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Effects of soil quality and air pollution on the rooting and survival of *Salix borealis* cuttings

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The rooting and survival of *Salix borealis* cuttings were studied in the surroundings of the Severonikel smelter (Kola Peninsula, NW Russia). In favourable habitats (birch and willow-dominated secondary communities), 22% of the 120 cuttings were successfully established, and 89% of the established cuttings survived up to the fourth year after planting. Cutting survival and vigour were affected by both soil quality and air pollution level. Cutting mortality during the two first weeks after planting depended on soil contamination; in metal-polluted soils it was seven times as high as in clean soils. The detrimental effects of ambient sulphur dioxide became visible after four weeks, whereas survival at the end of the growing season was almost exclusively determined by the nutritional quality of the soil. Cuttings from female genets were more resistant to pollutants than cuttings from male genets; cuttings from the unpolluted site were more tolerant of metals than cuttings from the heavily polluted site. The leaf size of the surviving cuttings was independent of soil, water or air contamination. These preliminary findings seem to indicate that cuttings of *S. borealis* can be used for the partial revegetation of industrial barrens even at high emission levels.

Introduction

Denuded landscapes adjacent to major emitters of sulphur dioxide and heavy metals are often called industrial barrens (Doncheva 1978, Kryuchkov and Makarova 1989, Tsvetkov 1991, Kozlov and Haukioja 1995, Winterhalder 1995). These landscapes are formed on acidic, metal-contaminated soils, void of large (> 3 m tall) trees and bushes, with a vegetation cover < 10%. The illuvial soil horizon is exposed on > 80% of the surface, which suffers from intensive water and wind erosion. From a functional point of view, an ecosystem becomes an industrial barren when the death of woody plants is not compensated by recovery for several years and the vegetation cover is in continual decline. Industrial barrens around the Severonikel smelter (Kola Peninsula, NW Russia) have expanded steadily during the past decades, and now cover some tens or even hun-

Fig. 1. Location of study sites (numbered 1 to 6) in relation to Severonikel smelter (marked with asterisk). Insert: location of study area on Kola Peninsula.

dreds of square kilometres (Kryuchkov 1993, 1994, Mikkola 1995, Rees and Williams 1997).

Six species of woody plants were found growing in the barren landscapes surrounding the Severonikel smelter (M. Kozlov, unpubl.). The sparsely growing low-stature mountain birches (*Betula pubescens* subsp. *czerepanovi* (Orlova) Hämet-Ahti), are severely stressed: leaf size and shoot length are 30%–60% of those measured in unpolluted area (Kozlov 1992, and unpubl.), and leaf fluctuating asymmetry is almost double that of the background level (Kozlov *et al.* 1996). The bush-like growth form was also observed in surviving Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* subsp. *obovata* (Ledeb.) Domin.) and aspen (*Populus tremula* L.), all of which show clear signs of pollution damage. In contrast, two willow species, *Salix caprea* L. and *S. borealis* (Fries.) Nasar., grow vigorously in industrial barrens: the leaf fluctuating asymmetry of both species in heavily polluted habitats is the same as that in unpolluted localities, while leaf size and shoot length in polluted areas are even larger than in the forests (Zvereva *et al.* 1997, and unpubl.).

Willows have been considered among the first candidates for the recovery of the barren landscapes around the Severonikel smelter, following soil amelioration and the establishment of a grass cover (Tsvetkov 1987, Tsvetkov and Chekrisov 1987, Tsvetkov *et al.* 1987). However, the reclamation program elaborated by Tsvetkov (1993) has never been implemented in the Kola Peninsula: the program presupposed both a substantial decline in emissions and a major financial input.

The annual emissions of sulphur dioxide from the Severonikel smelter reached a maximum of 278 000 tonnes in 1983, and then declined steadily to 98 000–129 000 t in 1994–1996 (Murmansk Regional Committee of Nature Protection 1996; V. Barcan, pers. comm.). This fact, together with the vigorous growth of extant willow clones at heavily polluted sites (Zvereva *et al*. 1997), suggested that a start could be made on restoring the denuded landscapes without the need for expensive measures to improve soil quality.

The purpose of this study was to investigate the sensitivity of cuttings of *S. borealis* to soil toxicity, soil fertility and airborne pollution, in order to develop practical methods of planting this willow species in heavily contaminated habitats around the nickel-copper smelter.

Materials and methods

Study sites and object

Six sites were selected for our experiments (Fig. 1). The plots were the same as those used in our earlier studies (Zvereva *et al*. 1995a, 1995b, 1997, Kozlov *et al*. 1995), and were relatively close to plots where the soil quality has been monitored for several years (Barkan *et al*. 1993, Chertov *et al*. 1993, Lukina and Nikonov 1996) (Table 1). Three plots close to the Severonikel smelter represented heavily contaminated barren landscapes with an original tree cover of pine (site 1) and spruce (sites 2 and 3); an intermediate plot (site 4) represented the birch and willow-dominated transitional community that has replaced the spruce forest killed by emissions a couple of decades ago. Two plots were established in spruce forest (site 5) and pine forest (site 6), within a zone of slight pollution damage. For more details about the vegetation on the study sites, *see* Koroleva (1993).

The study organism, *S. borealis*, is a willow species common throughout the study area, including the most severely affected industrial barrens (Zvereva *et al*. 1995b). It grows in the form of high bushes, sometimes tree-like, and usually has a few ramets. Along the Severonikel pollution gradient, the largest specimens of *S. borealis* (15–25 stems with maximum diameter ca 80 mm, up to 5 m high) were observed at the heavily polluted site 2 (Fig. 1).

Experiments

Establishment of cuttings in a favourable site

The purpose of the first experiment was to investigate the survival of cuttings planted in relatively fertile soil in a slightly polluted site. Ten cuttings (diameter 5–9 mm) were collected on 20 June 1995 (when the buds were ready to open) from each of 12 genets of *S. borealis* growing naturally along the Vudiavrjok, a river in the Khibiny Mountains (67°41´N, 33°39´E), the site with an approximately

Table 1. Basic characteristics of the study sites.

Nr.	Distance and direction from the smelter	Lat. N	Long. E	Metal concentrations in soil (µg g^{-1})						SO$_2$ in ambient air[c] (µg m^{-3})
				Total[a]		Exchangeable[b]				
				Ni	Cu	Ni	Cu	Ca	Mg	
1	3 km N	67°57´	32°51´	9 300	4 600	21.3	538.4	28.2	6.2	247 ± 17
2	1 km N	67°56´	32°49´	2 500–3 000	1 500–1 900	73.6	190.8	165.8	27.1	151 ± 21
3	9 km S	67°51´	32°48´	2 600–4 000	1 850–2 100	46.4	10.0	107.9	19.1	150 ± 9
4	14 km S	67°48´	32°47´	630	220	21.6	6.5	611.6	158.5	76 ± 6
5	36 km S	67°38´	32°46´	275–600	155–210	1.1	0.1	87.3	26.3	44 ± 4
6	47 km S	67°34´	32°35´	40–50	20–25	1.7	0.6	92.3	28.4	20 ± 2

[a] After Barkan *et al.* (1993); humus layer, data for 1988–1990; regional background levels are 15–40 µg g^{-1} Ni, 15–20 µg g^{-1} Cu.
[b] M. Kozlov, unpubl.; humus layer, data for 1995; levels at least polluted site: < 0.1 µg g^{-1} Ni, 0.3 µg g^{-1} Cu, 176.8 µg g^{-1} Ca, 41.8 µg g^{-1} Mg.
[c] M. Kozlov and E. Haukioja, unpubl.; data for 1994; concentrations estimated using passive lead-dioxide absorbers (method described by Barkan 1993); levels at the least polluted sites: 0.5–2.4 µg m^{-3}.

background level of environmental pollution.

The branches were cut with a sharp knife, placed in plastic bags containing a small amount of water, transported to the laboratory, and planted in plastic pots filled with ca 150 ml of unpolluted fertile soil (collected from a pea field near the town of Apatity). The cuttings were provided with sufficient water and grown in the laboratory for two weeks. The rooted cuttings were planted on 5 July 1995 at site 4; the survival of these cuttings was monitored on 6 August 1995, 16 June 1996, 22 June 1997 and 1 August 1998.

Sensitivity of cuttings to heavy metals

In the second experiment, we investigated the sensitivity of cuttings to heavy metals in relation to the site where the cuttings were collected (heavily polluted site 2 *versus* slightly polluted site 5), as well as to the sex and leaf size of the parental genets. Two cuttings (diameter 3–5 mm, length ca. 20 cm) were collected on 8 June 1997 from each of the 25 genets of *S. borealis* tagged at sites 2 and 5. The length of the largest leaf (usually the fourth, counting from the base of the annual shoot) was measured with a ruler, to the nearest 1 mm, on 20 July 1997, in ten shoots randomly chosen from the mid-crown of these genets and averaged for the genet-specific value.

In the laboratory, the cuttings were placed in 50 ml plastic vials. One of two cuttings from each clone was given clean water (taken from the Belaya River near Apatity, which is almost completely free of metal contamination from the Severonikel smelter); the other was given a solution of nickel and copper sulphates (10 mg l^{-1} Ni and 4 mg l^{-1} Cu) in the same water. The vigour of the cuttings was scored on 20 June as follows: dead (score 0), alive but poor appearance (score 1), or healthy-looking (score 2); the length of the fourth leaf was measured on five shoots of both live cuttings and the parent bushes.

Effects of soil and atmospheric environment on the establishment of cuttings

A two-way factorial experiment was established to test the relative importance of soil and atmospheric environment for the survival and vigour of the willows. Cuttings were taken from five clones of *S. borealis* growing on a slightly contaminated site (no. 5); in this experiment, clones were considered as replications. Cuttings (diameter 3–8 mm) were sampled on 8 June 1997; by this date the willow buds were approaching 15–20 mm in length, but the leaves were still folded on nearly all the bushes. We attempted to take branches with a minimum number of flower buds. The cuttings were planted within 3 hours after collection.

The soil was sampled on 7–8 June 1997 at sites 1, 3, 5 and 6. Within each site we combined 5 subsamples (ca. 2 kg each) taken from the A horizon at distances ca 10 m from each other, thoroughly mixed the site-specific sample, and filled plastic pots (20 per site) with ca. 150 ml of soil. The soil type (podzolic Al-Fe-humus soils) was similar at all the study sites, but the soil under pine-dominated forest (the sites 1 and 6) had lower concentrations of mineral nutrients, primarily calcium and magnesium (Lukina and Nikonov 1996).

The 80 pots were then distributed among the four sites in such a way that each site had five pots with soil from each of the four sites, and the five pots with an identical soil origin had cuttings from different willow clones (one cutting per clone).

The twenty pots at each site were randomly arranged in a block of 4 × 5 pots in an area ca. 3 m^2. The pots were inserted in the soil so that the rims of the pots were ca. 2 cm above ground level. In watering the cuttings, we used water from local ponds; each watering equalled about 10 mm rainfall. The cuttings were watered at the time of planting, the next day, and then every other day for ten days.

In surveys conducted on 22 June, 6 July, and 24 July 1997, all the cuttings were classified as either alive or dead. On 22 June, we measured the length of the fourth leaf on five shoots on both alive cuttings and the parent bushes.

Statistical analysis

Since the distributions of our data deviated from the normal, we used the non-parametric Kruskal-Wallis test (SAS NPAR1WAY procedure) for comparisons between the treatments, and calculated chi-square statistics for frequency data (SAS FREQ procedure). Spearman rank correlation coefficients for the scored data and Pearson correlation coefficients for the normally distributed measurements of leaf length were calculated using the SAS CORR procedure (SAS Institute 1990).

Results

Establishment of cuttings in a favourable site

Altogether 52 of the 120 cuttings (43.3%) rooted in the laboratory and started to develop leaves; the successful cutting rate varied among the parental genets from 0% to 100% (Fig. 2). At the end of the growing season in 1995, one half (26) of the rooted cuttings planted in the moderately polluted birch and willow-dominated community (site 4, *see* Fig. 1) had survived; 24 cuttings were still alive in summer of 1996, and 23 cuttings in the summers of 1997 and 1998. This gives an overall success rate of 19.2% in relation to the initial number of cuttings, with a survival rate of 0% to 50% for cuttings from different genets (Fig. 2).

Sensitivity of cuttings to heavy metals

The cutting mortality in Cu and Ni-contaminated water was four times higher than that in clean water (0.50 and 0.12, respectively; $\chi^2 = 19.9$, df = 1, $P < 0.0001$). Roots were formed by eight cuttings grown in clean water, but by none of the cuttings in metal-contaminated water ($\chi^2 = 8.70$, df = 1, $P = 0.003$). However, neither the vigour score ($\chi^2 = 0.24$, df = 1, $P = 0.63$) nor leaf length ($\chi^2 = 0.38$, df = 1, $P = 0.54$) of the survivors were affected by Cu and Ni.

Differences in survival between the metal-treated and control cuttings were more evident in willows originating from the heavily polluted site 2 ($\chi^2 = 17.2$, df = 1, $P < 0.0001$) than in cuttings from the slightly polluted site 5 ($\chi^2 = 5.89$, df = 1, $P = 0.053$). Metal-treated cuttings from female genets had slightly better vigour than those from male genets (sites 2 and 5 pooled; $\chi^2 = 3.77$, df = 1, $P = 0.05$).

In the case of willows from the heavily polluted site 2, there was no correlation between cutting vigour and leaf length of the parental genet ($r_S = 0.04$, $n = 25$ genets, $P = 0.84$). The vigour of cuttings from the less polluted site 5, on the other hand, decreased with increasing leaf length of the parental genets ($r_S = -0.64$, $n = 25$, $P = 0.0006$). Survival was not related to cutting diameter ($\chi^2 = 0.00$, df = 1, $P = 0.95$), but the length of cuttings which died during the experiment was ca 0.8 of the length of the surviving cuttings ($\chi^2 = 4.35$, df = 1, $P = 0.04$).

The length of leaves produced by the cuttings did not depend on the leaf length of their parental genets at either site 2 ($r = 0.23$, $n = 24$ genets, $P = 0.29$) or site 5 ($r = 0.06$, $n = 25$, $P = 0.76$).

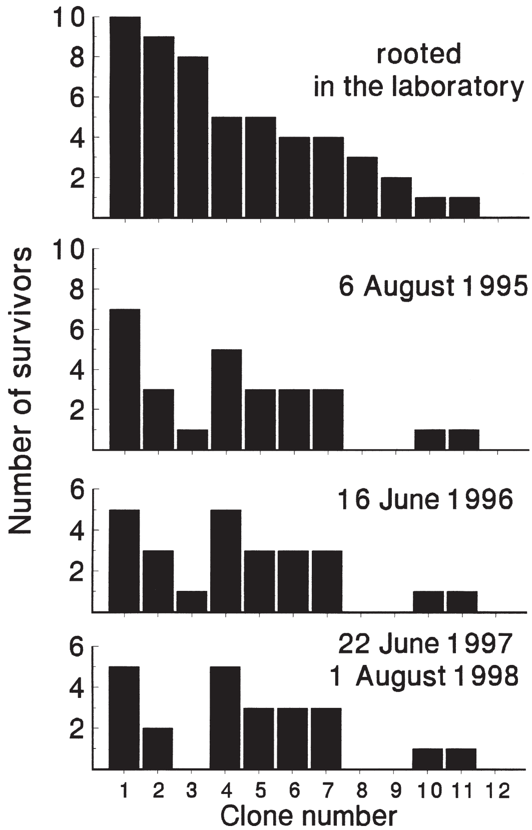

Fig. 2. Clone-specific survival of *S. borealis* cuttings at the birch- and willow-dominated transitional community (site 4) during the growing seasons in 1995–1998.

Effects of soil and atmospheric environment on the establishment of cuttings

The effect of soil origin on cutting survival was significant ($P < 0.01$) in all three field surveys,

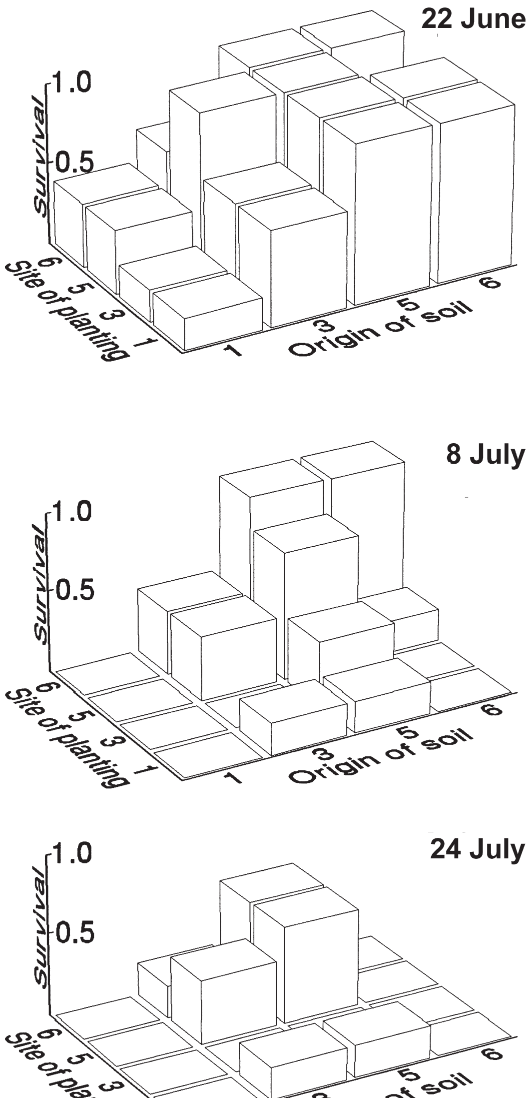

Fig. 3. Treatment-specific survival of *S. borealis* cuttings in a two-way factorial field experiment in 1997 in relation to soil origin and exposure site. Soils from sites 3 and 5 are more fertile than from 1 and 6; soils from sites 1 and 3 were strongly contaminated with heavy metals; airborne pollution was high at sites 1 and 3. For more details on soil quality and pollution loads, *see* Table 1.

although the pattern of survival changed during the study period (Fig. 3). Differences in survival between highly contaminated soils (sites 1 and 3) and relatively unpolluted ones (sites 5 and 6) were greatest in the first survey (22 June: 50% and 92.5%, respectively; $\chi^2 = 20.3$, df = 1, $P < 0.0001$), intermediate in the second survey (6 July: 12.5% and 45%, respectively; $\chi^2 = 12.3$, df = 1, $P = 0.0005$) and not significant in the third survey (24 July: 10% and 17.5%, respectively; $\chi^2 = 0.95$, df = 1, $P = 0.33$). In contrast, differences between soil originating from pine-dominated areas (sites 1 and 6) and spruce-dominated ones (sites 3 and 5) increased over time from the beginning of the experiment (22 June: 57.5% and 85%, respectively; $\chi^2 = 6.27$, df = 1, $P < 0.01$; 6 July: 15% and 42.5%, respectively; $\chi^2 = 6.27$, df = 1, $P < 0.01$; 24 July: 0% and 27.5%, respectively; $\chi^2 = 12.8$, df = 1, $P = 0.0004$).

The survival of cuttings exposed to highly contaminated ambient air (sites 1 and 3) and relatively unpolluted air (sites 5 and 6) was similar in the first survey (22 June: 70% and 72.5%, respectively; $\chi^2 = 0.25$, df = 1, $P = 0.62$). However, marked differences appeared in the second survey (6 July: 10% and 47.5%, respectively; $\chi^2 = 12.3$, df = 1, $P = 0.0005$), and were still pronounced in the third survey (24 July: 5% and 22.5%, respectively; $\chi^2 = 5.16$, df = 1, $P = 0.02$).

Neither soil toxicity ($\chi^2 = 2.01$, df = 1, $P = 0.16$), soil origin in terms of forest type ($\chi^2 = 0.24$, df = 1, $P = 0.62$), nor airborne contamination ($\chi^2 = 0.53$, df = 1, $P = 0.47$) affected the length of leaves produced by the cuttings. Among-clone variation in leaf length was significant ($\chi^2 = 13.8$, df = 4, $P = 0.0081$), but there was no correlation between the mean length of leaves produced by the cuttings and by their parent bushes ($r = 0.40$, $n = 5$ clones, $P = 0.51$).

Discussion

Although willows do not play an important role in the natural succession leading to the re-establishment of coniferous forests in our study area (Pushkina 1960), they can be used to slow down soil erosion at heavily disturbed sites (Kulagin 1982, 1991). The planting of willows could be especially relevant within industrial barren areas where the natural establishment of woody plants has not been observed during the past decade. Even if emissions of metals and sulphur dioxide from the Severonikel smelter were to cease, which is in fact a highly unlikely scenario, the existing level of soil toxicity will still prevent the establishment of Scots pine seedlings within an area of at least 200 km^2 (Kozlov 1997). It may take several decades before restoration of the climax community in this area begins.

Willows are successful colonists in the natural recovery of metal-contaminated areas around the Sudbury smelter in Canada (Winterhadler 1995), but they have not been used in the Canadian reclamation programme (Lautenbach *et al.* 1995). *S. borealis* was among the first plants which were (unsuccessfully) used for re-greening the industrial barrens around the Severonikel smelter in the mid-1970s (Kryuchkov and Liseenko 1988). Since then, three willow species (*Salix caprea* L., *S. triandra* L. and *S. phylicifolia* L.) have been recommended for the recultivation of the industrial barrens adjacent to the Severonikel smelter (Tsvetkov 1987, Tsvetkov and Chekrisov 1987, Tsvetkov *et al.* 1987). However, *S. triandra* does not belong to the native flora of the Murmansk region (Ramenskaya 1983) although it can withstand the local climate (Kazakov *et al.* 1993). One of two native willow species from this list, *S. phylicifolia*, is generally associated with moist habitats. It is relatively uncommon in heavily polluted areas (Zvereva *et al.* 1995b) and practically absent in the most severely devastated barren landscapes (M. Kozlov, unpubl.). Another native species, *S. caprea*, belongs to the group of tree-like willows, whose cuttings are generally less suitable for planting than the cuttings of bush-like willows (Kulagin 1991).

Cuttings of *S. borealis*, the species selected for our experiments due to its high resistance to pollutants and its bush-like growth form, demonstrated a moderate establishment ability in a favourable environment. The first field experiment suggested that cutting survival can be enhanced by proper choice of parental genets.

The effects of metal-contaminated soil and water on *S. borealis* cuttings were clearly negative; in the two-way factorial field experiment, these adverse effects were most pronounced within two weeks after planting. Combined with findings concerning the inhibition of root growth by nickel in several plant species (reviewed by Temp 1991), our results suggest that the rooting of *S. borealis* is disturbed by metal pollutants. This may explain the fact that only large willow bushes were found in the industrial barrens: seedlings had presumably been unable to establish themselves already decades ago, after the concentrations of metals accumulating in the soil began to exceed the toxicity limit. However, once a cutting has become successfully established, its subsequent survival does not seem to depend on soil toxicity.

Within-species variation in sensitivity to pollutants has been reported in a number of plants (Houston 1982, Macnair 1987). This variation has formed the basis for the appearance of metal-resistant populations in some grasses (reviewed by Macnair 1987, Winterhalder 1995), and it has been hypothesised that resistant genotypes of woody plants may also be "naturally" selected in a contaminated environment (Sinclair 1969, Houston 1982, Zvereva *et al.* 1997). However, cuttings from the heavily polluted site were even less resistant to metals than those from the relatively unpolluted site, suggesting that the survival of extant individuals of *S. borealis* in the industrial barrens did not correlate with the ability of cuttings taken from these genets to cope with metal toxicity.

Leaf length is considered to be one of the characteristics reflecting plant vigour, and the voluminous literature (Ensen 1982, Alexeeva-Popova 1991, Kozlowski *et al.* 1991) demonstrates that the long-lasting impact of different pollutants, including SO_2 and heavy metals, causes a reduction in the leaf size and shoot growth of woody plants. However, this was not the case with *S. borealis* growing along the Severonikel pollution gradients (Zvereva *et al.* 1997). Consistently with our earlier field observations, we did not find any effect of pollution on leaf length in the experimental cuttings.

Rather surprisingly, the leaf length of the cuttings was independent of the leaf length of their parental genets in both the laboratory and field experiments. Since pollution had no effect on leaf length either, these findings suggest a significant interaction between genotype and environment, e.g. a genet-specific response to contamination. However, our data do not allow the testing of this hypothesis.

Assuming that leaf size at the individual genet level indicates the intensity of the growth and differentiation processes, which are most easily destroyed by metal pollutants (Temp 1991), the better survival of cuttings collected from parental genets with small leaves may have resulted from their inherent slow growth rate. However, only one of the two populations involved in the laboratory experiment with solvents of heavy metals demonstrated this regularity, making further generalisation premature.

The differential sensitivity of male and female genets to pollutants has previously been reported for several willow species (Kulagin 1982). In this respect, *S. borealis* is similar to *S. viminalis* L.: in these species, female genets are more resistant to pollution. However, this is not always the case for willows, since in *S. cinerea* L. male plants were more resistant than female genets (Kulagin 1982).

In field experiments conducted in 1980–1984 around the Severonikel smelter, willow cuttings were planted in soils ameliorated with peat, cattle manure, limestone and fertilizers, which assured the establishment and long-term survival of some of the cuttings (no quantitative data available) at a heavily polluted site (Tsvetkov and Chekrisov 1987). Our experiment indicate that soil amelioration is not obligatory: by the end of the field observations in 1997, the mean survival rate of cuttings in the heavily contaminated soil from site 3 was 20%, which did not differ from the 35% survival in the less contaminated soil of the same type collected from site 5. Furthermore, this rate was similar to the survival of cuttings in 1995 on the relatively fertile soil (compare Table 1) at the moderately contaminated site 4 (Fig. 2). Thus the natural content of mineral nutrients in the soil from spruce-dominated forest appeared to be sufficient for the establishment of cuttings at the prevailing levels of metal contamination.

The establishment of cuttings can be improved by planting them in unpolluted soil. Successfully rooted cuttings may be transferred to industrial barrens along with a clod of clean soil. It may take up to four or five years before the metal concentrations in the transferred soil reaches the level characteristic of heavily polluted sites (Evdokimova and Mozgova 1993). This time is presumably sufficient for cutting establishment.

Conclusion

Some simple measures can assure the better performance of cuttings used for the partial revegetation of heavily polluted barren soils. Although limitations imposed by small sample sizes and short observation periods do not allow us to draw practical recommendations for reforestation measures, our findings suggest that cuttings should be collected from small-leaved female genets of *S. borealis* at an unpolluted site, grown for the first year in 0.5 to 1 l pots with uncontaminated, nutrient-rich soil at an unpolluted site, and then planted at the industrial barrens early the next season. The suggested method partly eliminates the financial obstacles which are critical for the practical rehabilitation of areas damaged by emissions from the Severonikel smelter, and can serve as the easiest "minimal intervention" (Skaller 1981) aimed at promoting the natural revegetation of industrial barrens.

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