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Article

Study of needle morphometric indices in Scots pine in the remote period after the Chernobyl accident

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Abstract – Biological effects in Scots pine populations experiencing chronic radiation exposure at doses up to 130 mGy per year as a result of the Chernobyl accident were studied in 2011 and 2013, using needle indices as endpoints. No relationships between the length, the mass of needles and the asymmetry in weight of paired needles and radiation exposure were revealed. The frequency of necrotic needles increases with the level of radiation exposure; however, the significance of these effects in different years was different. The index of fluctuating asymmetry in needle length significantly increases at annual doses of 90 and 130 mGy and correlates with the absorbed dose as well as ^{137}Cs and ^{90}Sr radionuclide activities in soils and cones at the study sites. The findings obtained are consistent with an international recommendation to consider radiation exposure of 100 mGy.y^{-1} as a margin for biota safety in chronic irradiation.

Keywords: *Pinus Sylvestris* L. / chronic radiation exposure / needle / Chernobyl accident / fluctuating asymmetry

1 Introduction

Contemporary forests undergo many physical, chemical and biological stresses. Radiation is a form of stress which elicits community responses often similar to those resulting from other forms of stress (Whicker and Fraley, 1974). 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 forests.

Conifers are particularly vulnerable, as predicted by Sparrow and Woodwell (1962), and this prediction was confirmed in a number of field experiments with irradiation of woody plants in the Brookhaven Laboratory (Sparrow *et al.*, 1968) and in the South Urals (Alexakhin *et al.*, 1994). Studies of the effects of radiation exposure on Scots pine populations in the initial period after the Chernobyl accident revealed death of sprouts, dying needles, reduced reproductive capacity, chromosomal aberrations and mutations in enzyme loci (Kozubov and Taskaev, 2002; Fedotov *et al.*, 2006). In the remote period, an increased mutational and cytogenetic variability in chronically irradiated pines was shown to be significantly associated with the levels of radiation exposure (Geras'kin *et al.*, 2011; Geras'kin and Volkova, 2014). Nevertheless, any stable effects on productivity, seed survival or adaptive capacity were not identified (Geras'kin *et al.*, 2011). Moreover, information on the possible alterations in morphometric traits of pine trees in the contaminated territories in the remote period after the accident is virtually absent. This work aimed at studying biological effects under relatively low levels of chronic radiation for pine trees using needle indices as endpoints.

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2 Materials and methods

2.1 Study sites

The study sites are situated in the Bryansk Region of Russia, contaminated as a result of the Chernobyl accident. The Scots pine populations under study have been growing in the radioactively contaminated areas for more than 25 years. The study sites are similar in soil properties, the stands of trees are homogeneous, and pine trees as an edificator species take up a significant part of the phytocenosis. The heavy metal contents in soils and cones are within background levels (Geras'kin *et al.*, 2011). There were two reference populations (Ref and Ref1). Monitoring of exposure dose rates and radionuclide activity concentrations in soils and pine cones at the study sites has been provided annually since 2003. Detailed information on the radioactive contamination of the study sites can be found in (Geras'kin *et al.*, 2011). Table 1 gives general information about the main radionuclide activities in soils and cones as well as the dose and dose rate levels at the study sites

Table 1. Radionuclide activities and exposure estimated at study sites.

Site	Annual dose for pine crowns, mGy (2008)	Exposure dose rate, μGy.h^{-1} (2010)	^{137}Cs activities in soils, Bq.kg^{-1}		Activities in cones, Bq.kg^{-1}	
			0–5 cm	5–10 cm	^{137}Cs	^{90}Sr
Ref	0.1	0.09	15.3	16.2	0.94	0.7
Ref1	0.3	0.08	69.2	43.1	3.9	1.6
VIUA	7	0.30	5191	2427	427	12.7
SB	23	0.35	23521	5273	202	13.9
Z	91	1.08	38077	11540	2536	53.2
Z1	130	0.88	42717	12187	1041	53.4

in 2010. A dosimetric model was developed to calculate the total (internal+external) radiation dose absorbed by the pine crowns (Spiridonov *et al.*, 2008). For the calculation, data obtained in 2008 were used.

2.2 Sampling

Two-year-old needles were sampled from 15 trees at each of 6 study sites; 20 and 40 paired needles per tree were collected in 2011 and 2013, respectively. The length and weight of the needles were measured with an accuracy of 0.5 mm and 0.1 mg, respectively, and the necrosis rank (healthy, partially damaged or heavily damaged needle) was determined. Developmental instability was estimated via indices of fluctuating asymmetry (*FA*), calculated from length (*L*) and weight (*W*) measurements (Palmer and Strobeck, 1986; Kozlov *et al.*, 2002):

$$FA_L = 2 \times \left|\frac{L_1 - L_2}{L_1 + L_2}\right|, \quad FA_W = 2 \times \left|\frac{W_1 - W_2}{W_1 + W_2}\right|.$$

FA is defined as non-directional variation between the left and right sides of a bilateral trait, and it may arise as a result of an inability to control development under genetic and environmental stress (Møller and Swaddle, 1997). In particular, differences in the Scots pine needle length and weight were used as a sensitive indicator of pollution impact (Kozlov *et al.*, 2002). In addition, among all types of morphological abnormalities, only triple needles were observed in 2013, while in 2011 we did not estimate the presence of abnormalities.

2.3 Data statistical analysis

The significance of the difference between the sample mean values was estimated with Student's t-test for independent variance. Primary data were checked for outliers, according to the Dixon criteria. In the Tables and Figures, mean S.E. values are given.

3 Results and discussion

3.1 Length and weight of the needles

The needle length of Scots pine in radioactively contaminated areas ranged from 67.0 ± 2.8 to 80.2 ± 2.3 mm in 2011

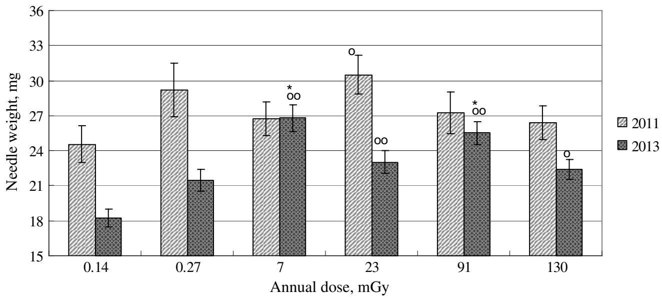

Figure 1. Needle weight in relation to the annual dose. Significant difference from Ref: ° $p < 5\%$; °° $p < 1\%$; significant difference from Ref1: * $p < 5\%$.

and from 64.8 ± 1.2 to 74.5 ± 1.7 mm in 2013. Reference values at Ref and Ref1 were 71.4 ± 2.3 and 77.6 ± 3.5 mm in 2011, and 66.9 ± 1.6 and 69.5 ± 1.5 mm in 2013, correspondingly. So, we failed to detect an increase in the needle length in response to radiation exposure ($r = -0.51$; $p > 5\%$ in 2011, and $r = -0.58$; $p > 5\%$ in 2013). An absence of changes in the needle size at annual doses of up to 130 mGy seems to be reasonable, as a prominent change in the needle size of pine in the Chernobyl NPP zone was observed at an acute dose of 0.7–1 Gy (Kozubov and Taskaev, 2002). This corresponds with Sparrow *et al.*'s (1965) observations of long-term effects of ^{60}Co irradiation on pitch pines at the Brookhaven National Laboratory. It was shown that dose rates of 2 mGy.h^{-1} and higher caused inhibition of needle growth, reduced seed production, and even tree mortality. On the other hand, exposure of one-year-old Scots pines in the range of 0.0025–0.078 mGy.h^{-1} during one growing season increased needle lengths, while 7 mGy.h^{-1} resulted in impaired seedling growth (Sheppard *et al.*, 1982).

The data on needle weight are shown in Figure 1. In 2011, radiation did not affect this variable significantly, except for the site with an annual absorbed dose of 23 mGy. In contrast, in 2013 the needle weight from all impacted sites was significantly higher than in both or at least in one of the reference populations. It is known that populations inhabiting polluted areas for a long time may show a decrease in sensitivity to acute impacts, such as changes in weather conditions. Significant changes between 2011 and 2013 in the parameters measured in the two reference populations may be explained by changes in weather conditions or other factors, whereas in

Table 2. Frequency of needles with necrotic damage.

Annual dose, mGy	Healthy, %		Partially damaged, %		Heavily damaged, %	
	2011	2013	2011	2013	2011	2013
0.1	70.2	54.2	28.7	42.8	2.8	3.0
0.3	56.3	32.0	37.8	51.3	5.8	16.7
7	68.5	72.3 °° **	28.5	26.6 °° **	3.0	1.2 **
23	54.2 °	81.1 °° **	38.8	17.3 °° **	7.0	1.7 **
91	50.0 °	42.8	41.5	44.0	8.5	13.2
130	40.5 °° *	17.8 °° *	44.2 °°	52.3	15.3 °	29.8 °°

Significant difference from Ref: ° $p < 5\%$, °° $p < 1\%$.
Significant difference from Ref1: * $p < 5\%$, ** $p < 1\%$.

Table 3. Correlation coefficients between the portion of needles damaged by necrosis and characteristics of radiation exposure, 2011.

Needle	Annual dose for pine crowns, mGy	^{90}Sr in cones	^{137}Cs in cones	^{137}Cs in soils	
				0–5 cm	5–10 cm
Healthy	–0.86*	–0.79	–0.53	–0.86*	–0.83*
Partially damaged	0.79	0.74	0.54	0.82*	0.78
Heavily damaged	0.92**	0.81*	0.46	0.86*	0.84*

* significant correlation, $p < 5\%$.

impacted populations this effect may not be so obvious because of their higher fitness for chronic stress conditions. The two-way ANOVA showed a significant effect of the sites of sampling on the weight of needles ($F = 3.41$, $p < 0.01$), but there was no significant impact of the year of sampling ($F = 2.02$, $p > 0.05$). An increase in needle weight was observed in the 30-km Chernobyl NPP zone in 1987, where 10- to 12-year-old pine trees were exposed to 9 mGy.d^{-1} (Sidorov, 1994). As shown by Kozubov and Taskaev (2002), doses below 0.1 Gy did not cause any visible damage to trees; however, at doses of 0.7–1 Gy the needle weight increased up to 153% of the control level.

3.2 Necrotic needles

Estimates of the necrotic needle proportion in the studied populations are presented in Table 2. On average, normal needles appeared in pine populations with a frequency of 18–81%, partially damaged by necrosis – 17–52%, and heavily damaged – 1–30%.

For 2011, the correlation between the portion of needles damaged by necrosis and the annual dose, ^{90}Sr activities in cones, and ^{137}Cs activities in soils and cones was analyzed (Table 3). The results obtained confirm that both radionuclides could impact necrosis appearance, but ^{137}Cs is supposed to be more significant. This is in line with our previous estimates that β-radiation contributed only 0.4–9.3% to the total dose at the study sites (Geras'kin *et al.*, 2011).

Also, a tendency to increase with the annual dose ($r = 0.76$, $p = 8\%$) was found for the percent of the most damaged needles in 2013. The frequencies of needles heavily damaged by necrosis in 2011 and 2013 correlate with each other ($r = 0.87$; $p < 5\%$). In the 4-km Chernobyl NPP zone in October 1987, about 5% of the seed-buds of *Pinus sylvestris* L. showed signs of necrosis (cumulative doses of 0.7 Gy at 700 μGy.d^{-1}) (Kozubov and Taskaev, 2002). Under conditions of industrial pollution, the frequency of dotted and terminal necrosis in Scots pine trees also increased, as shown in (Stewart *et al.*, 1973; Rautio *et al.*, 1998).

3.3 Fluctuating asymmetry indices

Developmental instability, most frequently detected by measurements of fluctuating asymmetry, has been widely used in environmental studies to examine the effects of anthropogenic and natural stressors (Beasley *et al.*, 2013). It is known that three types of asymmetry exist: directional, fluctuating and antisymmetry, and basically each species requires an examination of the asymmetry type. It is not necessary to provide the relevant analysis for well-known objects if an estimation has been carried out before because of the inherited determination and the adaptive importance of these traits. Such expertise has been provided for *Pinus sylvestris* L. in (Kozlov and Niemela, 1999).

In this study, the needle weight *FA* index changed between 0.040 and 0.054, and we did not find any dependence of FA_W on radiation exposure in the dose range examined.

The index of *FA* calculated from needle length varied between 0.005 and 0.012 (Figure 2). In 2013, the FA_L values at the two most contaminated sites were significantly higher than in the Ref1 population ($p < 1\%$).

There is an increase in the fluctuating asymmetry of the needle length in populations with the annual dose, which is significant in 2011 ($r = 0.97$, $p < 1\%$) and can be considered as a tendency in 2013 ($r = 0.75$, $p = 8\%$). So, in spite of their low values, the dose rates in the studied range can be

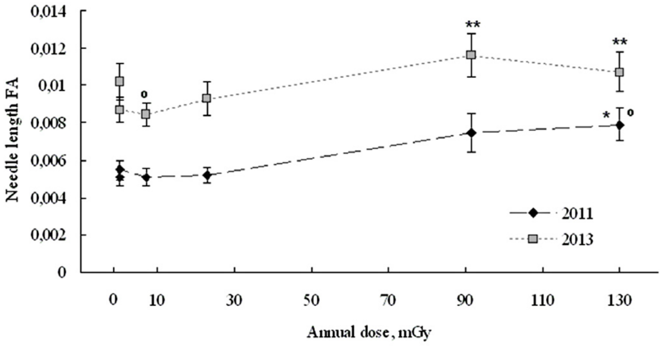

Figure 2. *FA* index estimated from needle length measurements in relation to the annual dose; significant difference from Ref: ° $p < 5\%$; significant difference from Ref1: * $p < 5\%$; ** $p < 1\%$.

considered as a factor which is able to modify developmental stability.

Our findings demonstrate that fluctuating asymmetry of pine needles may be used to evaluate conifer performance and detect environmental stress well before it affects plant growth or vigor.

3.4 Morphoses

It is well known that after the accident, the coniferous trees in the Chernobyl zone showed severe disturbances in growth and morphology; in the territory of 'minor damage' it was observed at external gamma-ray doses from 0.5 to 1.2 Gy (the dose rate was lower than 4.8 mGy.d^{-1} on the 1st of October 1986; the dose in needles was estimated to be lower than 10 Gy) (UNSCEAR, 1996).

In this study, nearly 30 years after the accident, the only type of morphosis found is triple needles. They were never found in either the two reference populations nor at the SB site (23 mGy.y^{-1}). In other impacted study sites triple morphoses sometimes appeared among 600 pairs of needles, which were studied in this experiment: 1 case at the VIUA (7 mGy.y^{-1}) and Z1 (130 mGy.y^{-1}) study sites, and 3 cases at the Z (91 mGy.y^{-1}) site.

4 Conclusions

This study contributes to data on consequences for biota of environmental contamination with radionuclides in the remote period of time after the accidental fallout. It was carried out with one of the most radiosensitive species among plants – Scots pine. Many previous studies of remote radiation-induced effects in pine trees have concentrated on genetic or cytogenetic consequences, while it is important to understand whether alterations in genetic traits, observed in the field, can affect tissue- or organism-level indices. It is found that such endpoints as the weight and length of pine needles do not show any stable or obvious relationship with radiation exposure; this is quite expected considering that the levels of radiation exposure in the study sites are rather too low to produce any serious damaging effect on biota. However, the frequency of necrosis and fluctuating asymmetry showed a correlation with the dose and radionuclide activities. Significant effects were observed at chronic environmental exposure at the dose rate of 90–130 mGy.y^{-1}. The findings are consistent with recommendations (ERICA, 2003) to consider radiation exposure of 100 mGy.y^{-1} as a margin for biota safety under chronic irradiation. The ICRP proposes to consider a dose range of 40–400 mGy.y^{-1} for screening studies of pine in chronic radiation exposure (ICRP, 2008), meaning that at higher exposures obvious effects on populations are possible.

This study supposes that needle length asymmetry can be used as an indicator of disturbance in the stability of needle development, and a degree of necrotic processes is a sensitive test for bioindication of pine forests' response to chronic radiation. Monitoring of these indices offers some opportunities to track populations over time and to evaluate when populations experiencing chronic exposure can reach critical thresholds that demand management action.

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