

Local adaptation of mountain birch to heavy metals in subarctic industrial barrens

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Abstract

Adaptation of short-lived plants to pollutants is a well documented phenomenon, but with long-lived trees pollution resistance has been found much less frequently. Mountain birch is a likely candidate for detecting resistance, as it is one of the few woody plants surviving in the heavily polluted industrial barrens of the Kola Peninsula, NW Russia. The current experiment was designed to find out whether 60 to 70 years of pollution impact from two nickel-copper smelters has caused selection of resistant birch genotypes and to investigate possible trade-offs between pollution resistance and competitive ability in clean conditions.

Seedlings from polluted sites outperformed seedlings from pristine sites in the heavy metal treatment, and vice versa, seedlings from pristine sites outperformed seedlings from polluted sites in the control treatment. I suggest that the superior performance of seedlings from polluted sites in the heavy metal treatment was a result of either heavy metal resistance or adaptive phenotypic plasticity, whereas the better performance of seedlings from pristine sites in the control treatment was a result of greater competitive ability.

Keywords: *Betula pubescens*, local adaptation, industrial barren, heavy metal resistance, competition, trade-off

1 Introduction

Adaptation to pollution stress is an extensively studied phenomenon with plants, and provides a well documented example of rapid evolutionary adaptation (BRADSHAW and MCNEILLY 1981; MACNAIR 1997). However, pollution resistance in populations of long-lived trees has been detected less frequently (RACHWAL and WIT-RZEPKA 1989; TURNER 1994; UTRIAINEN *et al.* 1997), possibly due to longer reproductive cycles or innate physiological reasons. It has thus been suggested that the survival of long-lived plants in heavily polluted environments is due to phenotypic acclimatization and plasticity rather than on evolution of resistance (DICKINSON *et al.* 1991; TURNER and DICKINSON 1993), especially as genetic adaptation to stress has often been shown to result in poorer competitive ability in pristine conditions (see for example HOFFMAN and PARSONS 1991; BEGON *et al.* 1996, and references therein).

Mountain birch (*Betula pubescens* subsp. *czerepanovii* [Orlova] Hämet-Ahti) is one of the very few woody plants surviving in the heavily polluted industrial barrens of the Kola Peninsula, NW Russia. Before human impact, the studied industrial barrens were either dominated by mountain birch (the area around Nikel, VALLE 1933), or were mixed forests of mountain birch, Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst.) (the area around Monchegorsk, BOBROVA and KACHURIN 1936). Nowadays birches in denuded landscapes around nickel-copper smelters have a stunted, bushy growth form

with small densely positioned leaves, in contrast to the high, slender trees with large, sparsely positioned leaves found in pristine habitats (KRYUCHKOV 1993). The stunted growth form and small leaf size can be considered either as a sign of stress (KOZLOWSKI *et al.* 1991), or alternatively, as a sign of adaptation to stress and the related trade-off in growth and, potentially, competitive ability (HICKEY and MCNEILLY 1975; HOFFMANN and PARSONS 1991; BEGON *et al.* 1996).

In the present paper I report the preliminary results of a greenhouse experiment with progenies of mountain birch from two pollution gradients. In this paper I aim to answer the questions: 1) has 60 to 70 years of pollution impact around two nickel-copper smelters resulted in mountain birch populations adapting to heavy metals, 2) does the possible adaptation carry a cost in performance in clean soil, and 3) are the results similar for seeds coming from two different impact zones, i.e. has the possible adaptation proceeded similarly in two different locations.

2 Materials and methods

2.1 Impact zones and source populations

The birch seeds used in the experiment originated from four source populations around two nickel-copper smelters in Kola Peninsula, NW Russia: Severonikel smelter in Monchegorsk (67° 55'N, 32° 48'E) and Pechenganikel smelter in Nikel (69° 24'N, 30° 16'E). The source populations were classified as “polluted”, 7 km S of Monchegorsk and 1 km E of Nikel, and “pristine”, 65 km SW of Monchegorsk and 40 km SE of Nikel. Both smelters, built in 1930s, are among the top European sources of aerial pollutants, mainly in sulphur dioxide and heavy metals (PEARCE 1994). In 1990, the Severonikel and Pechenganikel smelters emitted, respectively, 2.32×10^8 and 1.9×10^8 kg SO₂, 2.7×10^6 and 0.14×10^6 kg Ni and 1.8×10^6 and 0.09×10^6 kg Cu (BERLYAND 1991). Since that time, emissions have steadily declined, reaching (respectively for Severonikel and Pechenganikel) 0.42×10^8 and 0.61×10^8 kg SO₂, 0.002×10^6 and 0.15×10^6 kg Ni and 0.7×10^6 and 0.08×10^6 kg Cu in 2003 (MILYAEV and YASENSKIJ 2004). The emissions have resulted in extensive forest decline, followed by the destruction of ground vegetation and the uppermost soil layers (KRUYCHKOV 1993; RIGINA and KOZLOV 1999). The vegetation in both polluted sites is scarce; they are dominated by sparsely growing mountain birches and willows (*Salix caprea* L., *S. borealis* [Fries.] Nasar.). The ground vegetation consists of patches of dwarf shrubs (*Vaccinium myrtillus* L., *V. vitis-idaea* L., *Empetrum nigrum* ssp. *hermaphroditum* [Hagerup] Böcher) and grasses (mainly *Deschampsia flexuosa* [L.] Trin.) scattered over bare ground. The pristine sites are healthy looking subarctic forests; mixed forest of mountain birch, Scots pine and Norway spruce SW of Monchegorsk, and birch-dominated SE of Nikel. The field layer vegetation on both pristine sites covers almost 100 % of the ground, and consists mainly of *E. nigrum*, *Vaccinium* spp. and mosses.

2.2 Experimental design and measured variables

Mountain birch seeds were collected in September 2004 from the four source sites. Seeds were collected manually from five random seed-bearing mountain birches from both polluted and pristine sites. After collecting, seeds were stored in +3°C, and stratified in -20°C for one month starting two months before germination. Germination was started in a greenhouse on

March 15th 2005 in +22°C. To maximize root growth, non-fertilized fine-grained sand was used as growth medium. Seedlings were planted in two litre pots two weeks after germination, between March 30th and April 4th 2005. After planting, room temperature was kept between +12 and +18°C. The soil used in the experiment had 60 % fine-grained sand and 40 % *Sphagnum*-peat (N-P-K 14-4-20, Kemira Grow-How Oyj, Finland). Four seedlings from each source population (polluted and pristine sites of both Nickel and Monchegorsk) were planted in each pot to assess potential differences in growth and competitive ability. Seedlings from different mother trees were evenly distributed to different treatments. The experiment was replicated in 8 blocks, with four replicate pots per treatment in each block. The total number of pots was 64, with a total number of 256 seedlings. Light level was kept constant at 250 $\mu\text{mol s}^{-1} \text{m}^{-2}$ throughout the day, as the sun never sets in the source sites during the growing period.

Two weeks after planting I started adding heavy metals to the soil in half of the experimental pots. Heavy metals were added in 1 dl of water solution (586 mg l⁻¹ NiSO₄ \cdot 6 H₂O, 256 mg l⁻¹ CuSO₄ \cdot 5 H₂O) twice a week evenly across the surface, until the soil concentrations of nickel and copper were expected to have reached 170 mg kg⁻¹ Ni and 80 mg kg⁻¹ Cu per dry weight of soil, i.e. circa one third of what occurs in industrial barrens (BARCAN and KOVNATSKY 1998). Heavy metal addition was administered in order to find out how it would affect the competitive balance between seedlings of different origin.

Seedling performance indices were measured twice, between June 7th and 10th 2005 and between August 15th and 18th 2005. The first measurements were performed shortly after terminating the heavy metal addition, and the second measurements were performed shortly before starting to shorten the day length in preparation for winter. The variables measured were seedling height (to the nearest 5 mm) and the length of two largest leaves (to the nearest 1 mm). For analysis, average leaf lengths were calculated for each seedling. Larger height and larger leaves were considered to indicate better competitive ability and growth potential, and thus, fitness.

2.3 Statistical analyses

Repeated ANOVAs with measurement time as the repeated factor were performed for both measured variables. Heavy metal treatment, impact zone (Nickel or Monchegorsk), seed origin (polluted or pristine), and their interactions as well as measurement time \times heavy metal treatment interaction were used as fixed explanatory variables. Block, pot and mother tree were used as random variables. Pairwise comparisons were done with least squares means. To make sure, analyses were also performed separately for each impact zone. Seedling height was log-transformed prior to analysis to achieve normality of residuals. All analyses were conducted with procedure MIXED in SAS Institute 8.2 (LITTELL *et al.* 1996).

3 Results

Heavy metal treatment had a strong negative effect on both seedling height and average leaf length (Table 1, Fig. 1). Also the interaction between heavy metal treatment and seed origin was significant for both variables (Table 1). In the heavy metal treatment seedlings from polluted sites did better than seedlings from pristine sites and, vice versa, seedlings from pristine habitats performed better than seedlings from polluted habitats in control treatment (Fig. 1), indicating some degree of local adaptation. Analyses done separately for each

impact zone yielded similar results: the interactions between heavy metal treatment and seed origin were significant and similar for both Monchegorsk and Nikel ($p < 0.05$ for leaf length and $p < 0.001$ for seedling height). There was also some variation between impact zones (Table 1): Nikel seedlings were both shorter (Nikel 123.5 ± 5.1 mm, Monchegorsk 159.6 ± 6.3 mm) and had smaller leaves than Monchegorsk seedlings (38.8 ± 0.9 and 42.1 ± 1.0 mm, respectively). Heavy metal addition had a stronger negative effect on Nikel seedlings than Monchegorsk seedlings, but this effect was independent of seed origin (pristine vs. polluted) (Table 1). The interaction between measurement time and heavy metal treatment was significant for both measured variables (Table 1): the negative effects of the heavy metal treatment were more evident in the second measurement time.

Table 1. Repeated ANOVA results for factors affecting the measured performance characteristics.

Fixed variables	Seedling height			Average leaf length		
	df	F	P	df	F	P
Heavy metal treatment (HM)	1, 68.9	223.33	<0.0001	1, 87.7	265.21	<0.0001
Seed origin (polluted vs. pristine)	1, 19.6	5.60	0.03	1, 302	0.12	0.73
Impact zone (Monchegorsk vs. Nikel)	1, 20	46.38	<0.0001	1, 330	23.09	<0.0001
HM \square Impact zone	1, 315	10.95	0.001	1, 330	0.38	0.54
HM \square Seed origin	1, 311	31.96	<0.0001	1, 302	9.89	0.0018
Impact zone \square Seed origin	1, 19.6	3.08	0.09	1, 312	2.56	0.11
HM \square Impact zone \square Seed origin	1, 315	0.12	0.73	1, 312	0.05	0.82
Measurement time \square HM	1, 321	7.82	0.0055	1, 316	53.68	<0.0001
Random variables	V \pm SE	Z	P	V \pm SE	Z	P
Block	0.033 \pm 0.022	1.49	0.07	5.66 \pm 4.61	1.23	0.11
Pot	0.052 \pm 0.016	3.26	0.0006	10.73 \pm 4.56	2.35	0.0094
Mother tree	0.007 \pm 0.005	1.51	0.07	0	–	–
Repeated variable	V \pm SE	Z	P	V \pm SE	Z	P
Measurement time	0.122 \pm 0.010	11.19	<0.0001	64.06 \pm 5.41	11.85	<0.0001

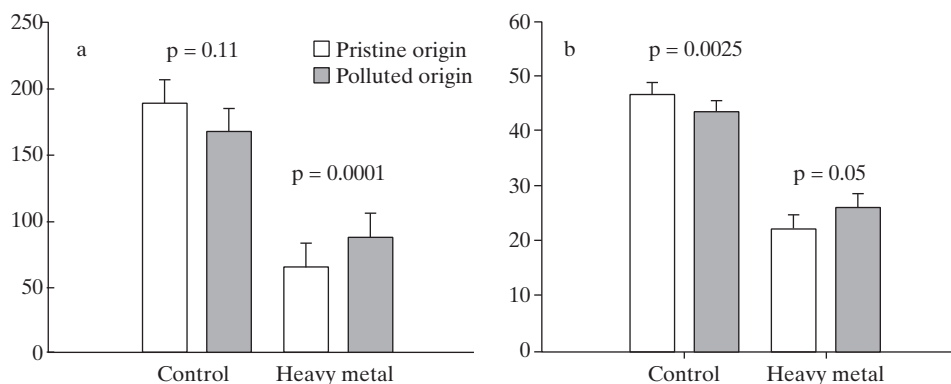


Fig. 1. The effect of heavy metal treatment on a) seedling height [mm] and b) average leaf length [mm]. P-values above bars denote differences within control and heavy metal treatments.

4 Discussion

The data suggests that emissions from the two studied nickel-copper smelters have resulted in mountain birch adapting to heavy metal stress in a timeframe of 60 to 70 years. In the heavy metal treatment seedlings from polluted sites did better, but in the control treatment seedlings from pristine sites were stronger. To our knowledge, this is one of the very few examples of long-lived trees adapting to anthropogenic pollution stress (although see BROWN and WILKINS 1985; WATMOUGH and DICKINSON 1995; PUNSHON *et al.* 1995; UTRIAINEN *et al.* 1997). The results are in accordance with the data from KOZLOV (2005), who discovered signs of local adaptation to pollution stress in a mountain birch population around the nickel-copper smelter of Monchegorsk, one of the impact zones used in the current study. An alternative explanation for the heavy metal resistance of seedlings originating from polluted sites could be adaptive phenotypic plasticity (VIA *et al.* 1995; SCHLICHTING and PIGLIUCCI 1998; KINGSOLVER *et al.* 2002): plasticity can help genotypes acclimate to various environmental conditions, but the formation and maintenance of the physiology and structures associated with plasticity can also be costly (DEWITT *et al.* 1998). If this is the case, it could explain the poor performance of seedling from polluted origin in control treatment. Another possible explanation is maternal effects (ROACH and WULFF 1987; SCHLICHTING and PIGLIUCCI 1998), i.e. the effect of the maternal parent's growth environment on offspring phenotype. Although we aimed to minimize maternal effects by using seeds and a controlled germinating environment, the possibility of maternal plant's growth environment affecting seedling performance can not be totally ruled out.

From our data it is not possible to distinguish whether it is adaptation to heavy metals, adaptive phenotypic plasticity or some other reason, like maternal effects, why the studied birch populations react differently to heavy metals; a more physiological/genetical approach would be required to tackle the question of mechanisms of adaptation. However, the evidence of rapid evolutionary adaptation could be put to use in restoration efforts by using seeds/seedlings from local mother trees in reforestation.

Interestingly, although seedlings from the two impact zones showed differences in performance, the aforementioned signs of local adaptation were evident for both pollution gradients. Although no final conclusions can be made from just two impact zones, the result raises the questions: could adaptation of trees to heavy metals be a more general phenomenon than previously thought, or could adaptation to anthropogenic stressors be more common in the naturally stressful subarctic areas?

Regardless of the physiological and evolutionary mechanisms behind heavy metal resistance, we hypothesize that survival selection (HOFFMAN and PARSONS 1991) is the reason behind the detected pattern. Very low tree densities in the industrial barrens indicate that mortality has been extremely high and seed germination very low, resulting in very strong selection pressure towards resistance/tolerance.

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