Adaptive Properties of the Dandelion (*Taraxacum officinale* Wigg. s.l.) under Conditions of Air Pollution by Motor Vehicle Exhausts

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Abstract—Microcalorimetric, gasometric, and nuclear magnetic resonance (NMR) methods have been used to evaluate heat production, gas exchange, and root membrane permeability in dandelion of different morphological forms from populations exposed to different levels of air pollution. The results show that these forms differ in the level of energy metabolism and that the form with a higher metabolic level (*T. off*. f. *dahlstedtii*) is more tolerant of air pollution with motor vehicle exhausts.

Pollution in urban areas has become an ecologically significant factor limiting the survival of many plant species. In response, plants realize as much as possible of their adaptive potential and modify metabolic processes, which allows them to improve resistance to anthropogenic stressors (Bezel' et al., 2001).

The dandelion is widely used as a test object in ecological research (Bezel' et al., 1998; Evseeva et al., 2002; Savinov et al., 2007; Shashurin and Zhuravskaya, 2007). Adaptive properties of dandelion populations are evaluated by criteria such as the viability of seed progeny (Pozolotina, 2001; Pozolotina et al., 2006), the level of correlation between morphometric characters (Zhuikova and Bezel', 2009), the degree of membrane lipid peroxidation (Savinov et al., 2007), etc. Showing two types of protective responses, plants are capable of ontogenetic switches from resistance to tolerance, thereby minimizing energy expenditures with age (Boege et al., 2007). An age-dependent increase in tolerance is characteristic of many plant species (Brandt and Lamb, 1994; Warner and Cushman, 2002).

This study is an attempt to reveal differences in protective responses evaluated by the correlation of heat production, respiration, and water permeability of "membrane sandwich" (plasmalemma + tonoplast) between dandelion (*Taraxacum officinale* s.l.) plants of two morphological forms (*T. off*. f. *dahlstedtii* and *T. off*. f. *pectinatiforme*) from urban cenopopulations.

MATERIAL AND METHODS

The dandelion, *Taraxacum officinale* Wigg. s.l., is a species of the genus *Taraxacum officinale* Wigg. from the family Asteraceae Dumort. (Compositae Giseke) (Bakin et al., 2000), a facultative apomict. The species includes a large number of apomictic groups (varieties, microspecies, or biotypes) morphologically differing from each other, which have lost the ability to cross pollinate and coexist in the same biotopes. In practical taxonomy, such groups are conventionally regarded as "normal" amphimictic species. The life form and structure of annual and perennial organs are the same in a; microspecies of the *Taraxacum officinale* Wigg. s.l. complex. The dandelion is a perennial herbaceous tap-rooted plant with a sympodial system of vegetative shoots, capable of facultative vegetative reproduction by root suckers (Ermakova, 1990). Its biotypes differ in the shape and degree of dissection of leaves, their lobes, and denticles (Zhuikova, 2009). We distinguished two morphological forms, *T. off*. f. *dahlstedtii* Lindb. fil. and *T. off*. f. *pectinatiforme* Lindb. fil., which could be readily identified at the generative stage of ontogeny. At the population level, these forms are known to differ in resistance to chemical pollution of the environment (Zhuikova and Bezel', 2009).

Young generative (q_1) plants were collected in cenopopulations from test plots (10×40 m) established in three different areas. The first cenopopulation was on a mixed forest margin 100 m from a dirt road near the village of Usady, 7 km from the city of Kazan. This cenopopulation was taken as control, assuming that it suffered a minimal impact from motor vehicles. The second and third cenopopulations were on city lawns at signalized intersections on Tatarstan and Gor'kovskoe Shosse streets, respectively. The amounts of emissions at these intersections were calculated following *Methodology of Motor Exhaust Measurement for*

Table 1. Specific levels of pollutant emissions by motor vehicles at signalized intersections, g/min (mean ± SD, $n = 4$)

Pollutant	Cenopopulation	
	Tatarstan	Gor'kovskoe Shosse
CO	6248 ± 93	16 120 ± 192
NO_x (calculated from NO_2)	203 ± 5.2	399 ± 2.9
CH	542 ± 9.2	1353 ± 18.2
SO_2	32 ± 0.74	68 ± 0.49
Formaldehyde	4.8 ± 0.13	8.8 ± 0.08
Lead	6.8 ± 0.09	18.36 ± 0.22
Benzo[a]pyrene	0.0044 ± 8.E-5	0.0105 ± 0.0001

Summary Calculations of Air Pollution in Cities (*Metodika...*, 1999) approved by the State Committee on Ecology and Use of Natural Resources (order no. 66 of February 16, 1999). According to these calculations, the above cenopopulations were classified as polluted (Tatarstan) and strongly polluted (Gor'kovskoe Shosse) (Table 1).

Seeds collected from the test plants were stored in paper bags in a dry place, at room temperature. Experiments were performed with full seeds distinguished by seed coat color, in two replications (50 seeds each). The seeds were germinated in Petri dishes with dechlorinated tap water (Martinkova et al., 2011). Germination energy was determined on day 7.

The method of spin-echo nuclear magnetic resonance (NMR) was used to evaluate transmembrane water exchange (Anisimov and Ratkovich, 1992). Apical segments of the main root (10 mm long) from four to six seedlings were placed in the probe of a NMR relaxometer to measure spin–lattice relaxation time T_1 (Idiyatullin et al., 1990) and spin–spin relaxation time T_2 (Carr and Purcell, 1954; Meiboom and Gill, 1958). Measurements were made at a proton resonance frequency of 19,2 MHz at room temperature, with an accuracy of over 95%. The complete set of relaxation data was used to determine the time of proton exchange (τ) between the vacuolar and cell wall water. The reciprocal of this parameter ($1/\tau$) characterized the rate of proton exchange through a semipermeable barrier, or membrane permeability (Anisimov and Ratkovich, 1992). The average $1/\tau$ values were calculated from three biological replications.

Heat production was measured in pooled samples of roots from three plants (30–40 mg) in an LKB-227 thermal activity monitor (Thermometric, Sweden) with 3-cm^3 microcalorimetric ampules. The sample was placed in an ampule containing 1 cm^3 of dechlorinated tap water and incubated for 30 min to adjust its temperature to 30°C, at which measurements were made (Alyabyev et al., 2007). The amplifier sensitivity range was 100 µV. One biological replication consisted of three ampules, with average values being calculated from three replications.

Respiratory gas exchange was analyzed in the Warburg apparatus (Semikhatova and Chulanovskata, 1965). A pooled sample of roots (150 mg from three plants, in three replications) was placed in a Warburg vessel, incubated at constant temperature for 10 min, and then oxygen intake was measured at 30°C. Its average values were calculated from three biological replications.

RESULTS AND DISCUSSION

The two morphological forms of dandelion show different survival strategies, which are characterized by prevalence of the protective component in *T. dahlstedtii* and of the combined stress-protective component in *T. pectinatiforme* (Zhuikova and Bezel', 2009). The type of strategy was determined from the level of correlation between morphometric characters. At the level of physiological processes, these forms proved to differ in the rates of respiration and hear production depending on pollution level (Table 2). These parameters were markedly lower in plants from Gor'kovskoe Shosse (strong pollution), compared to other cenopopulations, and in *T. off.* f. *pectinatiforme* than in *T. off.* f. *dahlstedtii*.

It is known that heat production is sharply reduced under conditions of extreme salinity (500 mM NaCl) (Alyabyev et al., 2007). This is a manifestation of protective response involving general retardation of metabolism (Melekhov, 1983). When there is competition for metabolic energy between damage and repair processes, retardation of metabolism promotes a shift

Table 2. Rates of respiration (µL O_2/g fresh weight h) and heat production (µW/g fresh weight) by the roots of young generative (g_1) dandelion plants of different morphological forms (mean ± SD, $n = 3$)

Cenopopulation	*T. off.* f. *ðectinatiforme*		*T. off.* f. *dahlstedtii*	
	respiration	heat production	respiration	heat production
Usady	179 ± 10	358 ± 10	273 ± 17	657 ± 24
Tatarstan	212 ± 46*	634 ± 65***	263 ± 25	831 ± 127**
Gor'kovskoe Shosse	128 ± 24**	282 ± 51**	170 ± 7.7***	482 ± 113**

Asterisks indicate that differences from the control are significant at * $p < 0.055$, ** $p < 0.01$, or *** $p < 0.001$.
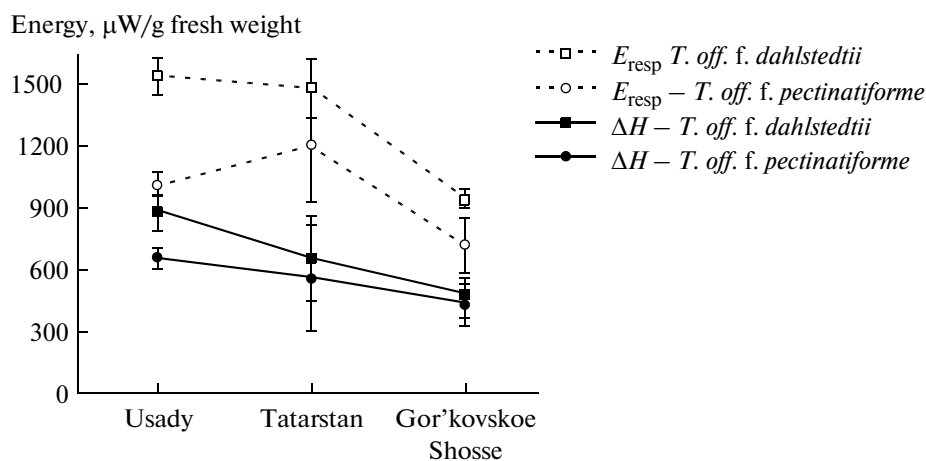
Energy, µW/g fresh weight

Fig.1. Changes in E_{resp} and ΔH of *T. off.* f. *dahlstedtii* and *T. off.* f. *pectinatiforme* plants depending on pollution level.

in the balance of energy in favor of its expenditure for repair (Criddle et al., 1989). The amount of this expenditure was estimated as follows. The total energy produced by respiration (E_{resp}) was calculated from change in enthalpy per nanomole of O_2 consumed for substrate oxidation (Hansen et al., 1994), using a coefficient of 455 µW/nmol. The result was used to determine the parameter of interest (ΔH) (Petrov, 1975), which was calculated as the difference between E_{resp} and the energy loss through heat production (Fig. 1).

In all cenopopulations, both E_{resp} and ΔH were higher in *T. off.* f. *dahlstedtii*. A significant difference in ΔH ($p < 0.001$) was revealed between *T. off.* f. *pectinatiforme* plants from the Usady (control) and Gor'kovskoe Shosse cenopopulations. Differences in ΔH between *T. off.* f. *dahlstedtii* and *T. off.* f. *pectinatiforme* from the Tatarstan and Gor'kovskoe Shosse cenopopulations lacked statistical significance. A relative increase in E_{resp} was noted in *T. off.* f. *pectinatiforme* from the Tatarstan cenopopulation ($p < 0.053$).
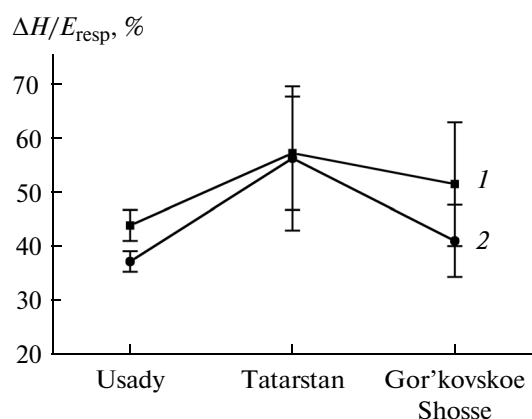
$\Delta H/E_{resp}$, %

Fig. 2. Changes in energy expenditures for metabolism in (*1*) *T. off.* f. *dahlstedtii* and (*2*) *T. off.* f. *pectinatiforme* plants depending on pollution level.

Changes in energy expenditures for metabolism were evaluated by calculating the $\Delta H/E_{resp}$ ratio (Fig. 2). The results show that both morphological forms in the control cenopopulations expend for metabolism less than 50% of E_{resp}, compared to over 55% in the Tatarstan cenopopulation ($p < 0.05$). It is known that stress causes excess stimulation of metabolism and can thereby activate general adaptive mechanisms providing for nonspecific plant resistance (Ishikama et al., 1995; Hughes and Dunn, 1996; Franco et al., 1999; Shakirova, 2001). Under strong pollution, a decrease in $\Delta H/E_{resp}$ was observed, which lacked statistical significance in *T. off.* f. *dahlstedtii* but was significant ($p < 0.01$) in *T. off.* f. *pectinatiforme*. It may well be that greater energy expenditures for metabolism in *T. off.* f. *dahlstedtii* plants provide for more effective adaptation to strong pollution.

Realizing as much as possible of their adaptive potential, plants modify metabolic processes so as to mobilize their energy resources for maintaining the structure and functions of biomembranes, with biosynthetic processes being minimized or ceased (Melekhov, 1985), which provides for the improvement of plant resistance to anthropogenic stressors (Bezel', et al., 2001). Physiological mechanisms of plant resistance are dependent on the state of biomembranes (Chirkova, 1997). Exposure to a stress factor (namely, pollution) first of all causes damage to the structural integrity of membranes, with consequent changes in their permeability (Bohnert et al., 1995; Tarchevskii, 2000). The diagrams shown in Fig. 3 characterize the permeability of membrane sandwich (plasmalemma + tonoplast) estimated from the rate of proton exchange ($1/\tau$) between the vacuolar and cell wall water.

In the Tatarstan cenopopulation, neither $1/\tau$ in root cells nor ΔH and $\Delta H/E_{resp}$ differed between the two morphological forms of dandelion. Under strong pollution (the Gor'kovskoe Shosse cenopopulation), membrane sandwich permeability for water in *T. off.* f. *dahlstedtii* increased only slightly, whereas in *T. off.* f.
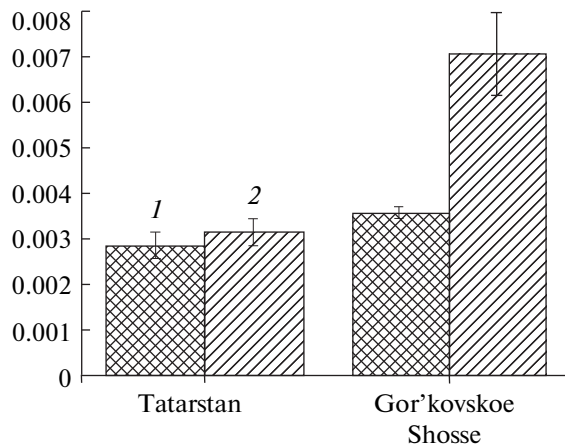
Fig. 3. Proton exchange rate in root cells of (*1*) *T. off.* f. *dahlstedtii* and (*2*) *T. off.* f. *pectinatiforme* plants from Tatarstan and Gor'kovskoe Shosse cenopopulations.
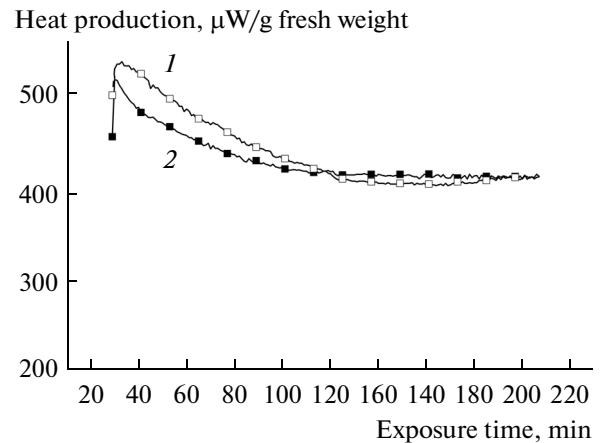
Fig. 4. Heat production by roots in (*1*) *T. off.* f. *dahlstedtii* and (*2*) *T. off.* f. *pectinatiforme* plants of old generative age (*3*) from Gor'kovskoe Shosse cenopopulation.

pectinatiforme it became twice higher (Fig. 3), with the $\Delta H/E_{resp}$ ratio being decreased (Fig. 2). It was logical to assume that the observed differences in physiological parameters would be reflected in the quality of seed progeny from q_1 plants.

When the seeds were prepared for germination, it was noted that they differed in the degree of seed coat pigmentation. By this criterion, the seeds from plants of each morphological form were divided into two groups, which proved to differ in germination energy (Table 3). Moreover, it was found that germination energy decreased with an increase in pollution level, but this trend was less apparent in case of *T. off.* f. *dahlstedtii* seeds with normal pigmentation.

These results show that the high level of metabolism in *T. off.* f. *dahlstedtii* plants allows them to maintain physiological functions of membranes (as estimated from parameters of their permeability) under conditions of pollution, which eventually provides for a higher quality of seed progeny.

The redistribution of energy resources in *T. off.* f. *pectinatiforme* from the Tatarstan cenopopulation allows them to maintain membrane permeability at the same level as in *T. off.* f. *dahlstedtii*, with the quality of seed progeny being slightly lower than in the latter

form. In the strongly polluted cenopopulation from Gor'kovskoe Shosse, a deficit of energy resources for repair processes leads to a twofold increase in $1/\tau$ and a significant reduction of germination energy.

As noted above, many plant species become increasingly tolerant with age (Brandt and Lamb, 1994; Warner and Cushman, 2002). Taking into account the ability of plants to minimize energy expenditures via ontogenetic switches from resistance to tolerance (Boege et al., 2007), we performed an analysis of heat production and membrane permeability in the roots of old generative plants (q_3). The results showed that, in this ontogenetic state, plants of both morphological forms from the Gor'kovskoe Shosse cenopopulation had similar $1/\tau$ values (1.6 ± 0.2 and 2.0 ± 0.4 ms) and practically did not differ in heat production (Fig. 4).

It appears that previously described differences in the survival strategy between the two morphological forms (Zhuikova and Bezel', 2009) depend on the input of energy resources for resistance to the impact of adverse environmental factors. These differences are manifested in young generative plants (q_1), which then develop similar mechanisms of tolerance and

Table 3. Germination energy of seeds differing in coat color from young generative (g_1) dandelion plants of different morphological forms from different cenopopulations, % (mean $\pm$ SD, $n = 6$)

Cenopopulation	*T. off.* f. *ðectinatiforme*		*T. off.* f. *dahlstedtii*	
	depigmented	normal	depigmented	normal
Usady	51 ± 7	62 ± 6	62 ± 6	77 ± 5
Tatarstan	33 ± 6	36 ± 11.5	50 ± 9.8	50 ± 11
Gor'kovskoe Shosse	3.3 ± 1.3	20 ± 5.8	15 ± 8.5	58 ± 11.3
R^2 (p)	$0.938**$	$0.788**$	$0.854**$	$0.212*$

Asterisks indicate that differences from the control are significant at * $p < 0.005$ or ** $p < 0.0001$.

thereby minimize energy expenditures by the old generative age (q_3).

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