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Dependence of Bio-Chemical and Physiological Indicators of the *Taraxacum officinale* Wigg State on the Intensity of the Traffic Flow.

G V Vorobyev[1]*, T I Ogorodnikova[2], A F Khamidullin[1], G Kh Akhmetzyanova[1], and V N Vorob'ev[1,2].

[1]Kazan (Volga) Federal University, ul. Kremlevskaya 16, Kazan, 420008, Russia
[2]Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Sciences, a/ya 30, ul. Lobachevskogo 2/13, Kazan, 420111, Russia

ABSTRACT

The effect of the environment pollution by cars on the peroxidation of lipids in the leaves able to seed germination in the cut blooms and breathing of germs of the *Taraxacum officinale* Wigg is considered. It was shown that germination is in direct relationship to the degree of the environmental pollution: the high pollution is the lower is the germination rate. Non-monotonous dependence of the lipid peroxidation (LP) was two-phase. At the first stage (21-1920 cars per hour) the growth in the car traffic resulted in decrease in this parameter (by 22% as compared to the control level, $p<0,05$); at the second stage (1920-5100 cars per hour), the concentration of malondialdehyde (MDA) was increased (by 133% as compared to the control level, $p<0,01$). The dependence of the germ breathing (ΔO_2) on the environmental pollution is two-phase. At the first stage (21-1920 cars per hour) following the increase in the traffic intensity the ΔO_2 growth is observed (by 137%, $p<0,001$ from the control level). At the second stage – reduction of ΔO_2 (by 39% from the control level, $p<0,001$). Analysis of dependencies of concentration of malondialdehyde and germ breathing on the car traffic intensity showed that by reduction of the peroxidation in the leaves of parent plants the intensity of the germ breathing is increased and upon increase in the LP the ΔO_2 is reduced.

Keywords: *Taraxacum officinale Wigg*, lipid peroxidation, breathing, germination, pollution of environment by the car traffic.

Corresponding author

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INTRODUCTION

Taraxacum officinale Wigg. is widely used as the test object in environmental studies [1-5]. It can be frequently found at urbanized areas along the roads with different traffic intensity. There are studies concerning the effect of slanting on the dandelion reproducibility in the environmentally favorable conditions [6]. However, the question how the reproductive capacities of a dandelion in conditions of operation of municipal services when the factor of the car emissions is overlapped with another one – periodic slanting – still remains open?

The objective of this study was establishment of dependence of the dandelion (*Taraxacum officinale* Wigg. s.l.) reproductive capacities in conditions of periodic slanting on the car traffic intensity.

MATERIALS AND METHODS

Object of the study

Taraxacum officinale Wigg. s.l. was selected as the object of the study – the plant of the Asteraceae Dumort. family (Compositae Giseke), genus *Taraxacum officinale* Wigg. [7].

Areas of the plant material collection

Collection of the plant material was performed at the end of May, 2013 at the areas located within 1-4 m along the roads with different level of pollution in the city of Kazan, Russia. The collection areas were selected so that the car traffic intensity varied within a wide range. The reference area was located within 7 km from the city at the territory of the gardeners' partnership 'Tatarstan' (Table 1).

Table 1: Traffic intensity at the plots being investigated (Kazan) (mean ± SD, n=10).

No.	Study plots	Traffic intensity, vehicles per hour
1	Kontrol	21±6
2	Halturin Street	330±54
3	Tatarstan Street	1920±211
4	Gor'kovskoe Shosse Street	5100±349

Evaluation of the pollution by motor vehicles

Pollution by motor vehicles was estimated by the traffic intensity (vehicles per hour). The vehicle counting was performed in the morning (from 8 till 10) and in the evening (from 17 till 19) [8]. The traffic intensity correlates with the content of the main contaminants (oxides of sulfur, nitrogen, carbon, benzine, kerosene, benzo [a] pyrene and formaldehyde) in the air along the motorways [4-5].

Evaluation of the germ reproduction and germination parameters

For analysis of the pollution impact the plants of the young generative (q_1) ontogenetic state were taken the floral shoot of which was cut on the next day after the blooming completion [6]. Ten blooms with the stem (10 cm) were cut in a random manner from the plots 10×40 m. Each bloom was placed in a separate paper bag. The ripened seeds were kept in a refrigerator at the temperature of -18^0C. The seeds were grown in the Petri dishes per 50 each with the use of the settled tap water. The counting of the germinated seeds was performed on the fourth day after germination [6].

Determination of the degree of the lipid peroxidation of bio-membranes

The degree of the lipid peroxidation was determined by accumulation of the LP product - malondialdehyde (MDA) in the leaves of 10 plants (q_1) collected in a random manner from each plot. The MDA content was assessed by the degree of accumulation of the product of reaction thereof with the thiobarbituric

acid (TBA) [9]. 500 mg of the plant tissue were homogenized with 1,5 ml of 20% trichloracetic acid (TCA). The obtained homogenate was centrifuged during 10 minutes at 12000 g and the temperature 4^0C in the centrifuge Rotina 380 (Andreas Hettich, Germany). The obtained supernatant was used as the sample for analysis. In the eppendorfs 1 ml of supernatant and 1 ml of the 0,5% TBA-solution were introduced. The samples were incubated in the water bath at the temperature 70°C within 1 hour and then cooled down to the room temperature. The measurements were performed with the use of the spectrophotometer Unico-2800 UV/VIS (United products & Instruments, USA) at the wavelength 532 nm as well as at 600 for correction of the non-specific absorption [10].

Germ breathing

The respiratory exchange of germs was recorded with the use of manometric method in the Warburg's apparatus [11]. The weighed portion of the four-day germs (150 mg each) was placed in the Warburg's vessels and measured after 10-minutes long thermostating the oxygen consumption (µl/h) at the temperature 30°C. The average values were calculated on the basis of the three biological replications.

Statistical analysis

The statistical analysis was performed with the use of the software OriginPro 9. For assessment of dependencies of the parameters being considered from the traffic intensity the regression analysis was used. The Shapiro-Wilk test was used for estimation of normality (does not differ from the normal (Gaussian) distribution across all distribution samples).

For multiple comparisons of the parameters investigated the one-sided ANOVA was used. The mean and standard deviation (SD) was used for graphical data representation.

RESULTS AND DISCUSSION

It is known than plants are able to modify the metabolic processes within their adaptive potential which allows increasing the resistance to anthropogenic stressors [12]. The most relevant non-specific reaction of aerobic organisms to unfavorable environmental factors is activation in the cells of the molecular oxygen with formation of its intermediate forms. This process and the lipid peroxidation induced by it are considered as the stress mediators [13].

In the Figure 1 the dependence of concentration of malondialdehyde (MDA) in dandelion leaves on the car traffic is presented.

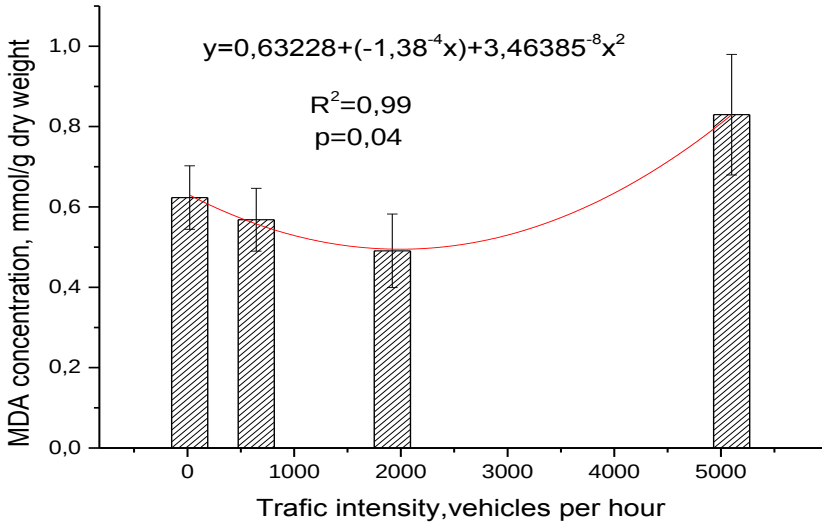

Figure 1: Variation of the MDA concentration in the dandelion leaves depending on the car traffic.

The dose-response relationship for MDA was double-stage. At the first stage (21-1920 vehicles per hour) the increase in the car traffic resulted in the reduction of this parameter (by 22% from the control level, p<0,05); at the second stage (1920-5100 vehicles per hour), the MDA concentration was increased (by 133% from the control level, p<0,01). This relationship may be referred to the paradoxical effects is expressed in the following: the toxic effect is increased by reduction of the toxicant concentration or dosage and vice versa the reduction of the toxic effect is observed along with the dose escalation [14-15].

At the present time the causes of non-monotonous response of living organisms remain underinvestigated. At the same time it was shown that at different stages of non-monotonous dose-response relationship an agent may affect different kinds of receptors or different signaling pathways of cells which determines the non-monotonous nature of dependence [16]. It makes sense to assume that nonmonotonicity of dependence of the MDA concentration on the pollution intensity (Fig. 1) is determined by the action of enzymes of the vegetative antioxidant which is compatible to the hypothesis of gradual involvement of different adaptive mechanisms in the process of phenotypic adaptation to the factor [17]. It was expected that these conditions will affect the quality of the seeds ripened after slanting.

The Figure 2 represents the dependence of the number of seeds sprouted on the fourth day after germination on the intensity of the environmental pollution by cars. In contrast to the MDA concentration dependence (Fig.1) the dependence of the number of sprouted seeds is monotone (r=-0.95; R^2=0.85, при p=0.048) (Fig.2). Monotone variation of different physiological and biochemical parameters of the seed plants is observed primarily under the influence of sublethal doses of different contaminants [18]. This kind of dependence apparently represents the degree of damage of germinal organs. More than a half of seeds were not sprouted at the high car traffic intensity (1920 – 5100) which may be associated with the increase of the benzo [a] pyrene and formaldehyde concentration in the air.

Adaptation to the environmental pollution by cars it related to the energy consumption by an organism [4]. It is obvious that in there in the bloom cut on the first day of maturation the seed-formation process shows lesser tolerance to anthropogenic stressor due to the limitedness of the energy resources. In these conditions accumulation of nutritional elements in the seeds shall be inversely proportional to energy consumption for adaptation to an anthropogenic stressor.

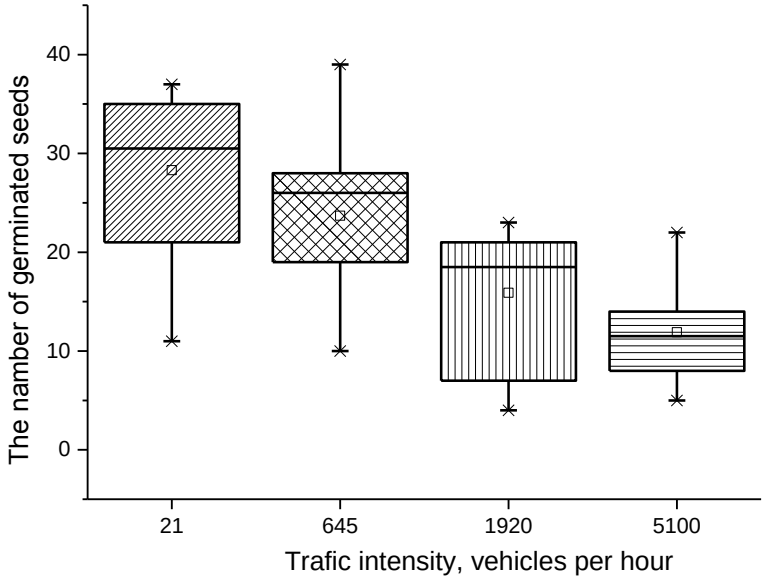

Figure 2: Dependence of the number of sprouted dandelion seeds on the car traffic.

This assumption is confirmed by differences in the germ breathing. High variability of the briefing figures in germs does not allow performing the comparative analysis of the breathing dependence on the degree of pollution. As an indicator of the breathing intensity the difference between the level of the oxygen

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consumption during the first and the second hour of the experiment (ΔO_2) in each replication (Fig. 3) was selected.

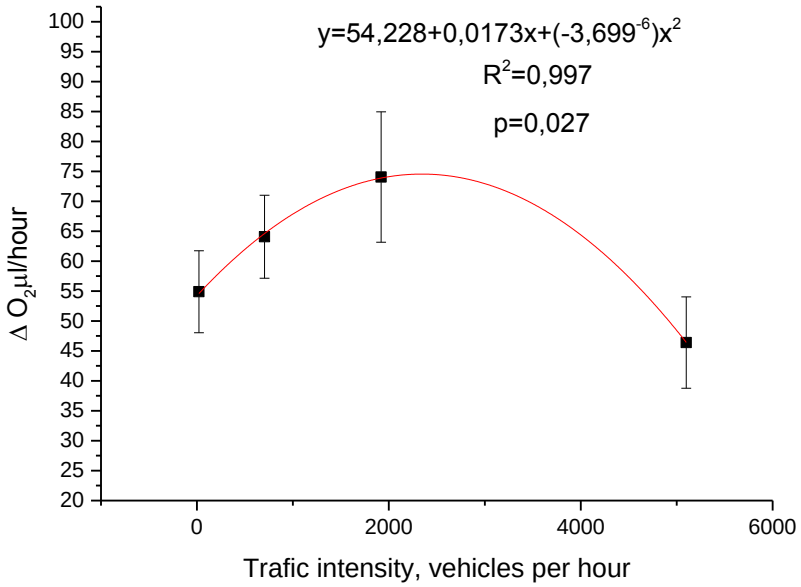

Figure 3: Dependence of the difference in the oxygen consumption -ΔO_2 in the dandelion germs on the car traffic

The ΔO_2 dependence on the car traffic intensity is double-staged. At the first stage (21-1920 vehicles per hour) the increase in the traffic intensity if observed along with the ΔO_2 growth (by 137%, $p<0,001$ as compared to the control). At the second stage – the drop in ΔO_2 (by 39% as compared to the control, $p<0,001$). The comparative analysis of dependencies of the MDA concentration (Fig. 1) and dependence of ΔO_2 (Fig. 3) on the car traffic intensity shows that along with the decrease in the LP the ΔO_2 grows and by increase in the LP the ΔO_2 is reduced ($r = -0,66$).

CONCLUSION

The results of our studies show that the dependence of the MDA concentration in the dandelion leaves on the degree of her the environmental pollution by car emissions is not monotone. At the average pollution (2000 vehicles per hour) the adaptive potential of the plants is implemented which is expressed in reduction of the LP and increase in the intensity of the germ breathing. In these conditions slanting of plants during the blooming period and at the beginning of maturation results in deterioration in the seed generation quality. Germination of seeds ripened after slanting is inversely proportional to the degree of the environmental pollution. In the conditions of the maximum pollution when the LP level exceeds the reference values by 33% and the germ breathing is lower by 39% the minimum number of seeds is sprouted. It is obvious that slanting of lawns during the blooming period and at the beginning of the dandelion maturation will significantly facilitate the municipal services in fighting this weed.

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