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Responses of Leaf-Eating Insects Feeding on Aspen to Emissions from the Middle Ural Copper Smelter

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Abstract—Foliar damage to aspen from leaf-chewing insects and miners has been assessed during a 4-year period in the environs of a large copper smelter in the Middle Urals. It has been shown that the total area removed, proportion of damaged leaves, and average area removed per damaged leaf are considerably smaller near the smelter than in the background zone. The degree of the effect is similar for all three parameters and remains stable with time. Both groups of leaf pests display lower trophic activity in the impact zone, but the effect of pollution on leaf-chewing insects is greater than on miners, while individual features of the tree and its environment affect miners more strongly than leaf-chewing insects.

Keywords: Middle Urals, leaf-chewing insects, miners, aspen, copper smelter, industrial pollution, foliar damage.

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The abundance and trophic activity of leaf-eating insects, which play an important role in the functioning of forest ecosystems, depend on many biotic and abiotic factors. Many studies on this dependence resulted in various hypotheses on interrelations in the host plant–leaf eater–entomophage system (Hain, 1987; Martem'yanov and Bakhvalov, 2007). Industrial pollution can be regarded as an additional strong abiotic factor of anthropogenic origin that acts on this system.

It is convenient to study the response of terrestrial biota to pollutant exposure in impact regions around a point polluter (Vorobeichik and Kozlov, 2012). Meta-analysis has revealed very strong heterogeneity in the effect of pollution: the degree of tree leaf damage near pollution sources is usually lower, but stimulation of leaf-eater activities at medium and high levels of pollution or lack of response are also observed in some cases (Kozlov, Zvereva, and Zverev, 2009; Zvereva and Kozlov, 2010). Therefore, it is necessary to accumulate more information on the responses of leaf-eaters to pollution. Possible causes of the observed variation in insect responses include characteristics of the polluter (type, amount of emission, and duration of action), specific features of living organisms (trophic group and life history), and environmental conditions (vegetation type and climate) (Kozlov and Zvereva, 2011). In our opinion, weather conditions in the environs of the pollution source should also be added to this list, at least as regards leaf-eating insects.

Interannual changes in the abundance of leaf-eating insects are believed to depend on the hydrothermal conditions of the current and previous growing seasons (Bogacheva, 1990; Reynolds et al., 2007; Meshkova, 2009; Bakhvalov, Koltunov, and Martem'yanov, 2010). However, the question about the influence of weather conditions on the effects of pollution has barely been studied, partly because of the lack of long-term surveys of leaf-eating insects in impact regions.

Damage to different tree species in impact regions has been studied extremely unevenly, which may explain bias in estimations of the effects of pollution on leaf-eating insects. Most of relevant studies have been performed on birch; while data on other tree species, including aspen, are scant (Kozlov, Zvereva, and Zverev, 2009).

The purpose of this study was to analyze interannual variation in foliar damage to aspen along the pollution gradient near a large copper smelter. We tested the hypothesis that the effect of pollution on leaf-eating insects remains stable with time. Aspen (*Populus tremula* L.) is a widespread forest-forming species in the taiga zone. Vegetative propagation, high growth rate, and photophily allow it to rapidly colonize disturbed forest areas, where it often occurs along forest edges, at roadsides, and in cutover sites.

MATERIAL AND METHODS

This study was performed in 2004–2006 and 2008 in the area affected by the Middle Ural Copper

Table 1. Relationship between leaf damage score and overall leaf area removal by leaf-eaters

Damage score	Area removal, %		Number of leaves
	Average value (95% confidence interval)	Lower and upper deciles	
1	0.6 (0.4–0.9)	0.1–1.3	20
2	2.3 (1.5–3.1)	0.6–4.5	20
3	20.4 (16.3–24.6)	8.6–31.8	20
4	51.7 (44.9–58.5)	33.7–68.7	20
5	79.2 (71.5–86.9)	60.8–93.3	9

Smelter (MUCS), in the environs of Revda (Sverdlovsk oblast). The smelter is one of the largest plants of the nonferrous metal industry in Russia. It has been in operation since 1940, emitting sulfur and nitrogen oxides, fluorine compounds, and polymetallic dust (Cu, Pb, Cd, Zn, Fe, etc.). Emissions reached a peak of 140 000 t per year in the 1980s and, beginning from the late 1990s, gradually decreased to 28 300 t in 2004 and 24 100 t in 2008. Three distinct zones were previously distinguished along the pollution gradient: the impact zone (up to 3 km west of MUCS); the buffer zone (3–6 km); and the background zone (7 km and farther) (Vorobeichik, Sadykov, and Farafontov, 1994). The state of various objects of the biota has been studied in the MUCS area for many years (Kaigorodova and Vorobeichik, 1996; Belskii and Lyakhov, 2003; Veselkin, 2004; Ermakov, 2004; Belskaya and Zinov'ev, 2007; Mukhacheva, 2007; Mikhailova, 2007; Zolotarev, 2009; Nesterkov and Vorobeichik, 2009; Trubina, 2009; Stavishenko, 2010; Smorkalov and Vorobeichik, 2011; Vorobeichik and Pishchulin, 2011), but the response of leaf-eating insects to pollution was considered only occasionally (Kozlov, Zvereva, and Zverev, 2009).

The material was collected in a dark coniferous forest in four test plots, 10 ha each. Two plots were in the background zone: one 30 km west of MUCS, in a raspberry–fern–wood sorrel spruce–fir forest with admixtures of aspen and birch, and the other 20 km west of MUCS, in a reed grass–wood sorrel fir–spruce forest with admixtures of birch, pine, and aspen; one plot was in the buffer zone, 6 km from MUCS, in a dead-cover spruce–fir forest with admixtures of pine, birch, and larch; and one more plot was in the impact zone, 1.5 km southwest of MUCS, in a dead-cover pine–spruce–fir forest with admixtures of birch and aspen.

In each plot, we randomly selected 30 (in 2004, 25) well-lighted aspen trees of about the same height (5–10 m) standing at least 10–20 m away from each other (usually along forest roads and glades). At the end of active growing period (late July to early August), one branch 20–30 cm long (on average, with about 40 leaves) was cut off from the southern side of each tree, 1.5–2.0 m above the ground, and subsequently studied in the laboratory. Such an approach was used to reduce variation in the degree of foliar damage depending on differences in microclimate within the plot and within the tree crown and to minimize the indirect effect of pollution via the thinning of tree stand. Both collection of the material and assessment of damage were performed by the same person. During the 4-year study period, a total of 18 211 leaves from 457 trees were examined (we failed to take into account two trees in 2005 and one tree in 2006).

Two damage types were distinguished: (1) hole feeding, including window feeding (leaf-chewing insects) and (2) mining (miners). The results of gall counts are not considered in this study. For each leaf, the damage score was estimated separately for each of the two damage types. The scale by Bogacheva (1979) was used: (0) no damage, (1) one small hole, (2) up to three larger holes or several smaller ones, (3) several large holes cut into principal veins, (4) multiple damage with less than 50% removal of leaf area, (5) more than 50% removal of leaf area, and (6) 100% removal of leaf area.

To evaluate the relationship between the damage score and the absolute area of the removed leaf lamina, 20 leaves with damage scores of 1 to 5 were selected. Preference was given to leaves with window-type damage, since they retained their contours, and this allowed more accurate measurements of the total leaf area and its proportion removed by the insects. The leaves were dried in a herbarium, scanned, and their images were processed in the program SIAMS Photolab (http://www.siams.com) to estimate the proportion of area removed from each leaf. The average proportion of removal corresponding to each damage score was used in subsequent calculations (Table 1).

The material for determining the species composition of leaf-eating insects was collected in 2008, simultaneously with samples for assessing foliar damage. Open-living larvae were photographed; mined leaves were herbarized.

In all cases, one tree was taken as an experimental unit for data analysis. Three parameters of foliar damage were considered: overall leaf area removal (C, the proportion of removed area in all leaves, both damaged and undamaged), extensity (E, the proportion of damaged leaves, regardless of the degree of damage), and intensity (S, the proportion of removed area only in damaged leaves). Both total damage (by the whole assemblage of leaf-eaters) and each of the two damage types separately (by leaf-chewing insects and by miners) were estimated. The extensity of damage depends mainly on the abundance of leaf-eaters, while its intensity (and, thus, overall leaf area removal) depends mainly on their species composition.

Average values of damage in different plots and years were compared by heteroscedacity-consistent two-way ANOVA with White–Huber correction for heterogeneity of variance, algorithm HC3 (Long and Ervin, 2000). Multiple comparisons were made by the Tukey test; variance was partitioned into components according to Snedecor. Variation coefficients were compared by *Z*-test, an analog of Student's *t*-test (Zar, 2010). In addition to ANOVA, the influence of pollution was estimated by calculating the mean effect size according to Hedges (Borenstein et al., 2009): the data were averaged by years using a model with random effects; confidence intervals were determined by bootstrapping (10000 iterations); and the significance of interannual heterogeneity (Q) was estimated using χ^2 distribution. Calculations were made in the program MetaWin 2.0.

Changes in the ratio between the components of overall leaf area removal along the pollution gradient were analyzed by calculating the response ratio by the formula $RR = \mathrm{Ln}(x_i/x_f)$, where x_i is the parameter value in plot i of the gradient and x_f is the parameter value in the background zone (30 km). This ratio has two features convenient for interpretation of the results: symmetry and additivity (Hedges, Gurevitch, and Curtis, 1999). Symmetry means the following: e.g., if $x_1/x_2 = 15/3$, then $RR = 1.61$; if $x_1/x_2 = 3/15$, then $RR = -1.61$; and if $x_1 = x_2$, then $RR = 0$. Additivity is especially useful for our purposes. The overall leaf area removal (%) for a given tree can be presented as the product of its intensity (S, %) and extensity (E, unit fraction), i.e., $C = S \times E$. Then if, e.g., $C_1/C_2 = (S_1 \times E_1)/(S_2 \times E_2) = (15 \times 0.15)/(3 \times 0.50) = 2.25/4.50$, then the response ratio for the overall removal $RR_C = 0.41$, which is precisely equal to the sum of response ratios for damage intensity ($RR_S = 15/3 = 1.61$) and intensity ($RR_E = 0.15/0.50 = -1.20$); i.e., $RR_C = RR_S + RR_E = 1.61 + (-1.20) = 0.41$. Additivity makes it possible to determine which particular component, extensity or intensity, is responsible for the change in the overall removal. When the values are averaged over several trees, additivity is retained only if geometric average is used; therefore, it was geometric average we used in calculations of response ratios.

Leaf samples for estimating the contents of metals (ten leaves per tree) were also taken simultaneously with those for damage assessment, from the same trees. The petioles were removed, and the leaves were dried at 60°C. Leaves from the same tree were pooled into one sample (about 0.1 g air-dry weight), which was digested in a mixture of 7 mL concentrated HNO_3 and 1 mL deionized H_2O in a Teflon bomb in a MWS-2 microwave digestion system (Berghof, Germany). Concentrations of metals (Cu, Pb, Cd, Zn, and Fe) were measured with an AAS vario 6 atomic absorption spectrometer (Analytik Jena, Germany). Our laboratory is certified for technical competence (certificate no. ROSS RU.0001.515630). A total of 120 samples were analyzed. Forest litter pollution was assessed in 2004 using the sampling and analysis methods described previously (Belskaya and Zinov'ev, 2007).

Data on weather conditions during the growing period were obtained from the nearest weather station (in the town of Revda).

RESULTS

The level of pollution in the study area, estimated from the concentrations of metals in the forest litter and in aspen leaves, increased with a decrease in distance from the source of emissions (Table 2). The concentrations recorded near the smelter were higher than those in the plot 30 km from the smelter, especially in cases of copper (by factors of 116.2 in the litter and 3.9 in leaves) and lead (30.0 and 43.1, respectively), while iron (10.8 and 3.3), cadmium (8.0 and 2.4), and zinc (4.5 and 2.6) were accumulated to a smaller degree. The distribution of plots with respect to metal concentrations in the litter corresponded well to our division of the study area into three zones: background (20 and 30 km from smelter), buffer (6 km), and impact (1 km). Similar, though less distinct clustering of plots was observed with respect to metal concentrations in the leaves.

Analysis of total foliar damage. Differences in the overall leaf area removal between the plots along the pollution gradient were statistically significant ($F = 17.5$, $p < 0.0001$, $df = 3$). In the background zone, leaf-eaters removed 8–10% of leaf area, as averaged over the study period (Table 3); significant differences between the plots located 20 km and 30 km from the smelter were recorded only in 2008 ($p < 0.0001$), with the overall removal in the former plot being greater by a factor of 1.8. In the impact zone, the overall leaf area removal was, on average, half smaller than in the background zone, but the difference between the plots at 1 km and 20 km from the pollution source was significant only in 2008 ($p < 0.0001$); conversely, spatial variation in the degree of leaf damage was higher. Indeed, the coefficient of variation recorded 20 km from the smelter was significantly lower than in the impact zone in 2004 and 2006 ($z = 2.56$ and $z = 2.07$, respectively; $p = 0.05$).

The values of overall leaf area removal significantly differed between years ($F = 18.5$, $p < 0.001$, $df = 3$). In particular, they decreased to a minimum in almost all zones in 2006 and reached a maximum in 2008; the interaction of factors "plot × year" lacked statistical significance ($F = 1.4$, $p = 0.183$, $df = 9$). Interannual differences contributed more strongly to variation in the overall removal (18.7% of variance) than did differences between plots along the gradient (8.0%), but the remaining variance (more than 70%) was due to

Table 2 . Concentrations (μg/g) of metals in forest litter (movable forms) and in aspen leaves (total content) at different distances from the smelter

Metal	Distance from smelter, km			
	30	20	6	1
	Forest litter			
Cu	47.6 (45–51)	53.1 (50–56)	1067.4 (813–1361)	5530.4 (5260–5969)
Pb	92.7 (87–97)	101.5 (90–108)	895.1 (809–947)	2780.9 (2589–3154)
Fe	1152.3 (1057–1309)	1177.8 (1000–1436)	2820.2 (2406–3133)	12425.5 (9683–17322)
Zn	245.8 (230–257)	296.8 (276–318)	706.3 (571–774)	1111.4 (915–1374)
Cd	3.5 (3.2–3.8)	3.5 (3.2–3.9)	20.8 (16.7–23.5)	28.1 (22.5–35.0)
	Leaves			
Cu	7.9 (5–11)	8.2 (5–10)	8.7 (6–12)	30.6 (18–68)
Pb	2.3 (0.4–5)	2.6 (0.3–6)	4.5 (0.6–8)	99.1 (23–275)
Fe	68.4 (55–88)	99.7 (69–127)	102.8 (73–143)	224.9 (151–419)
Zn	264.1 (146–301)	268.1 (159–435)	584.8 (308–832)	698.1 (263–1172)
Cd	2.7 (0.8–4.9)	2.9 (1.1–5.0)	5.8 (2.8–11.0)	6.6 (2.8–16.4)

Note: Arithmetic mean and (in brackets) maximum and minimum values are given; replications: for litter, $n = 3$ (test plots); for leaves, $n = 30$ (trees).

some other factors that were not taken into account (Fig. 1).

The extensity of foliar damage depended both on the plot ($F = 47.1$, $p < 0.0001$, $df = 3$) and the year ($F = 42.0$, $p < 0.0001$, $df = 3$), with the interaction "plot × year" being statistically significant ($F = 3.91$, $p < 0.0001$, $df = 9$). This parameter at a distance of 1 km from the smelter was lower than at 20 km (in 2004 and 2005, $p < 0.001$; in 2008, $p = 0.0006$) or 30 km (2004, $p = 0.0153$; 2005, $p = 0.0001$). In 2006, the proportions of damaged leaves were similar in all plots of the gradient. Variation in the extensity of damage in the impact zone was higher than at 20 km from the smelter in 2004, 2005, and 2006 ($z = 5.12$, $p = 0.001$; $z = 2.35$, $p = 0.05$; and $z = 5.59$, $p = 0.001$, respectively) and higher than at 30 km in 2006 ($z = 2.90$, $p = 0.05$).

Differences in the intensity of damage between the plots along the gradient were also significant ($F = 16.85$, $p < 0.0001$, $df = 3$) (Table 3), but a significantly lower value of this parameter near the smelter, compared to that at 20 km, was recorded only in 2008 ($p = 0.0004$). The significance of the effect of the year on the intensity of damage ($F = 14.87$, $p < 0.0001$, $df = 3$) is due to the fact that the values of this parameter in 2008 were high in all plots. Changes in damage intensity along the pollution gradient were of the same type in all years (the interaction "plot × year" lacked statistical significance: $F = 1.3$, $p = 0.235$, $df = 9$). Coefficients of variation in this parameter in the impact zone were higher than at 20 km and 30 km in 2004 ($z = 2.03$ and $z = 2.21$, respectively; $p = 0.05$) and higher than at 20 km in 2006 ($z = 2.17$, $p = 0.05$).

Comparison between contrasting plots (30 km and 1 km) by effect size clearly demonstrated the negative influence of pollution on all test parameters of foliar damage. The integrated effect size for all years was similar for all three parameters: –0.68 (confidence interval –0.86 to –0.38) for the overall leaf area removal; –0.66 (–0.99 to –0.37) for damage extensity; and –0.67 (–0.98 to –0.35) for intensity. The confidence intervals in all cases did not include zero, and

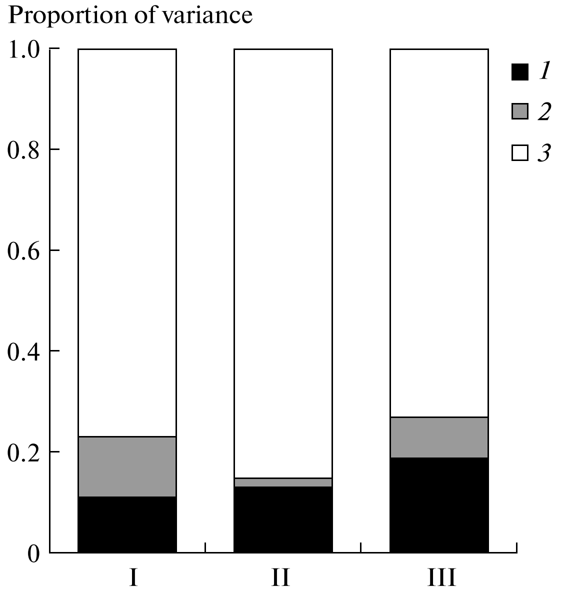

Fig. 1. Components of variance in the overall leaf area removal by (I) leaf-chewing insects, (II) miners, and (III) both groups. Proportions of variance determined by differences between (*1*) years or (*2*) pollution zones and (*3*) residual variance are shown.

Table 3. Measurements of foliar damage to aspen at different distances from the smelter in different years

Year	Distance from smelter, km			
	30	20	6	1
		Extensity, %		
2004	69.1 ± 3.4 **(32.9)	76.6 ± 3.3** (12.7*)	65.7 ± 16.3 (30.5)	49.5 ± 3.3 (45.7)
2005	59.3 ± 3.6** (37.0)	80.3 ± 1.9** (24.8*)	56.3 ± 3.1 (43.9)	41.2 ± 3.4 (44.8)
2006	57.9 ± 3.9 (19.5*)	57.3 ± 2.6 (15.5*)	51.6 ± 1.3 (21.4*)	50.1 ± 4.2 (35.4)
2008	77.4 ± 2.8 (28.5)	91.2 ± 2.6** (18.6)	73.1 ± 2.9 (30.2)	69.0 ± 4.5 (41.4)
AVG	65.9 ± 4.6 (13.8)	76.4 ± 7.1 (18.5)	61.7 ± 4.8 (15.6)	52.5 ± 5.9 (22.4)
		Intensity, %		
2004	11.5 ± 1.3 (55.7*)	9.7 ± 1.1 (59.0*)	8.8 ± 1.0 (55.3*)	5.7 ± 1.3 (117.2)
2005	10.2 ± 0.9 (48.8)	11.5 ± 1.6 (74.3)	8.2 ± 0.9 (60.2)	7.0 ± 0.8 (61.3)
2006	10.1 ± 1.3 (69.2)	8.8 ± 0.7 (44.1*)	8.6 ± 1.4 (89.1)	4.1 ± 0.6 (80.9)
2008	13.0 ± 1.7 (72.2)	20.4 ± 2.9* (76.7)	15.3 ± 1.7 (61.3)	11.3 ± 1.5 (73.8)
AVG	11.2 ± 0.7 (12.1)	12.6 ± 2.7 (42.2)	10.2 ± 1.7 (33.2)	7.0 ± 1.5 (43.9)
		Total removal, %		
2004	8.2 ± 1.2 (75.4)	7.3 ± 0.8 (57.2*)	5.9 ± 0.8 (67.4)	3.4 ± 1.2 (168.3)
2005	6.4 ± 0.8 (69.5)	9.4 ± 1.5 (82.5)	5.1 ± 0.7 (80.8)	3.1 ± 0.5 (89.4)
2006	6.1 ± 1.0 (87.6)	5.2 ± 0.5 (55.3*)	5.5 ± 1.3 (134.2)	2.4 ± 0.5 (116.1)
2008	10.9 ± 1.8 (88.3)	19.7 ± 2.9** (81.1)	12.0 ± 1.7 (75.6)	8.8 ± 1.5 (90.9)
AVG	7.9 ± 1.1 (27.9)	10.4 ± 3.2 (61.9)	7.1 ± 1.6 (45.8)	4.4 ± 1.5 (66.6)

Note: Average values ± error and (in brackets) variation coefficients (measure of variation between trees, %) are given; one tree is taken as a replication ($n = 25$ in 2004 and $n = 30$ in other years); AVG, average value ± error over all years ($n = 4$) and (in brackets) coefficient of variation (measure of interannual variation, %); significance of differences from impact zone: * $p < 0.05$; ** $p < 0.01$.

the effect size was stable in time: $Q = 2.9$, 3.0, and 2.9 for the overall removal, extensity, and intensity, respectively ($df = 3, p > 0.39$).

Analysis of damage types. According to our observations, leaves in the study area were damaged by lepidopteran larvae of the families Thyatiridae, Notodontidae, and Noctuidae, sawfly larvae of the family Tenthredinidae, and beetle larvae and adults, the most abundant of which were *Chrysomela populi* L., *Ch. tremulae* F., *Gonioctena decemnotata* (Marsh.), *G. viminalis* (L.) (Chrysomelidae), and *Byctiscus betulae* (L.) (Attelabidae). Pests found in leaf mines included lepidopteran larvae, primarily *Phyllonorycter apparella* (H.-S.) and also *Caloptilia stigmatella* (F.) and *Phyllocnistis labyrinthella* (Bjerk.) (Gracillariidae); dipteran larvae *Aulagromyza tremulae* (Hering) (family Agromyzidae), and sawfly larvae *Fenusella glaucopis* (Konow) (Tenthredinidae).

Two groups of leaf-eating insects are distinguished in this study: leaf-chewing insects and miners. The former group comprises insects living on the surface of leaves, both openly and semi-concealed (attelabids and tortricids); the latter comprises insects leading a concealed mode of life. In the study area, most of foliar damage was caused by leaf-chewing insects. The contribution of miners to the overall leaf area removal strongly varied from year to year (from 2 to 45%) but did not depend on the plot (Table 4). As averaged over all 4 years, the proportion of leaf area they removed varies from 0.9% in the impact zone to 2.6% in the background zone.

The responses of both insect groups to pollution (estimated by calculating response ratios) are shown in Fig. 2. In each of the 4 years, the total leaf area removed by each of the two groups was smaller in the impact zone than in the 30 km plot. In the buffer zone, the activity of leaf-chewing insects was also suppressed, whereas that of miners was found to be increased in 2005, 2006, and 2008. Such responses in different directions were also recorded in the 20 km plot: the total leaf area removed by leaf-chewing insects was greater than in the 30 km plot in 2005 and 2008, while the activity of miners was lower in those years.

The responses of components contributing to the overall leaf area removal changed along the pollution gradient either in the same or in opposite directions, with unidirectional responses differing in magnitude. As a rule, the responses of both groups of leaf-eating insects manifested in the intensity and extensity of damage were unidirectional, and the magnitude of oppositely directed changes was low. The contributions of damage intensity and extensity to changes in

Table 4. Contribution of miners (%) to the overall leaf area removal in aspen

Year	Distance from smelter, km			
	30	20	6	1
2004	17.3 ± 4.9	12.4 ± 3.4	8.4 ± 2.2	12.6 ± 3.7
2005	13.4 ± 4.1	2.0 ± 0.7	11.1 ± 2.6	18.6 ± 4.4
2006	28.7 ± 5.7	26.1 ± 5.5	45.0 ± 5.8	21.1 ± 4.5
2008	33.6 ± 5.8	23.1 ± 5.0	34.6 ± 5.8	27.6 ± 5.1

Note: Average values ± error are given; one tree is taken as a replication ($n = 25$ in 2004 and $n = 30$ in other years).

the overall leaf area removal did not depend on the level of pollution or year.

The contributions of interannual differences to variation in the overall leaf area removal by the two groups of leaf-eating insects were similar (Fig. 1): these differences accounted for 11.1% of the total variance in case of leaf-chewing insects, and for 12.6% in case of miners. At the same time, differences between the plots significantly contributed only to damage by leaf-chewing insects (11.6% of the total variance) but not to damage by miners (2.0%), which appeared to depend mainly on some factors that were not taken into account.

DISCUSSION

Despite the recent decrease in emissions, high levels of heavy metals in the soil account for a heavy toxic load on all components of ecosystems, including tree vegetation. Higher concentrations of metals in aspen leaves near the smelter (Table 1) show that the toxicants can directly act on leaf-eating insects. The lower levels of foliar damage observed every year in the strongly polluted zone could well be expected, since this result complies with the current concepts concerning the suppression of leaf-eating insects by increased concentrations of metals in food (Butler and Trumble, 2008; Massad and Dyer, 2010).

However, it would be wrong to explain the lower degrees of damage exclusively by the direct toxic action of heavy metals and other pollutants on leaf-eating insects, since significant correlations between concentrations of heavy metals in leaves and the levels of foliar damage are often absent (Kozlov, Zvereva, and Zverev, 2009). Leaf area removal is an integral parameter reflecting, among other things, the results of complex interactions in the plant–leaf eater–entomophage system. Pollution can act on each of its components (Butler, Trumble, 2008), shifting the balance one way or another. Therefore, theoretically it can be expected that the activity of leaf-eating insects could be either suppressed or stimulated in a polluted environment. It has to be emphasized that it is impossible to estimate the exact contributions of particular factors to changes in leaf-eater activity from the results of field studies alone, without any additional information. Consequently, all the following reasoning about this issue is largely speculative.

The quality of food is regarded as one of the basic factors having an effect on the abundance of leaf-eating insects (Rafes, 1968; Viktorov, 1971). Changes in the biochemical reactions of the plant caused by pollution (*Zagryaznenie*..., 1988) affects the contents of both nutrients and protective substances in leaves (Sitnikova, 1990; Loponen et al., 1998; Durand et al., 2010; Nikula, Vapaavuori, and Manninen, 2010) and, as a result, also influence the physiological state of phytophagous animals (Koricheva, Larsson, and Haukioja, 1998). Since no stimulation of trophic activity has been revealed in our study, it can be assumed that pollution in this case has not resulted in higher concentrations of nutrients or lower concentrations of secondary metabolites in the plants. However, the protective functions of the latter can be performed, to some extent, by heavy metals; at least, such a possibility in discussed for hyperaccumulator plants (Boyd, 2007).

The relationships between phytophagous animals and their natural predators and parasites have been poorly studied. Most publications provide evidence that entomophages either do not respond to pollutants or are suppressed under their effect (Heliövaara and Väisänen, 1986; Butler, Beckage, and Trumble, 2009). In cases of decreased predator/parasite activity in the environs of industrial enterprises, higher abundance of their prey/hosts can be expected, which should lead to higher levels of damage. This was the explanation proposed by Zvereva and Kozlov (2000, 2006) for the increase in the abundance of leaf-eaters feeding on birch in polluted areas. In our study, the levels of foliar damage in the impact zone were lower, which could not be related to suppression of leaf-eating insects.

The influence of industrial pollution on leaf-eating animals can also be indirect, for instance, through changes in microclimatic conditions of their environment. Many leaf-eating insect species prefer to colonize well warmed and lighted areas with low moisture supply (Kondakov, 2002; Meshkova, 2009). Changes in the light and hydrothermal regimes can lead to structural reorganizations in communities of leaf-eaters and their redistribution between trees and within the crowns of individual trees (Vorontsov, 1963). The

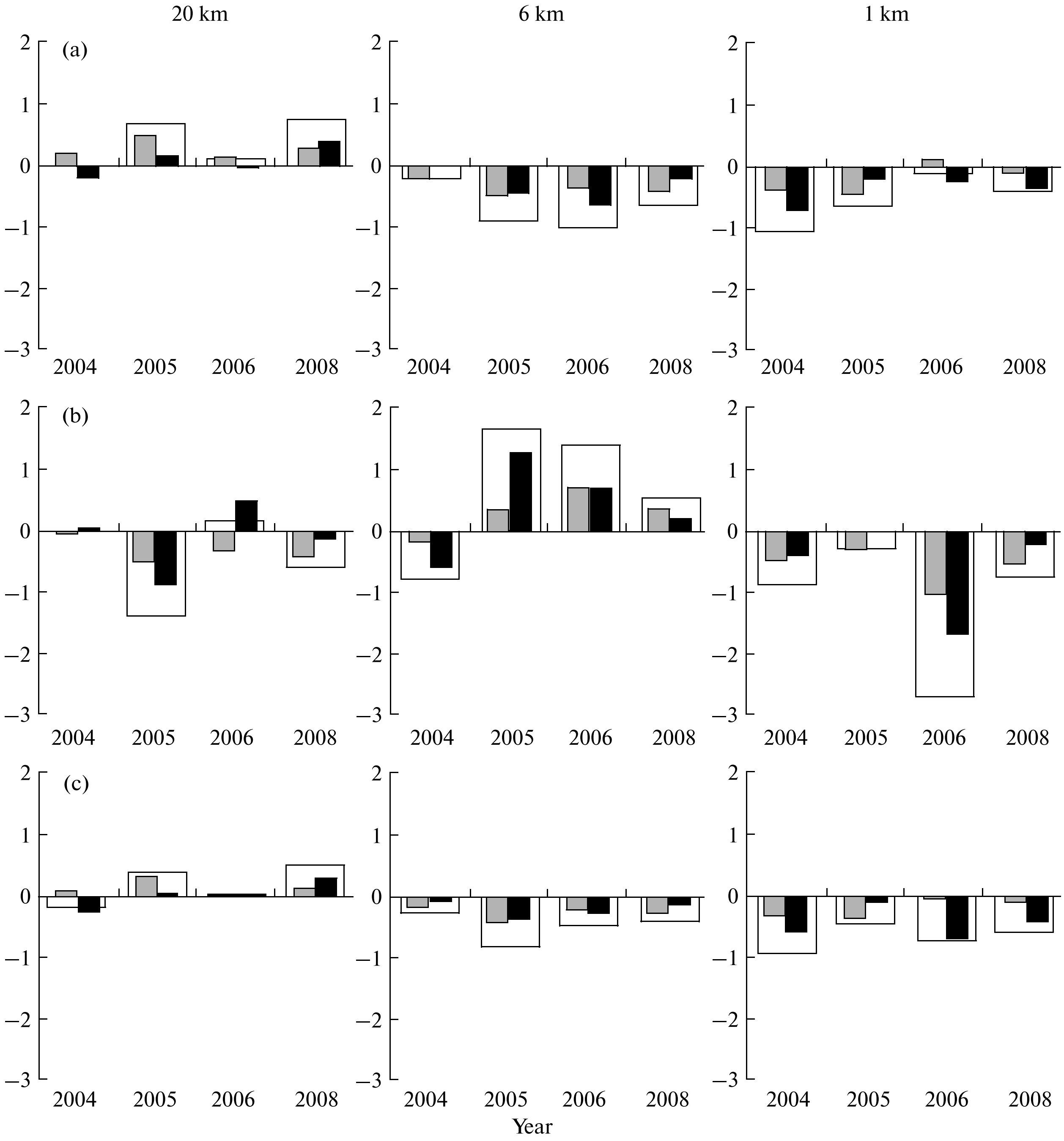

Fig. 2. Relative degrees of response to damage by (a) leaf-chewing insects, (b) miners, and (c) both groups of leaf eaters at distances of 20, 6, and 1 km from the smelter and in the background zone (30 km from the smelter) in different years. Gray bars, extensity of damage; black bars, intensity of damage; solid line, overall leaf area removal.

closer to the MUCS, the lower the stand density, basal area, timber volume, crown closure, and undergrowth density in the forest (Vorobeichik, Sadykov, and Farafontov, 1994), and, therefore, the higher the level of solar illumination in the tree stand. We tried to minimize phytoclimatic differences between tree stands of different pollution zones by taking censuses on well-lighted isolated trees. Therefore, the influence of this factor can be considered negligible, the more so that the trophic activity of leaf-eating insects prove to decrease in the strongly polluted zone.

The trophic activity of leaf-eating animals depends also on many abiotic factors, which determine considerable interannual variation in their activity. It is believed that the most important among these factors is air temperature, which directly acts on leaf-eaters

(Bale, Masters, and Hodkinson, 2002), so that their abundance positively correlates with summer temperatures (Bogacheva, 1990; Reynolds et al, 2007). The warmest summer during the period of our study was in 2008 (average temperature of July 19.9°C); the coolest one was in 2006 (15.4°C). The degree of damage recorded in these two years was the highest and lowest, respectively. The time span of this study is too short for accurately assessing the modifying influence of weather conditions of the year on the response of leaf-eating insects to pollution. However, the fact that the smallest difference between the degrees of damage in the background and impact zones were recorded in the warmest summer of 2008 does not comply with the conclusion by Kozlov and Zvereva (2011) that the effect of pollution on leaf-eating animals should increase with a rise in the average temperature of July. These authors used meta-analysis to compare several polluted areas on the basis of long-term data on the climate in the geographic area where the source of pollution was located. Possibly, the mechanism of interaction between climate and pollution along the latitudinal gradient is different from the mechanism of interaction between weather conditions and pollution in the area affected by a particular point source of emissions.

The conditions of the year had a significant effect only on changes in the extensity of damage. The intensity of damage and the overall leaf area removal showed no dependence on the interaction of factors "year × plot," which makes these parameters especially convenient for estimating the responses of leaf-eating insects to pollution, the more so that all three test parameters proved to be similar in effect size. Using the extensity parameter for this purpose can lead to biased estimations in meta-analysis if the level of damage near different sources of pollution is evaluated in different years.

The possible causes of high variation in the level of foliar damage deserve special consideration. According to Bogacheva (1990), the spatial variation of damage levels in plants has the following components: geographic (between different geographic regions), local (depending on abiotic conditions in different plots within the same geographic region), biotopic (depending on differences in microclimatic conditions within the same plot), and individual (depending on differences between individual trees). The factors considered in this study, pollution of the area and year, explain less than one-fourth of the total variance in the level of foliar damage (Fig. 1). Emphasis is made on individual aspects of variation: we have analyzed few large forest areas, taking censuses in many points within each plot and examining a large number of trees. Therefore, the group of factors unaccounted for may include the age of the tree, its genetic features determining the intensity of secondary metabolite production, degree of weakening, microclimatic conditions, and chemical properties of the soil at the place of its growth. The mechanisms of the effect of these factors on the level of foliar damage in polluted areas have barely been studied. The higher values of variation coefficients for foliar damage in the impact zone suggest that local and individual differences between environmental conditions in habitats of leaf-eating insects increase along the pollution gradient.

The responses of leaf-eating insects to the impact of environmental factors depend on the feeding guild (Koricheva, Larsson, and Haukioja, 1998; Vehvilaïnen, Koricheva, and Ruokomaki, 2007; Cornelissen, Wilson, and Vasconcellos-Neto, 2008). The results of this study show that differences between plots along the pollution gradient are more important to leaf-chewing insects than to miners, which, in turn, are more strongly affected by individual differences between trees. This result could well be expected, being determined primarily by differences in the mode of life between members of the two groups. Miners, the larvae of which feed inside plant tissues, are protected from the action of external factors such as temperature and sunlight (Baranchikov and Ermolaev, 1998; Pincebourde and Casas, 2006), and therefore weakly respond to pollution (Mulder and Breure, 2006; Zvereva and Kozlov, 2010). Unlike leaf-chewing insects, feeding miners do not consume toxic dust that settles on the leaf surface. On the other hand, individual features of trees, such as the asynchronous unfolding of leaves in spring, have a stronger effect on miners than on mobile open-living species (Forkner et al., 2008). Nevertheless, the direction of response to high pollution levels is the same in the two groups (Fig. 2). Furthermore, the degree of response is sometimes even stronger in miners than in leaf-chewing insects. However, the group of leaf-chewing insects contributes much greater to foliar damage, compared to miners (Table 4), and it is this group that determines the direction of response for the whole leaf-eating assemblage, while miners only modify this combined response. Differences in the direction of response between miners and leaf-chewing insects observed at intermediate pollution levels also point out to the aforementioned specifics features in the responses of the two groups of leaf-eating insects.

CONCLUSIONS

This study helps to close the gap in the ecology of impact areas regarding the analysis of the influence of industrial pollution on the foliar damage by leaf-eating insects in aspen. Comparison between the most contrasting plots has revealed a considerable decrease in the overall leaf area removal and the extensity and intensity of foliar damage to aspen in the area adjacent to the smelter. The magnitude of this effect was similar for all three parameters of damage and stable in time, despite strong interannual differences in absolute parameter values. The influence of the particular year on the effect of pollution was observed only for one parameter, damage extensity, and only in comparison with the entire gradient including all intermediate levels of pollution load. Therefore, the proportion of damaged leaves, which depends mainly on the abundance of leaf-eating insects, is the most readily measurable but also the most variable parameter of foliar damage. Consequently, using it in meta-analyses of one-year data can be an additional source if variation in the observed effect.

The increased variation in the degree of foliar damage revealed in the impact zone suggests that pollution results in higher heterogeneity of the environment for leaf-eating insects, which is determined by specific genetic features of trees, the degree of their weakening, and local microclimate. It is these factors, not taken into account in this study, that are responsible for most of variation in the overall leaf area removal, while differences between the plots account for less than 10% of this variation. The difference in the mode of life between the groups of leaf-eating insects also has certain effect on their response to pollution: the activity of both leaf-chewing insects and miners is lower in the impact zone, but pollution itself has a stronger effect on leaf-chewing insects, while the activity of miners strongly depends on individual features of the tree and its environment.

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