose–response dependences were outside the 95% confidence interval values; therefore, they were excluded from the regression analysis. Exclusion of such points from regression analysis is an accepted procedure in statistics (Glantz 2005). Sampling means with standard errors were used for presentation of graphical data. The least significant difference was used for multiple comparison at the *P* < .05 level between treatment and control means.

Results

Guaiacol Peroxidase Activity and LP Rate in B pendula *Leaf*

Regression analysis revealed dependences of LP rate and GPX activity on traffic intensity, which were most adequately described by quadratic polynomial equations. The increase in traffic intensity (up to 72-209 vehicles/h) caused an increase in LP rate (by 15%-46%) and GPX activity (up to 2 times) in comparison with the control (Figure 1a and b). However, a further increase in traffic intensity induced a normalization of LP level (Figure 1a). Apparently, this was due to a further increase in GPX activity (up to 2.88 times in comparison with the control; Figure 1b).

Guaiacol Peroxidase Activity and LP Rate in T cordata *Leaf*

Dependence of LP rate on traffic intensity had a complicated pattern that could not be approximated by 1 regression curve. Therefore, segmented regression (piecewise regression) was used, that is, this dose–response dependence was divided into

2 parts, and an appropriate regression model was chosen for each part. However, a statistically significant regression equation was obtained only for the first part of the curve (Figure 2a). The second part did not have enough points to be approximated adequately by the cubic polynomial equation. At first, an increase in traffic intensity caused a linear decrease in LP rate (maximum by 30% in comparison with the control). Then, this parameter normalized (at 2958-3793 vehicles/h) and reduced again in comparison to the control level at the highest traffic intensity (Figure 2a).

Regression analysis also revealed a complex dependence of GPX activity on traffic intensity, which was most adequately described by a cubic polynomial equation (Figure 2b). At first, an increase in traffic intensity caused an increase in GPX (by 25% in comparison with the control). Then, this parameter reduced (maximum by 75% in comparison with the control) at 3793 to 4350 vehicles/h and normalized at the highest traffic intensity (Figure 2b).

Discussion

Thus, we found 2 types of change in GPX activity in plant leaf under the action of traffic pollution:

(1) The first type was observed in *B pendula* leaf, and increase in traffic induced an increase in LP rate. This significantly enhanced GPX activity, which normalized peroxide homeostasis. As a result, GPX activity increased, and LP rate had a biphasic paradoxical effect under increase in traffic intensity (Figure 1a and b). Many authors also observed an increase in GPX activity in different species of trees and herbaceous plants under the action of traffic pollution (Seta-Koselska et al. 2014) and exposure to different pollutants (Maheshwari and Dubey, 2009; Papa et al. 2012; Song et al. 2012; Ivanov et al. 2013; Li et al. 2013).

(2) The second type was observed in *T cordata* leaf. Dose–response dependence of GPX activity was a multiphase paradoxical effect (Figure 2b). The LP rate dose–response dependence was also a paradoxical effect. However, there was not a
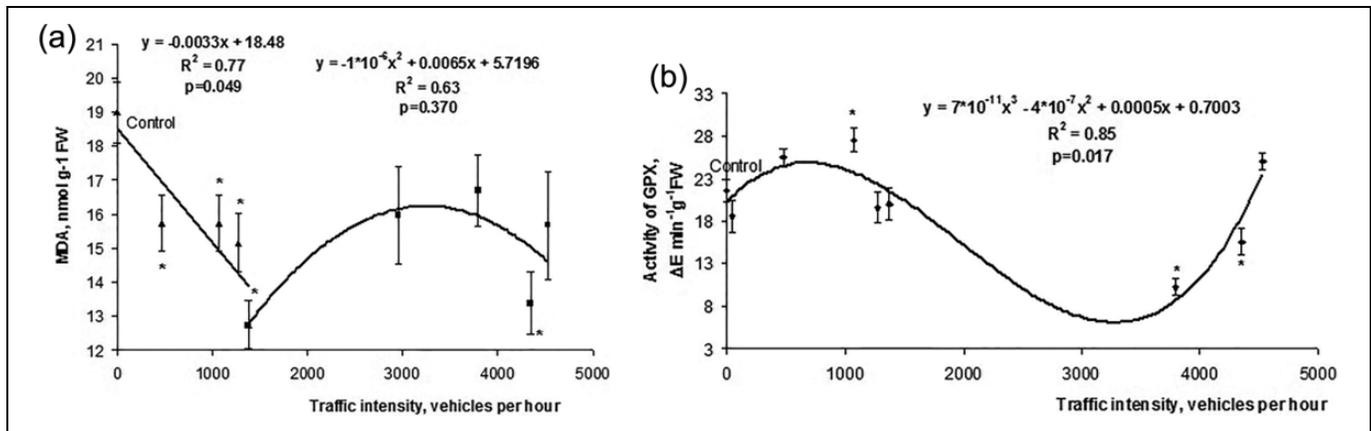
Figure 2. Dependence of lipid peroxidation rate (a) and guaiacol peroxidase activity (b) on motor traffic intensity in the *Tilia cordata* leaf. Values represent mean $\pm$ standard error (SE; n = 10). * indicates significant differences between treatments and control at $P < .05$. FW, fresh weight of leaf.

clear connection between change in GPX activity and LP rate under middle- and high-level traffic pollution. For example, at low traffic (1007 vehicles/h), increased GPX activity reduced the LP rate relative to the control level. However, under further increase in traffic load (up to 1274-1377 vehicles/h), GPX activity normalized but LP rate continued to decrease (Figure 2a and b). This indicates that other regulatory mechanisms instead of GPX were activated, which could control LP rate. A similar switch of mechanisms controlling LP rate was also observed at high traffic intensity (3793-4350 vehicles/h). In this case, a decrease in GPX activity was accompanied by normalization (3793 vehicles/h) and even a decrease (4350 vehicles/h) in LP rate (Figure 2a and b), although one would expect an increase in LP rate if it was controlled only by GPX. It is known that except GPX, LP level can be regulated by ascorbate peroxidase, catalase, and nonenzymatic antioxidants (Polesskaya 2007; Kreslavski et al. 2012; Sharma et al. 2012).

In comparison with *B pendula*, *T cordata* is a more resistant species to nitrogen and sulfur oxides (Smith 1981) which are contained in the exhaust of motor vehicles. Therefore, we did not find an increase in LP level relative to the control level (increase in LP level usually accompanies stress; Polesskay 2007) in *T cordata* leaf under traffic pollution. It is possible that the switch in mechanisms controlling the LP rate in *T cordata* leaf was due to the fact that activation of different regulators is more optimal for adaptation to certain pollution levels.

We can draw the following conclusions based on this study:

1. In *B pendula* leaf, GPX activity enhanced significantly (up to 2.8 times relatively control) under increase in traffic that induced biphasic paradoxical effect for LP rate. At the first phase, LP level increased relatively control, and at the second phase, it was normalized by enhanced GPX activity.
2. In *T cordata* leaf, dependences of GPX activity and LP rate were paradoxical effects. However, there was not a clear connection between change in GPX activity and

and LP rate under middle- and high-level pollution. The LP level reduced relatively the control or normalized even if GPX activity was lower than the control. This indicates that in *T cordata* leaf, other regulatory mechanisms instead of GPX were activated, which could control LP rate under middle- and high-level pollution.

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