Effects of radioactive contamination on Scots pines in the remote period after the Chernobyl accident

**Stanislav Geras'kin · Alla Oudalova · Nina Dikareva ·
Sergey Spiridonov · Thomas Hinton · Elena Chernonog ·
Jacqueline Garnier-Laplace**

Abstract A 6 year study of Scots pine populations inhabiting sites in the Bryansk region of Russia radioactively contaminated as a result of the Chernobyl accident is presented. In six study sites, ^{137}Cs activity concentrations and heavy metal content in soils, as well as ^{137}Cs, ^{90}Sr and heavy metal concentrations in cones were measured. Doses absorbed in reproduction organs of pine trees were calculated using a dosimetric model. The maximum annual dose absorbed at the most contaminated site was about 130 mGy. Occurrence of aberrant cells scored in the root meristem of germinated seeds collected from pine trees growing on radioactively contaminated territories for over 20 years significantly exceeded the reference levels during all 6 years of the study. The data suggest that cytogenetic effects occur in Scots pine populations due to the radioactive contamination. However, no consistent differences in reproductive ability were detected between the impacted and reference populations as measured by the frequency of abortive seeds. Even though the Scots pine populations have occupied radioactively contaminated territories for two decades, there were no clear indications of adaptation to the radiation, when measured by the number of aberrant cells in root meristems of seeds exposed to an additional acute dose of radiation.

Introduction

The impact of environmental pollution on the structure and function of ecosystems is a primary concern to environmental scientists, as well as to society in general. Release of toxic pollutants into the environment may have dramatic effects on ecosystems and influence not only biodiversity and community organization, but also the sustainable development of natural resources (Rands et al. 2010).

Forests cover 30% (4 billion hectares) of the Earth's land surface (FAO 2005) and are of immense ecological importance. Investigations over the last several decades (Mankovska and Steinnes 1995; Kozlowski 2000; Kozlov and Zvereva 2007) have demonstrated that forest ecosystems are vulnerable to human-induced environmental stresses, resulting in a progressive loss of biodiversity and general declines in forest health. Adverse impacts to the forest ecosystems lead to concomitant changes in the quality and extent of wildlife habitat (Galbraith et al. 1995). Such studies indicate a large potential for anthropogenically caused changes in forest environments. Over time, such losses will have more dramatic ecological and economic consequences.

Contemporary forests are subjected to many physical, chemical and biological stresses. Radiation is a form of stress which elicits community responses frequently similar to those resulting from other forms of stress (Whicker and Fraley 1974). At the ecosystem level, the direct effects of radiation on individual plants become intertwined with the indirect effects of many multiple interactions and secondary

S. Geras'kin (✉) · A. Oudalova · N. Dikareva ·
S. Spiridonov · E. Chernonog
Russian Institute of Agricultural Radiology and Agroecology,
Kievskoe shosse, 109 km, 249032 Obninsk, Russia
e-mail: stgeraskin@gmail.com

T. Hinton · J. Garnier-Laplace
Institute of Radioprotection and Nuclear Safety, Cadarache,
Bldg 159, BP 3, 13115 St Paul lez Durance Cedex, France

effects. As a result, the changes observed in a forest following irradiation are caused not only by radiation per se, but also by the inherent nature of forest ecosystem. Therefore, considerable insight into the basic nature of forest ecosystems and their ability to withstand or recover from stress can be obtained through observations of irradiated forest.

As a result of radiation accidents in the South Urals of Russia in 1957 and at Chernobyl in 1986, large forested areas were severely affected by radioactive contamination. Mass mortality of pine trees in these territories represent a striking example of radiation damage at the ecosystem level, and provide convincing proof of enhanced sensitivity of forest ecosystems to radiation exposure in comparison with other ecosystem types (Alexakhin et al. 2004). Coniferous trees generally have a high retention capacity and low turn-over rate for atmospherically dispersed contaminants. Indeed, the radionuclide interception fractions for pine stands were 2–3 times greater than for stands of deciduous trees (Ipatyev et al. 1999). As a result, the average contamination density of forests was found to be approximately 2–30% higher than that of adjacent open fields (Tikhomirov and Shcheglov 1994) and the normalized (per unit of ^{137}Cs deposition) Chernobyl-related γ-dose rate in forest locations significantly exceeds that of any location outside the forest (Ramzaev et al. 2008).

Conifers are particularly vulnerable, as predicted by Sparrow and Woodwell (1962) and confirmed in the course of field experiments with acute γ-irradiation of woody plants in the Brookhaven Laboratory (Sparrow et al. 1968) and in the South Urals (Alexakhin et al. 1994).

One of the major difficulties in the implementation of an ecological risk assessment is a lack of knowledge about the effects from chronic, low-level exposures to contaminants. The acute phytotoxic effects of many environmental factors are well known, but the effects of long-term chronic exposure to low pollutant concentrations is neither well understood nor adequately included in risk assessments. There is growing evidence (Theodorakis 2001; Peterson et al. 2003) that sublethal effects of chronic exposure to environmental contaminants led to population-level impacts. Such pressures may push many pine taxa beyond their tolerance thresholds. Although low dose rates may be obtained in the laboratory by protraction, these may not adequately represent true environmental radiation exposures. To properly understand the effects of normal, real-world contaminant exposures, one must consider actual field conditions. However, for most wildlife groups and endpoints there are very few studies that link accumulation, chronic exposure and biological effects in natural settings (Garnier-Laplace et al. 2004).

A primary goal of ecotoxicology is to relate effects manifested in individuals to changes in population size or structure. Considerable efforts have been spent searching for biomarkers of effects that might provide risk managers an early warning of pending damage. Cytogenetic monitoring is thought to be promising since cytogenetic tests are sensitive enough, may integrate the impacts of harmful agents in the environment, and provide meaningful information on biological damage. Documentation of cytogenetic effects holds such promise that the International Commission on Radiological Protection has recommended it as an endpoint in determining population level impacts to non-human biota exposed to radiation (ICRP 2009). It was hypothesized that cytogenetic abnormalities caused cell death in gametes and subsequent decrease in reproductive potential. Few studies, however, have actually linked cytogenetic damage to other endpoints known to have a direct impact on population levels, such as mortality or reproductive output (Anderson and Wild 1994; Hinton and Bréchignac 2005).

The Chernobyl accident caused dramatic and long-term increases in ambient radiation doses to many forest environments. Sites still exist in the Bryansk region of Russia, 20 years after the Chernobyl accident, where radioactive contamination significantly exceeds background. Long-term observations of coniferous tree populations within areas contaminated from radiation accidents present a unique opportunity to obtain quantitative data on the biological consequences of chronic radiation exposure including trends and dynamics of adaptation processes. The present study follows preceding investigations (Geras'kin et al. 2003, 2005) that showed the frequency of cytogenetic abnormalities in root meristems of germinated Scots pine seeds may be used to quantify pollution-induced stress, not only in areas with prominent damage to the conifers, but also in forests with slight or no visible symptoms of an impact. Such research is likely to increase in importance since very few studies have been carried out on natural populations exposed to chronic radiation at levels slightly higher than background. A better understanding of the long-term response of stressed forest ecosystems is vital for improving ecological risk assessment and management, developing protective measures, and preventing further forest degradation.

The aims of this study were to examine whether Scots pine trees have experienced stress in areas with relatively low levels of radioactive contamination and, if so, to quantify such an impact. Additionally, tests were run to determine if adaptation to the chronic exposure has occurred in the Scots pine populations that have occupied radioactively contaminated territories for decades.

Materials and methods

Test organism

Scots pine (*Pinus sylvestris* L.), the dominant tree species in North European and Asian boreal forests, was chosen as

a test organism for an assessment of the possible effects from the radioactive contamination. Scots pines are widespread in the area affected by the Chernobyl accident, and sampling can be obtained from trees growing in different contamination levels. The reproductive organs of coniferous trees, with their complex organization and long maturation period (Cairney and Pullman 2007), are among the most sensitive to damaging effects from a wide range of anthropogenic contaminants (Kozubov and Taskaev 1994; Micieta and Murin 1998).

Study sites

Four radioactively contaminated sites (VIUA, SB, Z1, and Z2) were chosen in 2003 at the territories of the Novozibkovskiy, Klintsovskiy and Krasnogorskiy districts of the Bryansk region (Table 1) about 200 km north-east of the Chernobyl nuclear power plant (Fig. 1). This area was significantly contaminated by the Chernobyl fallout with an initial ^{137}Cs ground deposition level more than 1 MBq/m^2 in some locations (Ramzaev et al. 2008). At the beginning of our studies (2003), the contribution from radionuclides other than ^{137}Cs to the radioactive contamination was negligibly small (Ipatyev et al. 1999). The γ-radiation dose rates were measured at the study sites every year with a DRG-01T dose rate meter ("Leninez", Russia), at a height of 1 m above ground. All dose rate values expressed in Roentgen units initially were converted to Gray units (Gy) by using a multiplication factor of 8.76×10^{-3} (Mashkovich 1982). Dose rates at the study sites ranged from 0.37 to 1.21 μGy/h, compared to 0.10 μGy/h at control sites (Table 1). Latitude and longitude records were made with a geographical positioning system (GPS). The soils of the study sites are sandy podzolic with similar granulometric composition, chemical and physical properties, and nutrition availability.

The reference sites (Ref and Ref1) were located in the Vigonichskiy and Unechskiy districts of the Bryansk region. They were selected based on their proximity to the impacted area and similarity of environmental properties. The secondary control, Ref1, was taken to test possible heterogeneity in populations unrelated to the radioactive contamination.

Collection of plant and soil samples

Pine cones were collected in November–December of 2003 through 2008 from 20 to 50 years old Scots pine trees. At each site, 30–50 cones were taken from each of 20–29 trees, at a height of 1.5–2.0 m above soil surface, within homogenous stands where pine trees were abundant. The cones were kept outside for maturing through the end of February, then moved into a laboratory and stored at room

Table 1 Location and exposure dose rate at the study sites in the Bryansk region of Russia

Notation	Location	Exposure dose rate (μGy/h)
Ref	Vigonichskiy district, N 53°1′ E 33°55′	0.10 ± 0.01
Ref1	Unechskiy district, N 52°46′ E 32°39′	0.09 ± 0.01
VIUA	Novozibkovskiy district, N 52°29′ E 31°50′	0.37[a] ± 0.03
SB	Klintsovskiy district, N 52°33′ E 31°44′	0.49[a] ± 0.16
Z1	Krasnogorskiy district, N 53°5′ E 31°42′	1.21[a] ± 0.23
Z2	Krasnogorskiy district, N 53°5′ E 31°42′	0.73[a] ± 0.18

[a] Difference from the reference sites Ref and Ref1 is significant ($p < 5\%$)

temperature under reduced humidity until dehiscing and seed removal. Seeds were de-winged manually.

In this work data on radioactive and chemical contamination determined in 2008 are presented. γ-Dose rates were measured under every tree from which cones were collected. Soil samples were taken from every site at a depth of 0–5, 5–10 and 10–15 cm at locations with the highest γ-dose rate level.

Measurements of radionuclides and heavy metals in soil and plant samples

Soil samples were air-dried and then passed through a 1 mm sieve. Basic chemical and physical properties of soils were measured in accordance with ISO/IEC 17025 (2005) (the general requirements for the competence to carry out tests and/or calibrations, including sampling). One randomly selected cone from each tree was taken to form a pooled sample of cones from each study site. Activity concentrations of ^{137}Cs in cones as well as ^{137}Cs, ^{40}K, ^{226}Ra, and ^{232}Th in soil samples were determined with γ-spectrometry using a desktop program-controlled MCA InSpector-1270 with coaxial germanium detector and spectroscopy software GENIE-2000 (Canberra Industries, USA). Minimal measurable activity concentrations at the 1 h measurement duration were 2, 10, 10, and 40 Bq/kg for ^{137}Cs, ^{226}Ra, ^{232}Th, and ^{40}K, respectively. To determine ^{90}Sr activity concentrations cones were ashed at 600°C and ^{90}Sr was extracted from a double treatment with boiling solution of 6 M HCl. Quantification limit was 0.05 Bq at the 1800 s measurement duration.

Total concentrations of heavy metals (Cd, Cu, Co, Cr, Mn, Ni, Pb, and Zn) were measured in soils following

Fig. 1 Location of the study sites
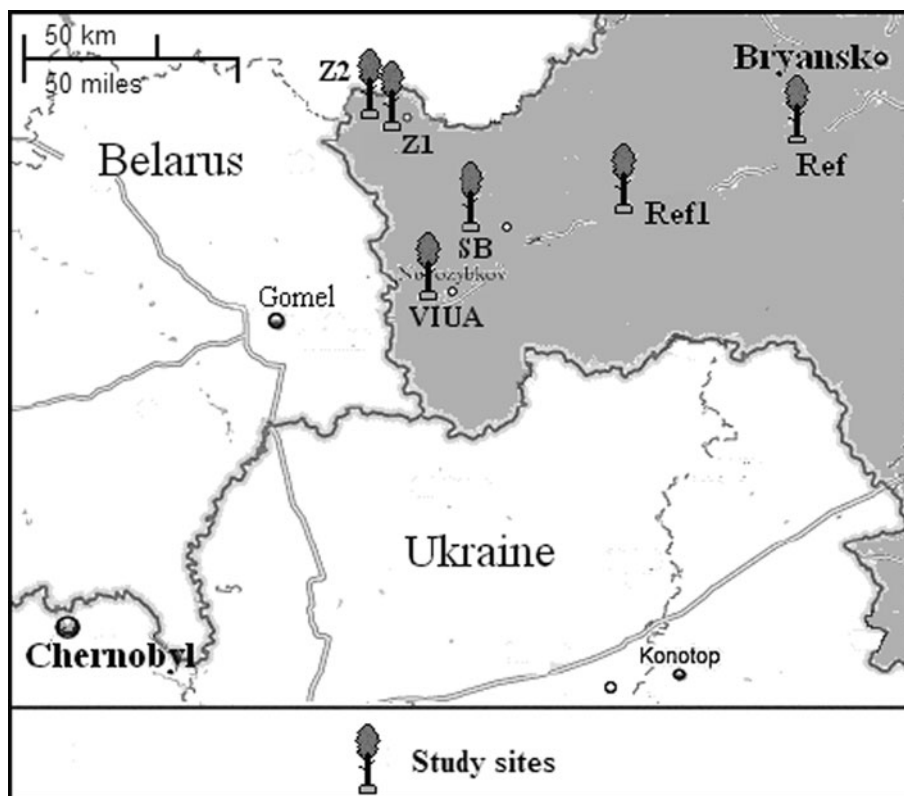
treatments with a HNO_3, HCl, and HF mixture according to Obuhov and Plehanova (1991). Metals content of cones were determined from samples ashed at 500°C. Metals were extracted from the ashed samples using 35% nitric acid. Concentrations of heavy metals in solution were determined using plasma optical emission spectrometer ICP-OES Liberty II (Varian, Australia/USA) in accordance with ISO 11047 (1998). Quantification limit was 1–10 μg/l.

Dose assessment

To calculate the radiation dose absorbed by reproduction organs (cones) of pine trees, a dosimetric model was developed. Figure 2 illustrates the conceptual scheme of the model. Several layers were defined (crown, under crown, and three soil layers at various depths). Each layer was treated as an infinite thick source. A uniform distribution of radionuclides within each layer was also assumed. To calculate the absorbed dose to the cones from γ-ray emitting radionuclides in a particular soil layer, the above layers were considered as shielding and attenuated a portion of the gamma energy. Doses were calculated for cones located within the "crown" layer.

The Taylor form for an accumulation factor was applied to take account for multiple scattering of radiation by the upper layers (Mashkovich 1982):

$$B(E_0, \mu d) = A \exp(-\alpha_1 \mu d) + (1 - A) \exp(-\alpha_2 \mu d), \quad (1)$$

where E_0—energy of γ-quantum, μ—linear attenuation factor, d—layer thickness, A, α_1 and α_2—constants.

The following equations were used to calculate the γ-dose rate from radionuclides located in the i-layer of soil (Spiridonov et al. 2008):
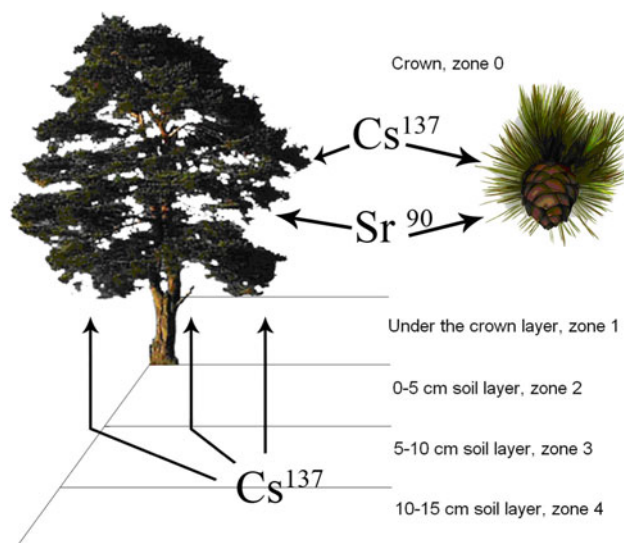

Fig. 2 Scheme of radionuclide-containing layers considered in an assessment of dose absorbed by generative organs of pine trees

$$D_i^\gamma = \frac{2\pi\Gamma\rho_s q_v^i}{\rho_i\mu_s}\left\{ E_2^*\left[\frac{\mu_s}{\rho_s}\left(\rho_0\Delta h_0 + \sum_{k=1}^{i-1}\rho_k h_k\right)\right] \right.$$
$$\left. - E_2^*\left[\frac{\mu_s}{\rho_s}\left(\rho_0\Delta h_0 + \sum_{k=1}^{i}\rho_k h_k\right)\right]\right\}, \quad (2)$$

$$E_2^*(z) = \frac{A}{1+\alpha_1}E_2[(1+\alpha_1)z] + \frac{1-A}{1+\alpha_2}E_2[(1+\alpha_2)z], \quad (3)$$

where Γ—gamma constant, q_v^i—^{137}Cs activity concentration in the i-layer, ρ_i—medium density in the i-layer, ρ_s—air density, μ_s—linear attenuation factor for γ-radiation in air, h_k—thickness of the k-layer, Δh_0—distance between the location of reproduction organs of pine trees and the upper limit of the zone 1, E_2—the King function (Mashkovich [1982]).

To calculate the γ-rays dose rate from radionuclides located in the tree crown, Eq. 2 was transformed to:

$$D_0^\gamma = \frac{2\pi\Gamma\rho_s q_v^0}{\mu_s\rho_0}\left\{ 2\left(\frac{A}{1+\alpha_1} + \frac{1-A}{1+\alpha_2}\right) \right.$$
$$\left. - E_2^*\left[\mu_s\frac{\rho_0}{\rho_s}(h_0 - \Delta h_0)\right] - E_2^*\left[\mu_s\frac{\rho_0}{\rho_s}\Delta h_0\right]\right\}, \quad (4)$$

where q_v^0—radionuclides activity concentration in crown, ρ_0—medium density, h_0—thickness of the crown layer.

To estimate β-radiation dose rate, data on the ^{137}Cs and ^{90}Sr (^{90}Y) activity concentrations in cones were utilized. Since a depth of β-particles penetration into biological tissue is much less than a cone size, the equation for an infinite source was used:

$$D_\beta = k\bar{E}_\beta q_\beta, \quad (5)$$

where $\bar{E}_\beta$—average energy of β-quantum, q_β—concentration of β-emitters, k—constant.

Analysis of reproduction potential

To estimate reproductive potential of the pine tree populations the numbers of abortive seeds within the cones were determined. All seeds available from the cones sampled were used. Numbers of both normal (matured) and abortive (hollow) seeds per cone were scored by visual inspection. Small seeds and seeds with morphological abnormalities, such as shrunken and irregular-shaped, were counted separately.

Cytogenetic analysis

For cytogenetic analysis, only seeds of good quality were used. Quality was determined by ease of removal from the cones and visual inspection. Seeds were soaked for 24 h in distilled water at 4°C in the dark to synchronize cell division and provide evenness of swelling at the beginning of germination. Seeds were germinated on damp filter paper in Petri dishes at a controlled temperature of 24°C. Preliminary studies (Geras'kin et al. [2003]) indicated that the peak incidence of first mitoses appears at a root length of about 7–10 mm, and that second mitoses occur at a length > 20 mm.

The seedling roots at the stage of first mitosis were fixed in a 1:3 solution of acetic acid and alcohol. Preparations of root apical meristems were made and stained with aceto-orcein. All slides were coded and technicians determined the cytogenetic damage blinded to the field site. In 20–100 slides, all the ana-telophase cells (4400–11500 cells per site per year) were scored to calculate the fraction of cells with cytogenetic alterations. Chromosomal aberrations in ana-telophases (chromatid (single) and chromosome (double) bridges and fragments) were distinguished from mitotic abnormalities (multipolar mitoses and lagging chromosomes).

Irradiation of seeds

Portions of the seeds collected at the study sites in 2003, 2004 and 2006 were acutely irradiated with γ-rays (15 Gy, 0.6 Gy/min) of ^{60}Co (Lutch Irradiator, Latenergo, Latvia) at room temperature. After irradiation, seeds were immediately submitted for germination. 10–24 slides were prepared of the root tips from seedlings, and 1000–4500 ana-telophase cells per site, per year, were scored for cytogenetic alterations.

Data statistical analysis

To assess aberrant cell frequency with a confidence probability of 95% at relative probable error of $\varepsilon = 20$–25%, a method of sample volumes optimisation was used in an interactive manner (Geras'kin et al. [1994]). The experimental data were checked for outliers, according to Sachs ([1972]) and Barnett and Lewis ([1984]). The F-test was used to test a hypothesis on the absence of differences between plots in physical–chemical properties and nutrition availability in the soils as well as levels of their contamination with radionuclides and heavy metals. To determine the significance of the difference between the sample mean values, the Student's t-test for independent variance was applied. MS Excel 2002 and MathCad 13 were used for calculations. In Tables and Figures, mean $\pm$ S.E. values are given.

Results

Radionuclides and heavy metals in soil and plant samples

The effects of environmental contaminants may be modified by associated factors such as the pH or nutritional

status of the soil (Dueck et al. 1987). Soil pH was low in all samples and ranged from 4.02 to 5.06 (Table 2), with the two reference soils having the extreme values. Nutrient concentrations and soil physical parameters are typical of the Central region of Russia (Kovda and Zyrin 1981). There are certain variations in physical and chemical properties of the soils, which is difficult to avoid in field studies. Thus, the cation exchange capacity, organic matter content and some mineral elements were somewhat higher at the SB, Z1, and Ref sites compared to other sites. The differences in soil properties from the reference sites, however, appeared significant only for the Z1 site ($p < 5\%$), as revealed with F-test.

Content of Cd, Cu, Pb, Zn, Ni, and Mn (Table 3) were below the corresponding allowable concentrations of chemicals in soils adopted in Russia (Hygienic standards 2006). F-test showed no significant difference between the reference and impacted sites in composition of chemical contaminants levels in soils, or in pine cones. Concentrations of the mobile forms of metals in soils from the study sites were previously analyzed (Geras'kin et al. 2008), and no violations of the maximum permissible concentrations were found for all tested metals (Co, Cr, Mn, Ni, Pb, and Zn). Ratios of chemical concentrations in cones and soils, C/S (Table 3) for Cu, Cd, Zn, and Ni amount to 0.5–0.8 which is up to 2 orders of magnitude higher than C/S = 0.003–0.03 for Pb, Co, and Cr. Moreover, at the VIUA site, Cu concentrations in cones were twice of that of the soil. There are not found significant difference between the sites in content of heavy metals in cones, according to F-test. In total, the data in Tables 2 and 3 confirm that the study sites did not differ substantially in soil properties, or chemical content of soil or pine cones.

Radionuclide concentrations, however, varied by orders of magnitude among the study sites (Table 4). Activity concentrations of radionuclides generally decreased with soil depth, suggesting that radioactive fallout was the initial source of the contamination. The contrast between these sites in radioactive contamination provides an ideal opportunity for in situ validation of the presumed mutagenic hazard. The main contribution to the radioactive contamination was from ^{137}Cs, with activity concentrations at the contaminated sites (averaged through 0–15 cm soil layer) ranging from 2.4 to 56.3 kBq/kg of soil. These activity concentrations exceed those at the Ref site by factors of 47-1081. Moreover, the ^{137}Cs content in soils at the SB, Z1 and Z2 sites surpassed the minimum significant activity of 10 kBq/kg issued in the Russian Radiation Safety Norms (2009). F-test revealed differences between the soils from the reference and contaminated sites on radionuclide levels that were significant in 22 of 24 paired comparisons ($p < 5\%$). In cones from the impacted populations, radionuclides activity concentrations were

Table 2 Physical–chemical properties and nutrition availability in soils of the study sites

Study site	pH$_{KCl}$	Humus (%)	N (%)	P$_2$O$_5$ (%)	K (%)	Ca (%)	Mg (%)	Cation exchange capacity, mg-equivalent/100 g	Hydrolytic acidity (mM/100 g)	P$_{Ref}$	P$_{Ref1}$
Ref	5.06 ± 0.06▲	2.05 ± 0.02▲	0.28 ± 0.01▲	0.082 ± 0.001▲	1.16 ± 0.01▲	0.32 ± 0.01▲	0.42 ± 0.01▲	19.88 ± 0.12▲	6.18 ± 0.20▲		0.43
Ref1	4.02 ± 0.03*	2.78 ± 0.03*	0.35 ± 0.01*	0.059 ± 0.003*	0.58 ± 0.02*	0.11 ± 0.01*	0.06 ± 0.01*	12.03 ± 0.65*	11.00 ± 0.70*	0.43	
VIUA	4.14 ± 0.02*▲	1.42 ± 0.03*▲	0.33 ± 0.01*	0.050 ± 0.002*	0.47 ± 0.01*▲	0.10 ± 0.01*	0.07 ± 0.01*▲	10.12 ± 0.14*	9.97 ± 0.13*▲	0.26	0.73
SB	4.02 ± 0.05*	6.93 ± 0.05*▲	0.72 ± 0.01*▲	0.085 ± 0.001*	0.52 ± 0.01*	0.17 ± 0.02*▲	0.09 ± 0.01*▲	21.70 ± 0.32*▲	20.4 ± 0.01*▲	0.38	0.10
Z1	4.76 ± 0.02*▲	11.60 ± 0.08*▲	1.36 ± 0.01*▲	0.218 ± 0.013*▲	0.74 ± 0.01*▲	0.54 ± 0.03*▲	0.11 ± 0.01*▲	48.50 ± 1.23*▲	28.9 ± 0.82*▲	0.01	0.00
Z2	4.60 ± 0.01*▲	4.53 ± 0.02*▲	0.29 ± 0.01	0.055 ± 0.002*	0.56 ± 0.01*	0.17 ± 0.05	0.07 ± 0.01*	10.34 ± 0.09*	7.50 ± 0.09*	0.17	0.54

* and ▲ significant difference from the Ref and Ref1 sites, respectively ($p < 5\%$)

P$_{Ref}$ and P$_{Ref1}$—significance level at testing of difference between the given plot and Ref or Ref1, respectively, according to F-test

Table 3 Concentrations of heavy metals in soils and pine cones

Study site	Element concentration, mg/kg								p_{Ref}	p_{Ref1}
	Cd	Cu	Co	Cr	Mn	Ni	Pb	Zn		
Soils										
Ref	0.045 ± 0.003▲	6.48 ± 0.04	8.43 ± 0.46▲	44.37 ± 1.95▲	323.5 ± 13.8▲	15.26 ± 0.66▲	9.08 ± 0.76	42.18 ± 2.28▲		0.45
Ref1	0.059 ± 0.006	3.42 ± 2.55	1.74 ± 0.22*	6.39 ± 0.56*	233.9 ± 2.9*	2.15 ± 0.01*	6.94 ± 0.36	18.77 ± 3.36*	0.45	
VIUA	0.058 ± 0.008	0.93 ± 0.07*	2.12 ± 0.02*	8.32 ± 0.22*	224.7 ± 3.7*	2.65 ± 0.04*▲	7.22 ± 0.08	14.76 ± 0.29*	0.39	0.92
SB	0.087 ± 0.007*▲	2.51 ± 0.21*	2.21 ± 0.10*	10.66 ± 0.52*	248.7 ± 0.5*▲	4.22 ± 0.27*▲	13.84 ± 0.08*▲	23.63 ± 0.30*	0.53	0.89
Z1	0.124 ± 0.016*	3.60 ± 0.23*	2.85 ± 0.01*	10.64 ± 1.33*	349.8 ± 5.3▲	4.46 ± 0.08*▲	7.34 ± 0.13	23.89 ± 0.30*	0.79	0.31
Z2	0.110 ± 0.009*	1.56 ± 0.07*	1.22 ± 0.01*	5.31 ± 0.06*	246.2 ± 37.0	2.65 ± 0.12*▲	7.16 ± 0.09	18.74 ± 0.21*	0.53	0.89
TAC (MAC)	0.5	33	–	–	1500	20	32	55		
Cones										
Ref	0.026 ± 0.007	1.38 ± 0.12	0.106 ± 0.008▲	0.015 ± 0.015	13.9 ± 1.1▲	3.48 ± 0.21▲	0.165 ± 0.025	9.21 ± 0.74▲		0.21
Ref1	0.026 ± 0.001	1.62 ± 0.17	0.021 ± 0.00*7	0.046 ± 0.019	23.6 ± 2.4*	2.40 ± 0.17*	0.295 ± 0.140	13.16 ± 0.24*	0.21	
VIUA	0.049 ± 0.004▲	2.15 ± 0.05	0.092 ± 0.005▲	0.007 ± 0.006	26.7 ± 0.3*	3.59 ± 0.12▲	0.312 ± 0.052	10.72 ± 0.15*▲	0.15	0.85
SB	0.039 ± 0.001▲	1.48 ± 0.06	0.061 ± 0.010*▲	0.017 ± 0.012	24.4 ± 0.9*	1.82 ± 0.19*	0.592 ± 0.302	10.06 ± 0.15*▲	0.21	0.98
Z1	0.032 ± 0.003	1.27 ± 0.08	0.047 ± 0.006*▲	0.023 ± 0.019	28.4 ± 3.7*	2.47 ± 0.16*	0.163 ± 0.020	8.79 ± 0.14*▲	0.12	0.74
Z2	0.030 ± 0.004	1.40 ± 0.03	0.058 ± 0.004*▲	0.023 ± 0.011	20.6 ± 0.5*	2.44 ± 0.11*	0.144 ± 0.100	9.58 ± 0.16*▲	0.39	0.67
C/S, (mg/kg)/(mg/kg)	0.47 ± 0.09	0.81 ± 0.32	0.027 ± 0.006	0.003 ± 0.001	0.088 ± 0.011	0.77 ± 0.18	0.031 ± 0.005	0.49 ± 0.08		

* and ▲ significant difference from the Ref and Ref1 sites, respectively ($p < 5\%$)

p_{Ref} and p_{Ref1}—significance level at testing of difference between the given plot and Ref or Ref1, respectively, according to F-test

TAC tentative allowable concentrations of chemicals in sandy and loamy sandy soils (Hygienic standards, 2006); for Mn, MAC (maximum allowable concentration in soil) is given

C/S ratio for heavy metal concentrations in cones and in soil, averaged through the study sites

Table 4 Activity concentrations of radionuclides in soils and pine cones

Study site	Depth (cm)	Radionuclides in soils (Bq/kg)				p_{Ref}	p_{Ref1}	Radionuclides in cones (Bq/kg)	
		^{137}Cs	^{40}K	^{226}Ra	^{232}Th			^{137}Cs	^{90}Sr
Ref	0–5	73.2 ± 9.6	518 ± 92	17.2 ± 3.9	30.2 ± 5.3		0.33	30.0 ± 3.7▲	1.32 ± 0.65
	5–10	39.7 ± 4.6	560 ± 86	19.0 ± 8.6	36.1 ± 6.0		0.24		
	10–15	43.5 ± 4.9	537 ± 84	23.6 ± 5.7	40.5 ± 3.9▲		0.38		
Ref1	0–5	170 ± 15.8*▲	275 ± 51	12.9 ± 2.0	11.7 ± 4.9	0.33		10.3 ± 2.1*	1.07 ± 0.71
	5–10	128 ± 12.2*▲	271 ± 51	12.8 ± 3.4	11.6 ± 1.9	0.24			
	10–15	52.6 ± 5.6	310 ± 56	15.8 ± 4.1	11.0 ± 3.6*	0.38			
VIUA	0–5	1570 ± 136.6*▲	210 ± 47	12.1 ± 3.8	9.4 ± 2.8	0.09	0.02	948 ± 13*▲	16.79 ± 1.23*▲
	5–10	3925 ± 404.3*▲	404 ± 92	11.6 ± 5.8	11.0 ± 5.2	0.01	0.00		
	10–15	1813 ± 190.4*▲	307 ± 52	38.1 ± 21.3	8.0 ± 2.6*	0.07	0.01		
SB	0–5	16900 ± 2061.8*▲	250 ± 48	24.8 ± 6.2	52.5 ± 14.0	0.00	0.00	342 ± 8*▲	20.81 ± 1.31*▲
	5–10	10040 ± 1024.1*▲	620 ± 127	11.0 ± 6.6	26.8 ± 7.1	0.00	0.00		
	10–15	3698 ± 384.6*▲	454 ± 115	29.0 ± 12.8	20.0 ± 8.2	0.01	0.00		
Z1	0–5	39190 ± 3997.4*▲	290 ± 65	54.3 ± 22.8	49.1 ± 24.9	0.00	0.00	3246 ± 32*▲	64.25 ± 2.20*▲
	5–10	38510 ± 3928*▲	383 ± 65	34.3 ± 12.2	58.4 ± 30.1	0.00	0.00		
	10–15	30780 ± 3139.6*▲	248 ± 69	99.0 ± 40.4	42.2 ± 29.5	0.00	0.00		
Z2	0–5	96900 ± 8721*▲	383 ± 173	30.0 ± 13.2	34.7 ± 26.0	0.00	0.00	1008 ± 18*▲	83.0 ± 2.19*▲
	5–10	42400 ± 5172.8*▲	258 ± 49	30.8 ± 8.6	10.1 ± 2.6	0.00	0.00		
	10–15	29700 ± 3623.4*▲	253 ± 47	15.0 ± 9.0	56.0 ± 10.6	0.00	0.00		

* and ▲ significant difference from the Ref and Ref1 sites, respectively ($p < 5\%$)

p_{Ref} and p_{Ref1}—significance level at testing of difference between the given plot and Ref or Ref1, respectively, according to F-test

Table 5 Estimated doses to generative organs of pine trees

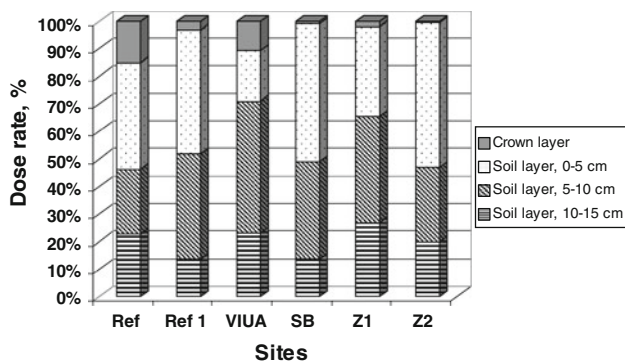| Study site	D_γ (mGy/year)	D_β (mGy/year)	D_{sum} (mGy/year)
Ref	0.124	0.013	0.137
Ref1	0.267	0.006	0.273
VIUA	6.62	0.344	6.96
SB	22.7	0.156	22.9
Z1	90.2	1.20	91.4
Z2	129.4	0.506	129.9

Fig. 3 The relative contribution from the radionuclide-containing layers to the total dose rate in the generative organs of pine trees, 2008

significantly higher than in samples from the Ref and Ref1 sites (Table 4). Maximum activity concentrations of ^{137}Cs and ^{90}Sr exceeded the reference level (Ref1 site) by a factor of 315 and 78, respectively. ^{137}Cs content in cones exceeded that of ^{90}Sr by at least one order of magnitude.

Doses absorbed by reproduction organs of pine trees

Data on activity concentrations of radionuclides in soils and cones, sampled in 2008, were used for the dose estimation. At the most contaminated Z2 site the annual dose to cones was 130 mGy (Table 5). According to our estimates, β-radiation contributed approximately 0.4–9.3% to the total dose at the impacted study sites. The greatest part of the dose absorbed by pine cones was due to ^{137}Cs located in the upper 10-cm soil layer. Among all the compartments, the aboveground biomass contained the lowest concentrations of radionuclides. The model predicts that radionuclides located in the crown deliver a lower dose to cones than radionuclides located in the upper soil layers. The contribution to the dose formed by the crown-located radionuclides is mainly made by β-radiation. The relative contributions of different layers to the total dose received by reproduction organs of pine trees are presented in Fig. 3.

Table 6 Aberrant cell frequency and alterations spectrum in root meristem of germinated seeds collected from pine trees at the study sites

Year	Study site	No. seedlings	AT-cells	No. alterations	AC (%)	No. different types of alterations		
						f' + m'	f'' + m''	g + mp
2003	Ref	102	11494	123	0.87 ± 0.07	14	49	60
	VIUA	47	6579	109	1.35 ± 0.13**	20*	45	44
	SB	53	8707	130	1.27 ± 0.09***	23	47	60
	Z1	38	6070	121	1.66 ± 0.12***	15*	53***	53*
	Z2	41	6752	118	1.68 ± 0.15***	14	53**	51*
2004	Ref	84	9525	98	0.74 ± 0.07	13	34	51
	VIUA	52	8043	145	1.58 ± 0.13***	19*	67***	59
	SB	44	6937	104	1.31 ± 0.10***	18	40*	46*
	Z1	37	5699	122	1.66 ± 0.12***	12	47**	63***
	Z2	46	6778	134	1.76 ± 0.16***	12	69***	53*
2005	Ref	49	11457	99	0.82 ± 0.06	48	21	30
	VIUA	48	9615	150	1.60 ± 0.15***	62	27*	61***
	SB	25	5593	75	1.23 ± 0.12***	28	7	40***
	Z1	19	4403	77	1.65 ± 0.17***	19	21*	37***
	Z2[a]	–	–	–	–	–	–	–
2006	Ref	36	9117	96	0.97 ± 0.09	35	31	30
	Ref1	30	9262	82	0.86 ± 0.06	32	20	30
	VIUA	27	7769	102	1.37 ± 0.10***▲▲▲	27	29	46*▲▲
	SB	24	7818	97	1.24 ± 0.10*▲▲	35	34▲	28
	Z1	24	8007	123	1.56 ± 0.09***▲▲▲	36	35▲▲	52***▲▲▲
	Z2	24	8055	121	1.63 ± 0.13***▲▲▲	33	39▲	49**▲▲▲
2007	Ref	30	7638	75	0.96 ± 0.08	38▲▲	9▲▲	28
	Ref1	28	7487	70	0.93 ± 0.08	16**	27**	27
	VIUA	30	7503	92	1.22 ± 0.12▲	25*	26*	41
	SB	28	6854	83	1.29 ± 0.10*▲▲	7***	25**	51***▲▲▲
	Z1	24	7413	108	1.50 ± 0.16**▲▲	15*	33***	60**▲▲
	Z2	27	6620	104	1.68 ± 0.13***▲▲▲	18*	35**	51**▲▲▲
2008	Ref	30	7583	72	0.82 ± 0.13	19	27	26
	Ref1	37	9006	75	0.74 ± 0.05	24	22	29
	VIUA	28	7642	90	1.16 ± 0.11*▲▲	19	22	49*▲▲
	SB	27	7591	85	1.14 ± 0.11▲▲	4**▲▲▲	31	50**▲▲
	Z1	28	7678	105	1.39 ± 0.14**▲▲▲	17	26	62**▲▲▲
	Z2	27	6176	95	1.44 ± 0.12***▲▲▲	4*▲▲	41*▲▲▲	50***▲▲▲

AT ana-telophases scored, *AC* aberrant cell frequency, *g* chromosome laggings, *mp* multipolar mitoses, *f'*, *m'* chromatid fragments and bridges, *f''*, *m''* chromosome fragments and bridges

Significant difference from the frequency at the Ref site: * $p < 5\%$, ** $p < 1\%$, *** $p < 0.1\%$

Difference from the frequency at the Ref1 site: ▲ $p < 5\%$, ▲▲ $p < 1\%$, ▲▲▲ $p < 0.1\%$

[a] In 2005 there was impossible to collect enough seeds for cytogenetic analysis at the Z2 site

Cytogenetic effects in pine tree populations

In the study reported herein, cytogenetic effects in pine populations have been investigated for 6 years, and the findings are presented in Table 6. There were no significant differences in frequencies of cytogenetic abnormalities, observed at the same study site from year to year, except for some data associated with 2004: (i) aberrant cell occurrence in the Ref site in 2004 was lower than in 2007, and (ii) at the VIUA site there were differences between 2004 and 2007, as well as 2004 and 2008. The distinctions found are significant at the statistical level of 5% but not 1%.

Aberrant cell frequencies in root meristems of germinated seeds from the impacted populations exceeded the corresponding reference levels in all years of the study (Table 6). The highest frequencies of aberrant cells were
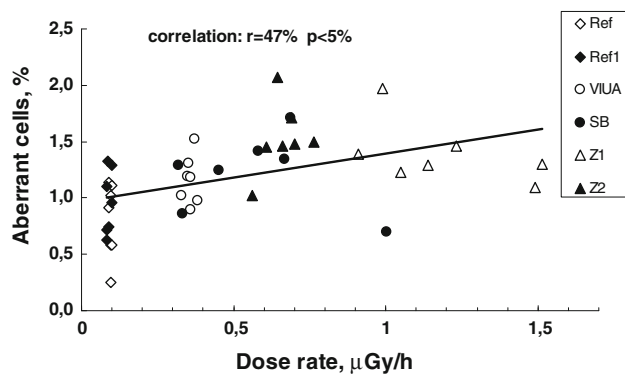
Fig. 4 Average level of aberrant cells frequency within a tree and exposure dose rate measured under that tree, 2008
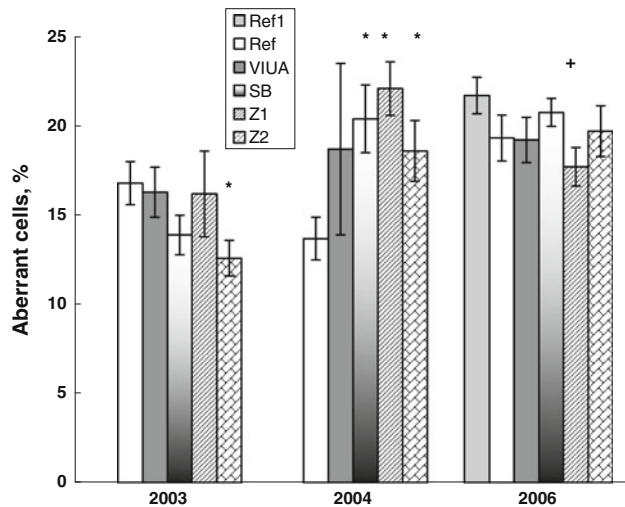

Fig. 5 Aberrant cell frequency in root meristem of Scots pine seedlings after acute exposure of seeds to 15 Gy of γ-rays. *, Difference from the Ref site is significant ($p < 5\%$); +, difference from the Ref1 site is significant ($p < 5\%$)

found in seedlings from the most contaminated Z1 and Z2 sites. There was significant correlation ($r = 0.47$, $p < 5\%$) between an average level of aberrant cells frequency within a tree and exposure dose rate measured under that tree (Fig. 4). Cytogenetic abnormalities in the impacted populations exceeded the levels observed in both reference populations at the significance of $p < 5\%$; except between the Ref and VIUA sites in 2007 was $p = 7\%$, and between the Ref and SB sites in 2008 was $p = 6\%$. Also, the occurrence of cytogenetic abnormalities in 2006–2008 increased with the dose absorbed ($r = 0.83–0.93$, $p < 5\%$) and ^{137}Cs specific activity in soil ($r = 0.84–0.93$, $p < 5\%$). For aberrant cell occurrence in 2003–2005, there was also a tendency to increase with the estimated dose, but it was not significant. It is not surprising, however, since doses absorbed by reproduction organs of pines (Table 5) were estimated from the data on radionuclide activities in soils and cones measured in 2008, and they would vary in the
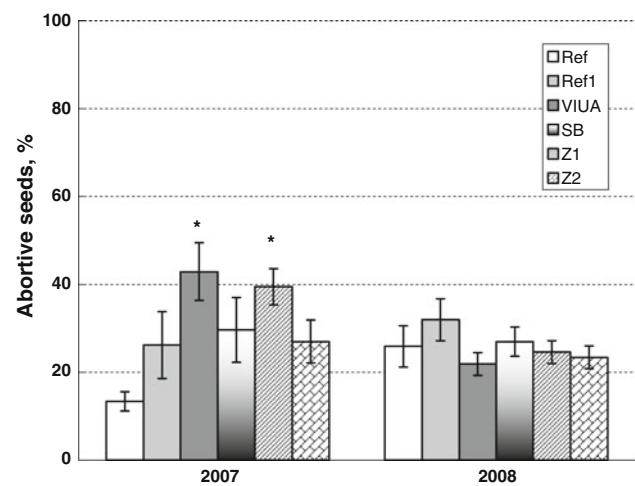

Fig. 6 Proportion of abortive seeds in the Scots pine populations at the study sites. *, Difference from the Ref is significant, $p < 5\%$

course of time because of radioactive decay and migration of radionuclides. In our previous contribution (Geras'kin et al. 2008) doses to pine tree cones were estimated from data on radionuclides activities measured in 2003, and a significant correlation between frequency of aberrant cells and dose was shown. Overall, the data show that chronic, low level radiation exposure results in an increase in cytogenetic abnormalities in Scots pine populations.

Spectrum of cytogenetic alterations

In the Scots pine populations at the most contaminated sites (Z1 and Z2) the occurrence of severe types of cytogenetic alterations, such as chromosomal aberrations (double fragments (f'') and bridges (m'')) and mitotic abnormalities (chromosome laggings (g) and multipolar mitoses (mp)) is above the corresponding reference levels during all 6 years of the study, and this excess is significant in nearly all cases (Table 6).

Test of adaptation to radiation exposures

There was not found a reliable increase in radioresistance of the seeds from the chronically exposed Scots pine populations to an additional acute γ-ray exposure (Fig. 5). Therefore, the test used failed to reveal any significant distinction in adaptive ability between populations experiencing different levels of radiation exposure.

Reproductive ability

In Fig. 6 the proportion of abortive seeds is presented as an indicator of reproductive potential in the Scots pine populations. In 2007, frequency of abortive seeds in the impacted populations was higher than in the reference

ones, and this enhancement was even significant when comparing the VIUA and Z1 sites to the Ref (but not Ref1) site. However, in 2008 there was no effect of chronic exposure on reproductive ability.

Discussion

Forest stands are usually long-lived, and over time can accumulate large amounts of biomass thereby acting as a biological sink for radionuclides. Pine trees readily take up many radionuclides from the soil; pines have the third largest soil-to-plant transfer factor for ^{90}Sr and ^{137}Cs among woody plants, after aspen and birch (Ipatyev et al. 1999). Pine trees used in our study have been growing on radioactively contaminated territories for over 20 years; therefore, a high concentration of radioactivity is expected in both vegetative and reproductive organs of these trees. As a whole, the activity concentrations of both ^{137}Cs and ^{90}Sr in the cones from the study sites increased as a function of radioactive contamination in the soil (Table 4). Some exceptions, however, occurred. For example, ^{137}Cs activity concentrations in cones collected from the SB and Z2 sites did not correlate to a radioactive contamination in the soil. We suspect that this relates to significant spatial heterogeneity of radioactive contamination in the study sites. Moreover, soil samples in 2008 were taken under the most contaminated trees to get maximum estimation of radiation impact within the site. So, the calculated doses refer to the upper border of possible radiation exposure. In addition, there are also biological processes that change radionuclides distributions within plants. Thus, a high internal mobility of ^{137}Cs from the older tissues (2-year-old cones, for example) to physiologically active organs is typical for trees (Thiry et al. 2009) and the dynamics can be influenced by a number of factors, including competition with stable nutrient analogs, resulting in an inverse relationship between stable potassium and ^{137}Cs uptake; and between stable calcium and ^{90}Sr uptake.

Generally, ^{90}Sr uptake by trees is 5–10 times higher than that of ^{137}Cs because the mobile fraction of ^{90}Sr in soil is higher than ^{137}Cs (Ipatyev et al. 1999). Despite the higher mobility, however, we found ^{90}Sr specific activity in cones to be 1–2 orders of magnitude less than that of ^{137}Cs (Table 4), because of considerably lower ^{90}Sr content in the soil.

One of the most difficult aspects of any field study is a choice of an appropriate control. In this study, two reference sites were selected to account for problems related to spatial heterogeneity, as well as to have an additional source of reference samples in case of failure at one of the sites due to poor cone harvest, fire, timber harvesting, etc. The reference sites were not completely identical, of course. At the Ref site, the content of some mineral elements (K, Mg) and heavy metals (Cu, Co, Cr, Zn) in soil was higher than in the Ref1 and other test sites. Their levels, however, were within the range typical for soils of this region (Kovda and Zyrin 1981), and well below the maximum allowable concentrations adopted in Russia. On the other hand, the Ref1 site did not differ from others in nutrient content, soil physical–chemical properties and metal concentrations, but had two times higher ^{137}Cs activity concentration than the Ref site. Still, this ^{137}Cs level does not exceed the background variations from the global fallouts and is below the activity value at the least contaminated VIUA site by a factor of >20.

Estimated doses absorbed in reproduction organs of pine trees 22 years after the Chernobyl NPP accident (Table 5) were about thirty times lower than the 0.4 mGy/h guideline proposed by IAEA as safe for terrestrial plants (IAEA 1992). On the other hand, dose rates at the most contaminated Z1 and Z2 sites exceeded the ERICA generic predicted no-effect value of 10 μGy/h, applicable as an incremental dose rate in addition to the background for generic ecosystems (Andersson et al. 2009). This comparison suggests that radiation levels at the study sites were high enough to induce cytogenetic rather than morphologic abnormalities in the exposed populations. Indeed, the aberrant cell frequency, as well as the occurrence of severe types of cytogenetic alterations in root meristems of germinated seeds collected from the impacted populations, exceeded the reference level during all six years of study (Table 6).

A parallel study of allozymic variation in the same pine populations (Geras'kin et al. 2010) revealed that several indexes of genetic variability (heterozygosity, frequency of polymorphic loci, number of phenotypes), as well as frequencies of mutations for loss of enzymatic activity significantly increased with dose absorbed by reproduction organs of the trees. In total, these data suggest that a high level of mutation occurs in the progeny of these pine populations and that their genetic diversity is conditioned by radiation exposure.

Although great progress has been made in understanding the nature of mutations, too little is yet known about the way in which mutations can lead to observable effects in life traits of organisms and populations. A high level of mutational variability could result in an acceleration of microevolutionary processes in the populations. Because plants lack a germ line, epigenetic and genotypic changes are readily generated and selected in the soma and can be transmitted to progeny (Walbot 1996). In particular, this fundamental property underlies the remarkable phenotypic and genotypic plasticity of plants, and their elasticity to rapidly changing environments, including high levels of man-made pollution (Macnair 1993).

The effect of severe stress on populations is often thought to eliminate the most susceptible individuals

(Pitelka 1988; Prus-Glowacki et al. 1999). However, an alternative effect is to change the number and vitality of offspring produced by individuals (Valladares et al. 2007). In many wild plant species, especially trees, the selection pressure of pollution has led to the natural evolutionary development of tolerant plant genotypes in response to wide range of various pollutants (Dickinson and Turner 1991). It is true that a much larger number of seeds are produced than that which develop into adult plants, and that the changes in frequency of the different genotypes are due to a greater death of some genotypes than others. In such a way, a greater percentage of tolerant offspring would be expected from trees subjected to pollutants. This is a form of response to selection, and a very powerful one.

Reproduction is a key life history trait linking individual responses to population effects and is considered to be more sensitive to radiation exposures than the endpoint of mortality (IAEA 1992). In the first year after the Chernobyl accident a significant decrease in reproductive ability of pines (reduction of seed mass and their number per cone, as well as increase in portion of abortive seeds) was observed at doses over 1 Gy (Ipatyev et al. 1999; Fedotov et al. 2006). Eleven years after the accident this tendency still persisted. In 1997, the portion of abortive seeds from pine populations that had received doses of 10–20 Gy in 1986 significantly exceeded the corresponding reference level (Fedotov et al. 2006). The effect of radioactive contamination on reproductive ability of pine trees was also observed at the South Urals radioactive trail. Chronic exposure of pine trees at dose rates of 4.2–6.3 μGy/h resulted in a significant decline of seed mass, as well as an increase in the fraction of abortive seeds (Kalchenko and Spirin 1989). At a lower dose rate of 0.8 μGy/h, the enhancement in percent of abortive seeds was not observed. Even 15 years after the accident, in 2000–2001, decrease in pollen viability as well as increase of the number of anomalous pollen grains in Scots pine populations from the Bryansk region were detected at dose rates of 1.8–5.4 μGy/h (Scock et al. 2005). In contrast to the results mentioned above, we failed to find any clear and stable linkage between reproductive ability and doses absorbed by reproduction organs of pine trees (Fig. 6). So, the increased cytogenetic abnormalities found in this study (Table 6) had no effect on the reproductive ability of the exposed populations.

An appearance of some standing factors (either of natural origin or man-made) in the plants' environment may activate genetic mechanisms, changing a population's resistance to a particular stress. Laboratory studies of repair inhibitors, dose–effect relationships for low- and high-LET radiations, measurements of unscheduled DNA synthesis and an efficacy of the single strand breaks recovery (Shevchenko et al. 1992) suggest that the divergence of

populations in terms of radioresistance is connected with a selection for changes in the effectiveness of the repair systems. Another study on the possible mechanisms of adaptations to radioactive contamination (Kovalchuk et al. 2004) showed extremely low (more than 10-fold) recombination level, and a higher level of global genome methylation in chronically irradiated plants that may have prevented extensive genome rearrangements. Although our understanding of mechanisms of adaptation is far from complete, these studies give good evidence that the response of biological systems to stress have been produced by normal evolutionary processes in relation to the environments in which the populations occur. This is, of course, a wasteful system since metabolic energy has to be used to produce the adaptive trait even when it is not required. Actually, increased fitness in unfavorable environments is associated (Hickey and McNeilly 1975) with decreased fitness in favorable environments. As a result, there are situations (Hoffmann and Hercus 2000) when resistance to environmental changes has not evolved or has not persisted. Moreover, adaptation is often observed in one species but not found in others, despite equivalent opportunity and exposure conditions. Indeed, at the South Urals radioactive trace, radioresistance increased 3–4 times in radiosensitive plants, but remained practically unchanged in radioresistant species (Shevchenko et al. 1992). It seems that if we examine radio-adaptation in nature we find as much evidence of it is not occurring, as we do of it is existing. It quickly becomes obvious that the role of microevolutionary processes in a natural population's response to low-level chronic exposure is still not clearly understood.

The response of a population exposed to low dose rate irradiation depends on both the type of organism and the biophysical properties of radiation (relative biological effectiveness, linear energy transfer, dose rates etc.). Contrary to the increased radioresistance of seeds from plant populations inhabiting radioactively contaminated territories described in Shevchenko et al. (1992) and Kalchenko and Fedotov (2001), the findings of this study do not testify to increased resistance of seeds collected from the impacted Scots pine populations to the subsequent γ-ray exposure (Fig. 5). Similarly, acute γ-irradiation of seeds from a wild vetch population that had been inhabiting a site with enhanced levels of natural radioactivity in the north of Russia for long time, resulted in a rather high radiosensitivity in the assays of "frequency of cytogenetic disturbances in meristematic root tip cells", "rate of germination" and "survival rate at the end of vegetation period" (Geras'kin et al. 2007). An improved DNA repair capacity and ability to germinate under abiotic stresses (salinity and accelerated ageing) was shown in seed embryos of evening primrose growing near the Chernobyl

NPP on sites contaminated with γ- and β-emitters, while on the α-, β- and γ-contaminated site such an improvement was not found (Boubriak et al. 2008). This is in line with data on the successful adaptation of wild vetch populations living on sites most highly contaminated by β-emitters, but not by α-emitters (Syomov et al. 1992). Consequently, there are good theoretical and practical reasons for more attention being paid to the mechanisms by which populations become more radioresistant, and to those situations where radio-adaptation appears not to be taking place.

Twenty years after the Chernobyl NPP accident pine populations growing under chronic radiation show an increased level of cytogenetic effects in root meristems of germinated seeds that increases with radionuclide contamination. Results presented here are in agreement with those obtained in our previous studies on cytogenetic effects in Scots pine populations under chronic exposure to radionuclides (Geras'kin et al. 2003) or man-made pollution (Geras'kin et al. 2005). However, the higher rate of mutation had no effect on reproductive ability of these trees. In spite of the fact that the Scots pine populations have occupied radioactively contaminated lands for a long time, there are no clear indications of radio-adaptation when the "aberrant cells in root meristem of γ-irradiated seeds" assay was used.

The effects of chronic radiation on living organisms and populations remain poorly explored, and represent a much needed field of research as a global increase in energy production from nuclear reactors is forecast (Grimes and Nuttall 2010). Because controversy often surrounds prediction of low-dose effects in environmental risk assessment, further mechanistic research could have important implications in environmental managements. Much more is to be elucidated in our understanding before we will be able to provide realistic predictions of the biological consequences of chronic, low-level radiation exposures to natural plant and animal populations.

References

Alexakhin RM, Karaban RT, Prister BS, Spirin DA, Romanov GN, Mishenkov NN, Spiridonov SI, Fesenko SV, Fyodorov YeA, Tikhomirov FA (1994) The effects of acute irradiation on a forest biogeocenosis; experimental data, model and practical applications for accidental cases. Sci Total Environ 157:357–369

Alexakhin RM, Buldakov LA, Gubanov VA, Drozhko YeG, Ilyin LA, Kryshev II, Linge II, Romanov GN, Savkin MN, Saurov MM, Tikhomirov FA, Kholina YuB (2004) Large radiation accidents: consequences and protective countermeasures. IzdAT Publisher, Moscow, Russia

Anderson SL, Wild GC (1994) Linking genotoxic responses and reproductive success in ecotoxicology. Environ Health Perspect 102:9–12

Andersson P, Garnier-Laplace J, Beresford NA, Copplestone D, Howard B, Howe P, Oughton D, Whitehouse P (2009) Protection of the environment from ionizing radiation in a regulatory context (protect): proposed numerical benchmark values. J Environ Radioact 100:1100–1108

Barnett V, Lewis T (1984) Outliers in statistical data. Wiley, Chichester, UK

Boubriak II, Grodzinsky DM, Polischuk VP, Naumenko VD, Gushcha NP, Micheev AN, McCready SJ, Osborne DJ (2008) Adaptation and impairment of DNA repair function in pollen of *Betula verrucosa* and seeds of *Oenothera biennis* from differently radionuclide-contaminated sites of Chernobyl. Ann Bot 101: 267–276

Cairney J, Pullman GS (2007) The cellular and molecular biology of conifer embryogenesis. New Phytol 176:511–536

Dickinson NM, Turner AP (1991) How do trees and other long-lived plants survive in polluted environments? Funct Ecol 5:5–11

Dueck ThA, Tensen D, Duijff BJ, Pasman FJM (1987) N-Nutrient fertilization, copper toxicity and growth in three grass species in the Netherlands. J Appl Ecol 24:1001–1010

FAO (2005) Global forest resources assessment 2005: progress towards sustainable forest management. FAO forestry paper 147, Food and Agriculture Organization of the United Nations (FAO)

Fedotov IS, Kalchenko VA, Igonina EV, Rubanovich AV (2006) Radiation and genetic consequences of ionizing irradiation on population of *Pinus sylvestris* L. within the zone of the Chernobyl NPP. Radiat Biol Radioecol 46:268–278 (in Russian)

Galbraith H, LeJeune K, Lipton J (1995) Metal and arsenic impacts to soils, vegetation communities and wildlife habitat in Southwest Montana uplands contaminated by smelter emissions: I. Field evaluation. Environ Toxicol Chem 11:1895–1903

Garnier-Laplace J, Gilek M, Sundell-Bergman S, Larsson CM (2004) Assessing ecological effects of radionuclides: data gaps and extrapolation issues. J Radiol Prot 24:A139–A155

Geras'kin SA, Fesenko SV, Chernyaeva LG, Sanzharova NI (1994) Statistical method of empirical distribution analysis of coefficient of radionuclids' accumulation in plants. Agric Biol 1: 130–137 (in Russian)

Geras'kin SA, Zimina LM, Dikarev VG, Dikareva NS, Zimin VL, Vasiliyev DV, Oudalova AA, Blinova LD, Alexakhin RM (2003) Bioindication of the anthropogenic effects on micropopulations of *Pinus sylvestris* L. in the vicinity of a plant for the storage and processing of radioactive waste and in the Chernobyl NPP zone. J Environ Radioact 66:171–180

Geras'kin SA, Kim J, Oudalova AA, Vasiliyev DV, Dikareva NS, Zimin VL, Dikarev VG (2005) Bio-monitoring the genotoxicity of populations of Scots pine in the vicinity of a radioactive waste storage facility. Mutat Res 583:55–66

Geras'kin SA, Evseeva TI, Belykh ES, Majstrenko TA, Michalik B, Taskaev AI (2007) Effects on non-human species inhabiting areas with enhanced level of natural radioactivity in the north of Russia: a review. J Environ Radioact 94:151–182

Geras'kin SA, Dikareva NS, Oudalova AA, Spiridonov SI, Dikarev VG (2008) Cytogenetic effects in Scots pine populations from the Bryansk region radioactively contaminated as a result of the Chernobyl NPP accident. Radiat Biol Radioecol 48:584–595 (in Russian)

Geras'kin SA, Vanina JC, Dikarev VG, Novikova TA, Oudalova AA, Spiridonov SI (2010) Genetic variability in Scotch pine populations of the Bryansk region radioactively contaminated in the Chernobyl accident. Biophysics 55:324–331

Grimes RW, Nuttall WJ (2010) Generating the option of a two-stage nuclear renaissance. Science 329:799–803

Hickey DA, McNeilly T (1975) Competition between metal tolerant and normal plant populations; a field experiment on normal soil. Evolution 29:458–464

Hinton TG, Bréchignac F (2005) A case against biomarkers as they are currently used in radioecological risk analyses: a problem of linkage. In: Bréchignac F, Howard BJ (eds) Scientific trends in radiological protection of the environment. IRSN, Cadarache, France, pp 123–136

Hoffmann AA, Hercus MJ (2000) Environmental stress as an evolutionary force. Bioscience 50:217–226

Hygienic standards HN 2.1.72041-06, 2.1.72042-06 (2006) Maximum (MAC) and tentative (TAC) allowable concentrations of chemicals in soils. Rospotrebnadzor, Moscow, Russia (in Russian)

IAEA (1992) Effects of ionizing radiation on plants and animals at levels implied by current radiation protection standards. Technical reports series no. 332. International Atomic Energy Agency (IAEA), Vienna

ICRP (2009) Environmental protection: the concept and use of reference animals and plants. ICRP publications 108. International Commission on Radiological Protection (ICRP)

Ipatyev V, Bulavik I, Braginsky V, Goncharenko G, Dvornik A (1999) Forest and Chernobyl: forest ecosystems after the Chernobyl nuclear power plant accident: 1986–1994. J Environ Radioact 42:9–38

ISO 11047 (1998) Soil quality. Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc: Flame and electrothermal atomic absorption spectrometric methods. International Organization for Standardization. http://www.iso.org/iso/catalogue_detail.htm?csnumber=24010

ISO/IEC 17025 (2005) General requirements for the competence of testing and calibration laboratories. International Organization for Standardization. http://www.iso.org/iso/catalogue_detail.htm?csnumber=39883

Kalchenko VA, Fedotov IS (2001) Genetics effects of acute and chronic ionizing radiation on Pinus sylvestris L. inhabiting the Chernobyl meltdown area. Russ J Genet 37:427–447

Kalchenko VA, Spirin DA (1989) Genetics effects revealed in populations of Pinus sylvestris L. growing under exposure to small doses of chronic irradiation. Russ J Genet 25:1059–1064

Kovalchuk I, Abramov V, Pogribny I, Kovalchuk O (2004) Molecular aspects of plant adaptation to life in the Chernobyl zone. Plant Physiol 135:357–363

Kovda VA, Zyrin NG (eds) (1981) Microelements in soils of the USSR. MGU Publishers, Moscow, Russia (in Russian)

Kozlov MV, Zvereva EL (2007) Industrial barrens: extreme habitats created by non-ferrous metallurgy. Rev Environ Sci Biotechnol 6:231–259

Kozlowski TT (2000) Responses of woody plants to human-induced environmental stresses: issues, problems, and strategies for alleviating stress. Crit Rev Plant Sci 19:91–170

Kozubov GM, Taskaev AI (1994) Radiobiological and radioecological studies of woody plants. Nauka, St Petersburg, Russia (in Russian)

Macnair MR (1993) The genetics of metal tolerance in vascular plants. New Phytol 124:541–559

Mankovska B, Steinnes E (1995) Effects of pollutants from an aluminum reduction plant on forest ecosystems. Sci Total Environ 163:11–23

Mashkovich VP (1982) Shield from ionizing radiation. Energoatomizdat, Moscow, Russia (in Russian)

Micieta K, Murin G (1998) Three species of genus Pinus suitable as bioindicators of polluted environment. Water Air Soil Pollut 104:413–422

Obuhov AI, Plehanova IO (1991) Atomic-absorption analysis in soil for biologists. MGU Publishers, Moscow, Russia (in Russian)

Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB (2003) Long-term ecosystem response to the Exxon Valdez oil spill. Science 302:2082–2086

Pitelka LF (1988) Evolutionary responses of plants to anthropogenic pollutants. Trends Ecol Evol 3:233–236

Prus-Glowacki W, Wojnjcka-Poltorak A, Oleksyn J, Reich PB (1999) Industrial pollutants tend to increase genetic diversity: evidence from field-grown European Scots pine. Water Air Soil Pollut 116:395–402

Radiation safety norms (RSN-99/2009): SanPiN 2.6.1.2523-09 (2009) Ministry of Health, Moscow, Russia (in Russian)

Ramzaev V, Botter-Jensen L, Thompsen KJ, Andersson KG, Murray AS (2008) An assessment of cumulative external doses from Chernobyl fallout for a forest area in Russia using the optically stimulated luminescence from quartz inclusions in bricks. J Environ Radioact 99:1154–1164

Rands MRW, Adams WM, Bennun L, Butchart SHM, Clements A, Coomes D, Entwistle A, Hodge I, Kapos V, Scharlemann JPW, Sutherland WJ, Vira B (2010) Biodiversity conservation: challenges beyond 2010. Science 239:1298–1303

Sachs L (1972) Statistische Auswertungsmethoden. Springer-Verlag, Berlin, Germany

Scock AV, Glasoun IN, Samoshkin EN (2005) Influence of radioactive contamination on pollen viability and anomaly in Scots pine from Bryansk region. Forest J 5:7–11 (in Russian)

Shevchenko VA, Pechkurenkov VL, Abramov VI (1992) Radiation genetics of natural populations: genetic consequences of the Kyshtym accident. Nauka, Moscow, Russia (in Russian)

Sparrow AH, Woodwell GM (1962) Prediction of the sensitivity of plants to chronic gamma irradiation. Radiat Bot 2:9–26

Sparrow AH, Rogers AF, Schwemmer SS (1968) Radiosensitivity studies with woody plants. I. Acute gamma irradiation survival data for 28 species and predictions for 190 species. Radiat Bot 8:149–186

Spiridonov SI, Fesenko SV, Geras'kin SA, Solomatin VM, Karpenko YeI (2008) The dose estimation of woody plants in the long-term after the Chernobyl accident. Radiat Biol Radioecol 48:432–438 (in Russian)

Syomov AB, Ptitsyna SN, Sergeeva SA (1992) Analysis of DNA strand break induction and repair in plants from the vicinity of Chernobyl. Sci Total Environ 112:1–8

Theodorakis CW (2001) Integration of genotoxic and population genetic endpoints in biomonitoring and risk assessment. Ecotoxicology 10:245–256

Thiry Y, Colle C, Yoschenko V, Levchuk S, Hees MV, Hurtevent P, Kashparov V (2009) Impact of Scots pine (Pinus sylvestris L.) plantings on long term ^{137}Cs and ^{90}Sr recycling from a waste burial site in the Chernobyl red forest. J Environ Radioact 100:1062–1068

Tikhomirov FA, Shcheglov AI (1994) Main investigation results on the forest radioecology in the Kyshtym and Chernobyl accident zones. Sci Total Environ 157:45–57

Valladares F, Gianoli E, Gomez JM (2007) Ecological limits to plant phenotypic plasticity. New Phytol 176:749–763

Walbot V (1996) Sources and consequences of phenotypic and genotypic plasticity in flowering plants. Trends Plant Sci 1:27–32

Whicker FW, Fraley L (1974) Effects of ionizing radiation on terrestrial plant communities. Adv Radiat Biol 4:317–366