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Long term effects of ionising radiation in the Chernobyl Exclusion zone on DNA integrity and chemical defence systems of Scots pine (*Pinus sylvestris*)

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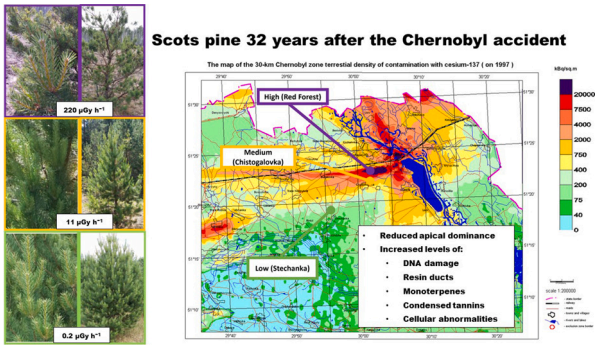
HIGHLIGHTS

- Ionising radiation still affects Scots pine in Chernobyl 32 years after the accident.
- Shoot tips and needles showed elevated levels of DNA damage at ≥11 μGy h^{-1}.
- Increased numbers of resin ducts and subcellular abnormalities in needles at ≥11 μGy h^{-1}.
- Increased levels of monoterpenes and condensed tannins in needles at 220 μGy h^{-1}.

GRAPHICAL ABSTRACT



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ABSTRACT

The Chernobyl Nuclear Power Plant (ChNPP) accident in 1986 resulted in extremely high levels of acute ionising radiation, that killed or damaged Scots pine (*Pinus sylvestris*) trees in the surrounding areas. Dead trees were cleared and buried, and new plantations established a few years later. Today, more than three decades later, gamma and beta-radiation near the ChNPP is still elevated compared with ambient levels but have decreased by a factor of 300 and 100, respectively. In the present work, Scots pine-trees growing at High (220 μGy h^{-1}), Medium (11 μGy h^{-1}), and Low (0.2 μGy h^{-1}) total (internal + external) dose rates of chronically elevated ionising radiation in the Chernobyl Exclusion zone were investigated with respect to possible damage to DNA, cells and organelles, as well as potentially increased levels of phenolic and terpenoid antioxidants. Scots pine from the High and Medium radiation sites had elevated levels of DNA damage in shoot tips and needles as shown by the COMET assay, as well as increased numbers of resin ducts and subcellular abnormalities in needles.

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Needles from the High radiation site showed elevated levels of monoterpenes and condensed tannins compared with those from the other sites. In conclusion, more than three decades after the ChNPP accident substantial DNA damage and (sub)cellular effects, but also mobilisation of stress-protective substances possessing antioxidant activity were observed in Scots pine trees growing at elevated levels of ionising radiation. This demonstrates that the radiation levels in the Red Forest still significantly impact the plant community.

1. Introduction

Following the Chernobyl Nuclear Power Plant (ChNPP) accident on April 26th, 1986, the release of 3–4 tons of radioactive fuel resulted in extremely high levels of ionising radiation, which during the first month after the accident originated predominantly from decay of short-lived gamma and beta emitting radionuclides (e.g., ^{136}Cs, ^{95}Nb, ^{140}La, ^{95}Zr and ^{144}Pr, ^{91}Y, ^{89}Sr, ^{140}La, Kashparov et al., 2018, 2020; Steinhauser et al., 2014; Kashparova et al., 2020). The coniferous forests close to the damaged reactor were exposed to the initial plume and to radioactive particles deposited on tree and soil surfaces. The consequences of this very high radiation exposure manifested as the 'Red Forest'; comprising a 4.5 km^2 zone of total death of Scots pine (*Pinus sylvestris*) trees near the ChNPP and a 120 km^2 zone of sub-lethal and moderate damage at further distances (Tikhomirov and Shcheglov, 1994; Yoschenko et al., 2018; UNSCEAR, 1996). It has been estimated that the needles of pine trees that died were exposed to gamma doses of 80–100 Gy (Kashparova et al., 2020). These dead trees were later cleared by bulldozers and buried, along with heavily contaminated topsoil and forest litter, into sub-surface storage trenches and covered with about 30 cm of clean sand (Yoschenko et al., 2018). New plantations of Scots pine, birch, oak and some shrub species were established during 1988–1989 (Kashparov et al., 2012).

Today, most trees in the area close to the ChNPP are either planted or naturally regenerated after the accident. After 30 years, the intensity of gamma and beta-radiation from the fuel component of the radioactive fallout near the ChNPP had decreased by a factor of 300 and 100, respectively (Kashparova et al., 2020; Holiaka et al., 2020). At the end of May 2016, the dose rate in the air 1 m above the ground level at highly contaminated sites in the Chernobyl exclusion zone (ChEZ) was about 40 μGy h^{-1} (Kashparova et al., 2020) due to long lived radionuclides such as ^{137}Cs.

Exposure to ionising radiation may affect plant growth and development in multiple ways, ranging from stimulatory effects at low doses, to increasingly harmful effects for vegetative growth at medium levels, and pronounced decreases in reproductive success and yield at high radiation levels (Jan et al., 2012). The relationship between exposure dose and biological effects depends on the species, age, seasonality, plant morphology, the amount of photosynthetically active tissue, physiology, and genome organization (Holst and Nagel, 1997; Fesenko et al., 2022). Field and laboratory studies have indicated that acute high doses of ionising radiation between 10 and 1000 Gy can be lethal to plants, while large field studies have demonstrated that chronic radiation at 100 μGy h^{-1} over multiple years is harmful to and cause persistent effects in conifers (Amiro and Sheppard, 1994; Caplin and Willey, 2018). Several studies of Scots pine and Norway spruce (*Picea abies*) have shown increased occurrence of abnormal morphogenesis, such as loss of apical dominance, as well as genetic and epigenetic changes as a result of releases during the ChNPP accident (Zelena et al., 2005; Geras'kin et al., 2008; Yoschenko et al., 2011; Volkova et al., 2017).

Under controlled laboratory conditions, exposure to gamma radiation from a ^{60}Co-source for 6 days resulted in a dose-rate dependent ($\geq$20 mGy h^{-1}) reduction in growth in young seedlings of Scots pine and Norway spruce (Blagojevic et al., 2019a, 2019b). Furthermore, during a post-irradiation period of about 1.5 months, adverse effects including increasingly disorganized apical meristems, visible deformation and mortality were observed (Blagojevic et al., 2019a, 2019b). Notably, Scots pine trees exposed to total doses estimated to 8.6–13.2 Gy did not show significant reduction in annual ring growth in 1986 and 1987 as compared to sites with background-level radiation, but the growth decreased later (Holiaka et al., 2020).

Similar to other stress factors, ionising radiation leads to the formation of free radicals (by splitting water molecules) (Caplin and Willey, 2018). ROS may cause oxidative damage to biomolecules such as proteins, lipids and nucleic acids, and compromise integrity of both cell membranes and DNA (Woodwell and Rebuck, 1967; Azzam et al., 2012; Esnault et al., 2010; Caplin and Willey, 2018). Ionising radiation also causes cellular damage by direct ionisation of essential macromolecules (Caplin and Willey, 2018). Thus, ionising radiation could induce a plethora of DNA damages ranging from single strand and double strand DNA breaks, complex clustered base lesions, deletions and chromosomal rearrangements (Caplin and Willey, 2018; Lomax et al., 2013). In the study of Blagojevic et al. (2019a), seedlings of the herbaceous, short-lived *Arabidopsis thaliana* showed long-term dose-rate-dependent DNA damage after exposure to $\geq$10 and 1 mGy h^{-1} of gamma radiation for 144 h and 360 h, respectively, which persisted 1.5 months post-irradiation, but had minimal adverse effects at organismal and cellular levels. Comparatively, at the end of 144 h irradiation with $\geq$1 mGy h^{-1}, young seedlings of Scots pine and Norway spruce also showed dose-rate-dependent DNA damage that was similar 1.5 months post-irradiation, but with much more pronounced effects on growth and mortality (Blagojevic et al., 2019a, 2019b). This is consistent with the hypothesis that DNA damage in response to ionising radiation may depend on genome organization, with smaller genomes/nuclei being less susceptible than larger ones (135 Mb in *A. thaliana* and over 20 Gb in Scots pine) (Bowen, 1961; Baetcke et al., 1967). Information about the dose-threshold for induction of DNA damage under chronic ionising irradiation field conditions is limited but is also likely to depend on other environmental conditions affecting the protective systems of plants. Thus, knowledge regarding dose and dose rate levels that cause stress-induced acclimation or adaptative processes in plant populations remains rudimentary (Geras'kin et al., 2013).

The extent of the cytotoxic damage due to stress-induced ROS, ultimately depends on the balance between ROS production and detoxification by an array of antioxidants. These include both antioxidant enzymes and low molecular mass antioxidants (Roldan-Arjona and Ariza, 2009; Alscher and Hess, 2017; You and Chan, 2015). Induction of antioxidants and genes encoding antioxidant enzymes in response to ionising radiation exposure has been reported for both herbaceous and woody plant species, including Scots pine (Vandenhove et al., 2009; Van Hoeck et al., 2017; Volkova et al., 2018). In addition to antioxidant systems common to most plant species, plants produce a cocktail of secondary compounds, which vary strongly in composition and content between plant species and families. Flavonoids, an abundant group of phenolics, have repeatedly been shown to function as antioxidants (Agati et al., 2012). Condensed tannins, phenolic polymers composed of flavonoids, are important antiherbivore defences that can precipitate proteins and interfere with herbivore digestion but are also strong antioxidants (Barbehenn and Constabel, 2011). Terpenoids, including mono-, and di- and sesquiterpenes, are the main ingredients in conifer oleoresin that is stored in resin ducts in the bark, sapwood, and needles. The functional roles of terpenoids have largely been ascribed to herbivore defence (Celedon and Bohlmann, 2019), but in recent years it has become clear that terpenoids also improve the ability of plants to deal with oxidative stress, regardless of the external stressor (Vickers et al.,

Table 1
Position and description of sampling sites, activity concentrations, internal, external and total dose rates of Scots pine needles at each site in Chernobyl, (mean ± SE, $N = 5$).

Site ID	Coordinates		Description	Activity concentration of Scots pine needles (DW), kBq kg^{-1}		Dose rate of Scots pine needles, μGy h^{-1}		
	°N	°E		^{137}Cs	^{90}Sr	Internal	Ambient (External)	Total
High (E7-16-S Real Red Forest)	51.382381	30.033179	Naturally regenerated trees on the previous Red Forest lands	46 ± 3	1520 ± 150	179 ± 22	41.3 ± 1.4	220 ± 22
Medium (E7-16-C Chistogalovka)	51.372305	30.029117	Naturally regenerated trees on previously cultivated farmland	13 ± 3	45 ± 3	6.2 ± 0.7	4.9 ± 0.3	11.2 ± 0.8
Low (Stechanka)	51.331068	29.939888	Naturally regenerated trees on previously cultivated farmland	0.34 ± 0.13	0.32 ± 0.07	0.06 ± 0.01	0.14 ± 0.02	0.20 ± 0.02

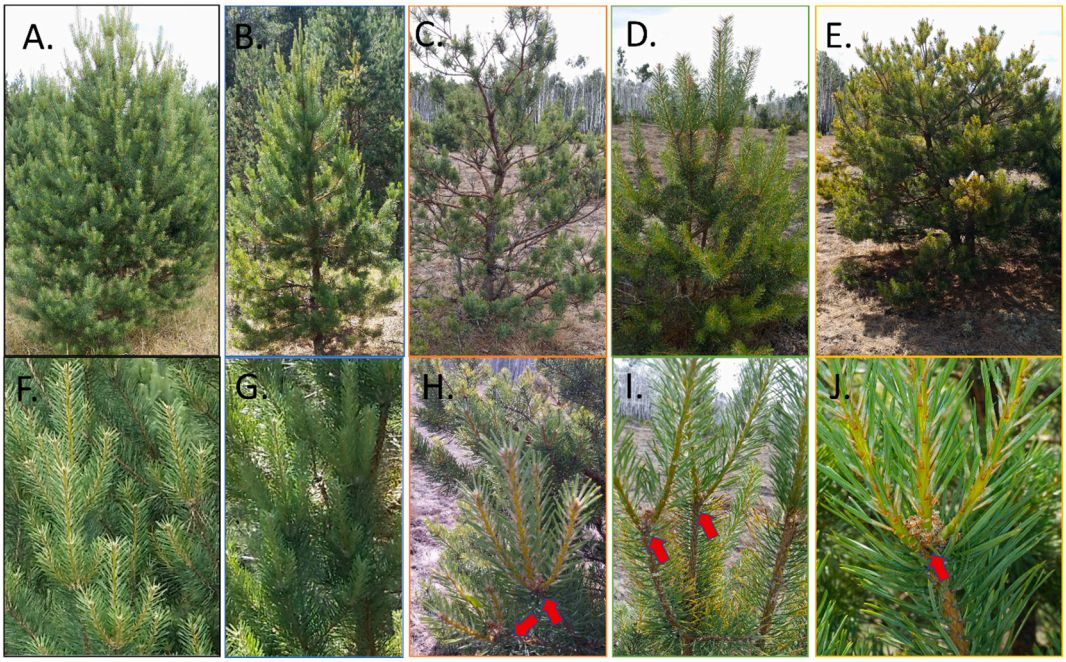

Fig. 1. Morphology of young, 2–3 m high Scots pine trees from Low (A), Medium (B) and High (C, D, E) radiation sites in the Chernobyl nuclear power plant accident area in June 2018. Normal apical dominance in Low (F) and Medium (G) sites and loss of apical dominance at the High site (H, I, J). Red arrows show examples of loss of apical dominance with multiple secondary shoots from the base of the damaged apical meristem.

2009). A higher level of terpenoids under stressful conditions is often reflected in increased resin flow (Lombardero et al., 2002). In many conifers, traumatic resin ducts are well known to be induced under various abiotic and biotic stress, i.e., primarily in the xylem of the wood but can also be found in the phloem (Krokene et al., 2008). However, the information about the effect of stress on the number of resin ducts in conifer needles is limited. Also, the dynamics of this part of the plant defence apparatus has, to our knowledge, not yet been studied in relation to ionising radiation.

Previous investigations have shown that Scots pine and other conifer trees exposed to long-term chronic ionising radiation show biochemical and morphological effects, such as reduced apical dominance (Caplin and Willey, 2018: Yoschenko et al., 2011, 2016, 2018; Konoplev et al., 2020). The aim of the present study was to investigate whether Scots pine-trees growing at chronically elevated ionising radiation within the Chernobyl Exclusion Zone (ChEZ) show evidence of damage at the DNA and cellular level and potential upregulation of defence mechanisms more than three decades after the nuclear plant accident. To achieve this, we sampled pine needles and shoots from three sites with contrasting radiation levels and did analyses of DNA damage, histology and ultrastructural analyses by microscopy as well as of secondary metabolites with antioxidant activity.

2. Material and methods

2.1. Sampling sites

We investigated Scots pine of approximately the same size (2–3 m height) from three sampling sites within the ChEZ that represent Low, Medium and High levels of exposure to ionising radiation (Table 1, Supplementary Figs. 1 and 2, Supplementary Table 1). These trees were naturally regenerated after the accident in 1986, on previous forest or agricultural land (Table 1). We do not have exact measurements of age. The Medium (Chistogalovka) and High (Red Forest) exposure sites have been used in several previous studies (Kashparov et al., 2018, 2020; Kashparova et al., 2020; Yoschenko et al., 2011), while the Low dose rate (Stechanka) site was chosen for this study to match the environmental characteristics of the two other sites. All sites were on flat, dry areas with soddy-podzolic, sandy soils (Podzoluvisol) with scattered young Scots pines (Fig. 1). We did not sample soils for this study, but contamination activities of ^{90}Sr and ^{137}Cs measured previously are given in Supplementary Table 1. Beresford et al. (2020a, 2020b) measured Pb concentrations in soil samples from various vegetation types in the same area and found all values to be below the median of 22.6 mg kg^{-1} for top soil collected from semi-natural ecosystems across the European Union

(Salminen et al., 2006). The ground vegetation was dominated by grasses in the Low and Medium dose rate sites, while the High site largely had no ground vegetation except from scattered lichens.

2.2. Sampling

On June 8 and 9, 2018, before noon, fully developed current year pairs of needles and shoot tips were sampled from five Scots pine trees at each site at approximately 2 m height from the ground and at the northern side of the trees. We made sure to only sample trees with no visible signs of previous herbivore attacks (for example shoots bitten off by moose). Needles for analyses of phenolics and terpenoids were immediately put into cryovials and flash-frozen in liquid-N_2 in the field, while samples of young needles for microscopy were put into fixative as described below. The samples were transported in liquid N_2 or fixative to the Norwegian University of Life Sciences, Ås, Norway, and the frozen samples were kept at −70 °C and the fixed samples dedicated to microscopy were kept at 4 °C until analyses. Fresh shoots tips and young needles for the COMET assay to determine DNA damage were used in a field laboratory close to the sampling sites. Detailed methods for the different analyses are described below. In parallel, pine needles were sampled from the same trees for measurements of activity concentrations of ^{137}Cs and ^{90}Sr.

2.3. Activity concentration of ^{137}Cs and ^{90}Sr in samples

After drying and homogenizing the needles, activity concentrations of ^{137}Cs (Table 1) were measured in cylindrical polyethylene containers with a volume of 100 cm^3, using a low-level gamma-spectrometer, equipped with a multichannel analyser ASPEC-927; passive protection device; a high-purity germanium detector GEM-30185 (EG&G Ortec, USA) and GammaVision32 software (Kashparova et al., 2020). The activity concentrations of ^{90}Sr (Table 1) were estimated after digestion of the plant samples at a temperature of 450 °C through the measurement of its daughter product ^{90}Y, using a beta-spectrometer SEB-01 (AKP, Ukraine) (Yoschenko et al., 2011).

Internal dose rate calculations were then done by using dose conversion coefficients for ^{90}Sr and ^{137}Cs to estimate the internal dose rates in pine needles from the ^{90}Sr and ^{137}Cs activity concentrations (Table 1; Kashparova et al., 2020).

2.4. External dose rate measurements

At each sampling site, the equivalent dose rate in the air, 1 m above ground, was determined by a radiometer-dosimeter (Automess AD6/H GmbH, Ladenburg, Germany) in direct proximity to all five pine trees on both the north and the south sides (Table 1).

2.5. Microscopy

For histological analyses, about 3 mm long samples from the basal, middle part and the tip of young needles of Scots pine (Supplementary Fig. 1) from the three different sites were fixed in a 4 % paraformaldehyde solution and stored at 4 °C, as recommended by Wu and Hu (1997). The preparation of the samples was done according to Lee et al. (2017) and briefly included the following steps: After fixation, the samples were washed with phosphate buffered saline solution (PBS, pH 7.0) for 1 h and dehydrated using a graded ethanol series (30 %, 50 %, 70 %, 90 % and 100 %, 1 h in each). After dehydration, the samples were infiltrated in sequence in a 1:1 and 1:2 mixture of hard grade London Resin (LR) white resin (London Resin Company, Hatfield, UK) and ethanol, followed by embedding in pure LR white resin at 60 °C overnight. The embedded samples were sectioned into 1 μm thick sections, stained with toluidine blue O (Sigma-Aldrich, St. Louis, MO, USA) to visualise the cells, and examined using a Leica light microscope (Leica DM6B, Wetzlar, Germany).

The ultrastructural analyses using transmission electron microscope (TEM) were described in Lee et al. (2017). Briefly, the samples were fixed and embedded in LR white resin as described above for the light microscopy, but 70 nm thick sections were made for the TEM analysis. The sections were mounted on formvar and carbon-coated 100-mesh copper grids (EMS, Hatfield, UK) and stained with 4 % uranyl acetate and 1 % $KMNO_4$, washed with water several times and examined using a FEI Morgagni 268 TEM (FEI, Hillsboro, Oregon, United States).

2.6. Comet assay

To assess DNA damage (strand breaks), the COMET assay was performed separately in shoot tips and associated young needles from three trees per site according to Gichner et al. (2003) and Blagojevic et al. (2019a). We used 1–2 shoot (10 mm) tips for each shoot tip sample and 3–5 whole needles for each needle sample. To prevent light-induced ROS-formation inducing DNA damage, the assay was performed under inactinic red light. Cell nuclei were isolated from each sample by chopping the plant materials vigorously for 30 s in a petri dish with 400 μl extraction buffer (PBS, pH 7.0 and 200 mM EDTA). Thereafter, 75 μl of the nuclei solution were mixed with 50 μl of 1 % low-melting point agarose (NUSieve GTG Agarose, Lonza, Basel, Switzerland). Ten microliters of this suspension were pipetted on pre-made agarose-coated microscope glass slides and dried for 1 min. The slides were immersed in a high-pH denaturation buffer (1 mM Na_2EDTA and 300 mM NaOH, pH $>$ 13) for 15 min. After denaturation, electrophoresis was performed at 20 V (300 mA) for 7 min at 4 °C. Thereafter, the slides were washed in distilled water for 1 min followed by neutralization in PBS for 5 min twice. The slides were again washed briefly in distilled water, followed by fixation in 95 % ethanol for 10 s. The fixed slides were dried in a slide rack overnight and stained with SYBR Gold (Life Technologies Ltd., Paisley, UK; dilution 1:5000) for 20 min. Cell nuclei with DNA damage, visible after the electrophoresis as elongated COMET-like nuclei, were scored using Comet IV (Perceptive Instruments Ltd., Bury St. Edmunds, UK) and an Olympus BX51 fluorescence microscope with a CCD camera (Olympus, Tokyo, Japan). The DNA damage was quantified on basis of the intensity and length of the elongated cell nucleus ("COMET"), relative to the head after the electrophoresis, and presented as % tail DNA. For each biological replicate we used three technical replicates and scored totally 80–90 nuclei.

2.7. Extraction and analyses of low-molecular phenolic compounds

The freeze-dried needles were ground to fine powder using a Retsch MM400 ball mill (Retsch, Haag, Germany) at 30 revolutions s^{-1} for 30–180 s. From the resulting powder sub-samples of ca 10 mg were extracted with methanol as described in Nybakken et al. (2018). The residues were stored in a freezer for further analysis of MeOH-insoluble condensed tannins.

The dried extracts were dissolved in 200 μl MeOH and diluted with 200 μl ultra-clean water (USF ELGA Maxima HPLC; Veolia Water Technologies, Saint-Maurice, France) and analysed using an HPLC system (Agilent Series 1200, Agilent Technologies, Waldbronn, Germany) with a G1312A binary pump, a G1329A autosampler, a G1316A thermoregulated column heater, and a G1315D diode array detector. As the stationary phase a Thermo Scientific column type was used (Thermo Fisher Scientific Inc., Waltham, USA), with a 50 × 4.6 mm internal diameter and filled with ODS Hypersil (3 μm) particles. The samples were eluted by way of a gradient of two eluents: 1.5 % tetrahydrofuran + 0.25 % acetic acid in Milli-Q ultrapure water (Eluent A) and 100 % MeOH (Eluent B) (Julkunen-Tiitto and Sorsa, 2001). The injection volume was 20 μl. The absorption spectra at 270 and 320 nm, along with the respective retention times, were used to identify the individual compounds and to calculate the contents by comparing with commercial standards (all purchased from Sigma-Aldrich, St. Louis, USA).

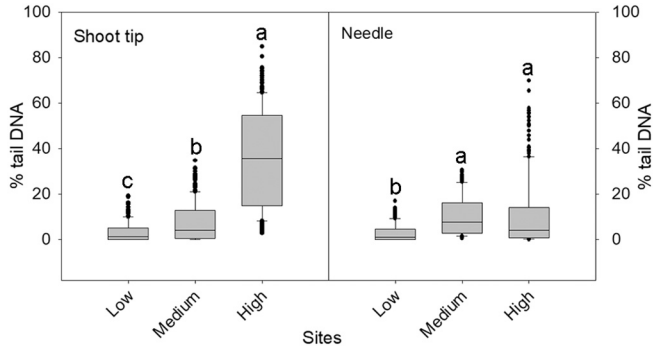

Fig. 2. DNA damage (COMET analysis) in shoot tip and young needles of Scots pine trees collected in June 2018 from Low, Medium and High radiation sites within the Chernobyl Exclusion Zone. Different letters within a subfigure indicate significant differences ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's test. The line in each box represents the median value for 3 biological replicates per site, based on 3 technical replicates per sample, totally 80–90 nuclei scored per biological replicate. The lower and upper box boundaries represent 25 and 75 percentiles. Error bars represent 10 and 90 percentiles with the data points outside shown as dots.

2.8. Analysis of condensed tannins (CT)

The contents of both MeOH-soluble and MeOH-insoluble CTs were identified using the acid butanol assay for proanthocyanidins described in Hagerman (2002). The HPLC-vials were removed from the auto sampler maximum 24 h after analysis and from these 50–100 μl were used to determine the amounts of MeOH-soluble CTs. The amount of MeOH-insoluble CTs were analysed from the residues left after the extraction process. The samples were put in 10 ml glass tubes along with enough MeOH to equal 0.5 ml in total (0.5 ml MeOH for MeOH-insoluble tannins), then further mixed with 3 ml butyric acid (95 % butanol, 5 % hydrochloric acid), and 100 μl iron reagent (2 M HCL with 2 % ferric ammonium sulfate). The glass tubes were properly sealed, mixed, and placed in boiling water for 50 min. Duplicate samples was prepared when extract amounts allowed. After cooling, the light absorption at 550 nm was determined using a spectrophotometer (UV-1800; Shimadzu Corp., Kyoto, Japan). The average between duplicate samples was used as one data value. Purified tannins from spruce needles were used as standards to calculate concentrations.

2.9. Extraction and analyses of terpenoids

Two-three freeze-dried needles were extracted in 1 ml solution of hexane with pentadecane (100 μg ml^{-1}, Sigma-Aldrich) on a planary shaker for about 24 h. The extract was transferred to a new vial and stored at −20 °C before further analyses. Extracted needles were dried in the fume hood and weighed to get exact dry weight.

The hexane-extracts were diluted 1:10 with hexane and analysed by GC (HP Varian 3400) equipped with a DW-wax capillary column (30 m × 0,25 mm × 0,25 mm, J&W Scientific, CA, USA) coupled to a Finnigan SSQ 7000 mass spectrometer. Individual mono- and sesquiterpenes were identified by using the program AMDIS and database library of NIST (National Institute of Standards and Technology). Scots pine contains a large number of different terpenes; thus, compounds were selected individually for further analyses based on quantity and representation in the samples between sites. Relative concentrations were calculated by normalizing peak areas of terpenes to the peak area of pentadecane and needle dry weight.

2.10. Statistical analyses

Effects of ionising radiation and DNA damage (COMET assay) and the number of resin ducts per needle sample were analysed by a one-way ANOVA in the general linear model mode using the Minitab statistical software (Minitab 19, Minitab Inc., PA, USA) ($p \leq 0.05$). To detect

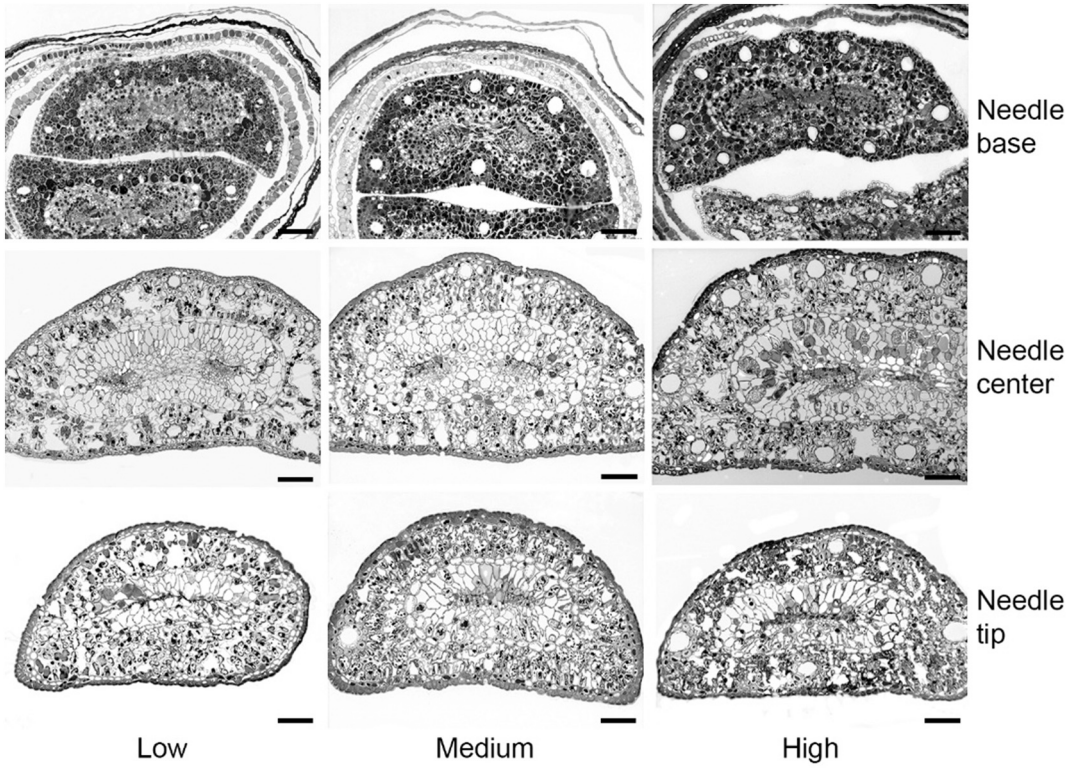

Fig. 3. Histology of representative samples from different positions of needles from young Scots pine trees from Low, Medium and High radiation sites within the Chernobyl Exclusion Zone. Scale bars: 100 μm.

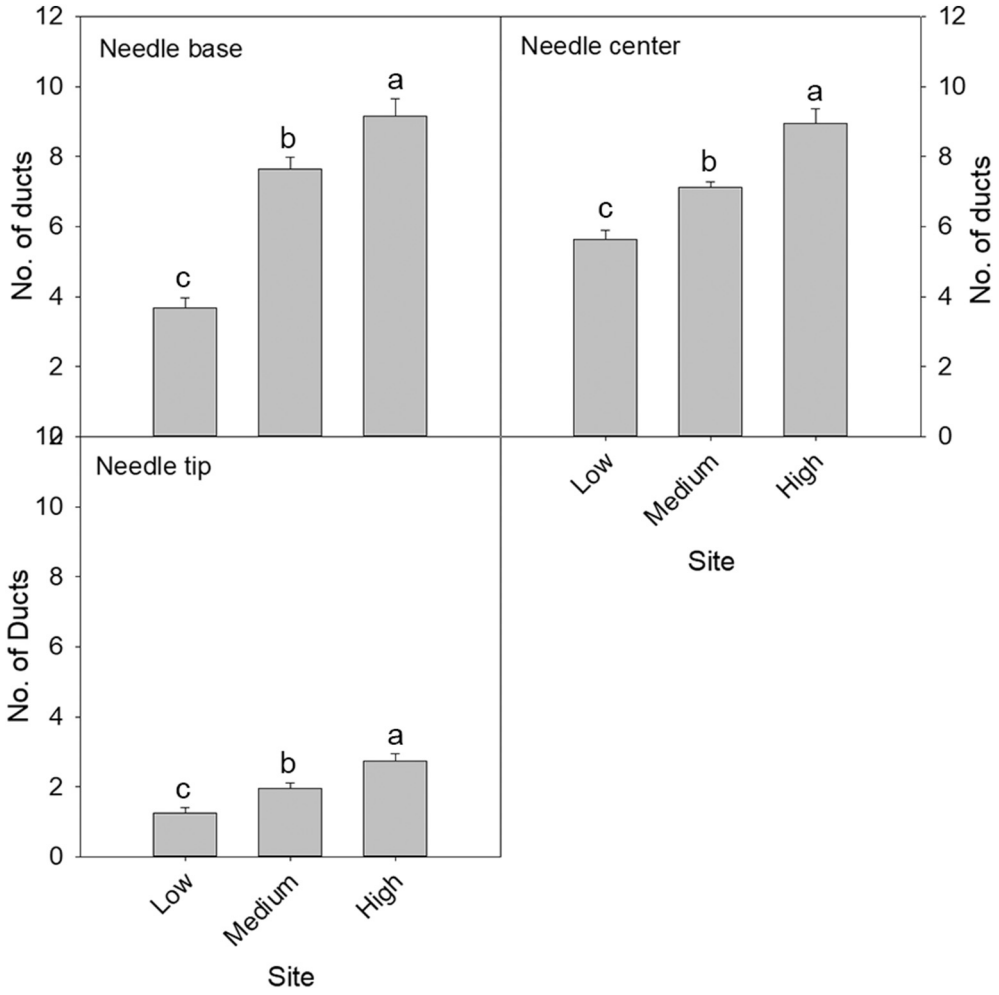

Fig. 4. Number of resin ducts in samples from different positions of needles from young Scots pine trees from Low, Medium and High radiation sites within the Chernobyl Exclusion Zone. Different letters within a subfigure indicate significant difference ($p \leq 0.05$) based on one-way AOVA followed by Tukey's test. The values are mean ± SE of sections from 5 needles from each of 5 plants per site.

differences between groups, Tukey's test was used. The data were first checked by normality and equal variance using the Ryan-Joiner and Levene's test, respectively (Minitab 19). All individual low molecular phenolics and terpenes as well as compound groups and condensed tannins were analysed with one-way ANOVAs in SigmaPlot 14 (SysStat Inc.). When there was a statistically significant difference between groups ($P < 0.05$), an All Pairwise Multiple Comparison Procedure after the Holm-Sidak method was performed. The data were first checked for normality and equal variance using the Shapiro-Wilk and the Brown-Forsythe tests, respectively (SigmaPlot 14).

3. Results

The Scots pine trees at the Low and Medium level radiation sites showed no visible signs of stress and had regular apical dominance and normal shoot growth. By contrast, all the Scots pine trees at the High radiation site in the Red Forest showed classical features of radiation effects with impaired length and thickness growth and loss of apical dominance combined with irregular and short shoots (Fig. 1).

The COMET assay revealed a significantly higher degree of DNA damage in the cells of shoot tips collected from plants from the Medium and High radiation sites compared with those from the uncontaminated Low radiation site. The samples from the High and Medium sites showed 36 % and 8 % of the DNA in the COMET tail, respectively, whereas those from the Low site had 3 % tail-DNA (Fig. 2). Although there was no significant difference in DNA damage in young needles from the Medium and High radiation sites, needles from both these sites showed significantly more DNA damage with up to around 10 % tail-DNA, as compared to those from the Low radiation control site, which had 3 % tail-DNA.

Histological analyses by light microscopy revealed a significantly higher number of resin ducts in all parts of the young needles from the High and Medium radiation sites compared with those from the Low. The needles from the High radiation site had significantly more resin ducts than the ones from the Medium site (Figs. 3 and 4). The needle base from the Medium site contained about twice the number of resin ducts (7.6 ± 0.3) compared to the needle base from the Low site (3.7 ± 0.3), and the needle base from the High site had 1.6-fold more resin ducts than that from the Medium site. For the other needle positions the difference between the Medium and Low sites was smaller, but the number of resin ducts appeared to increase with increased radiation. The needle base and the needle centre had more resin ducts (about 3.7–9.2, depending on site) compared to the needle tip (1.3–2.7).

Although ultrastructural studies of the basal part of apical needles revealed that the organelles in the majority of the cells were normal in the Medium and High radiation sites, subcellular abnormalities were observed in some cells from these sites unlike in cells from the Low site (Fig. 5). These abnormalities included some disruption of nuclear membranes, disruption of outer and inner (thylakoids) chloroplast membranes, enlarged starch grains in the chloroplasts, disrupted outer mitochondria membrane and unstructured mitochondria cristae (Fig. 5).

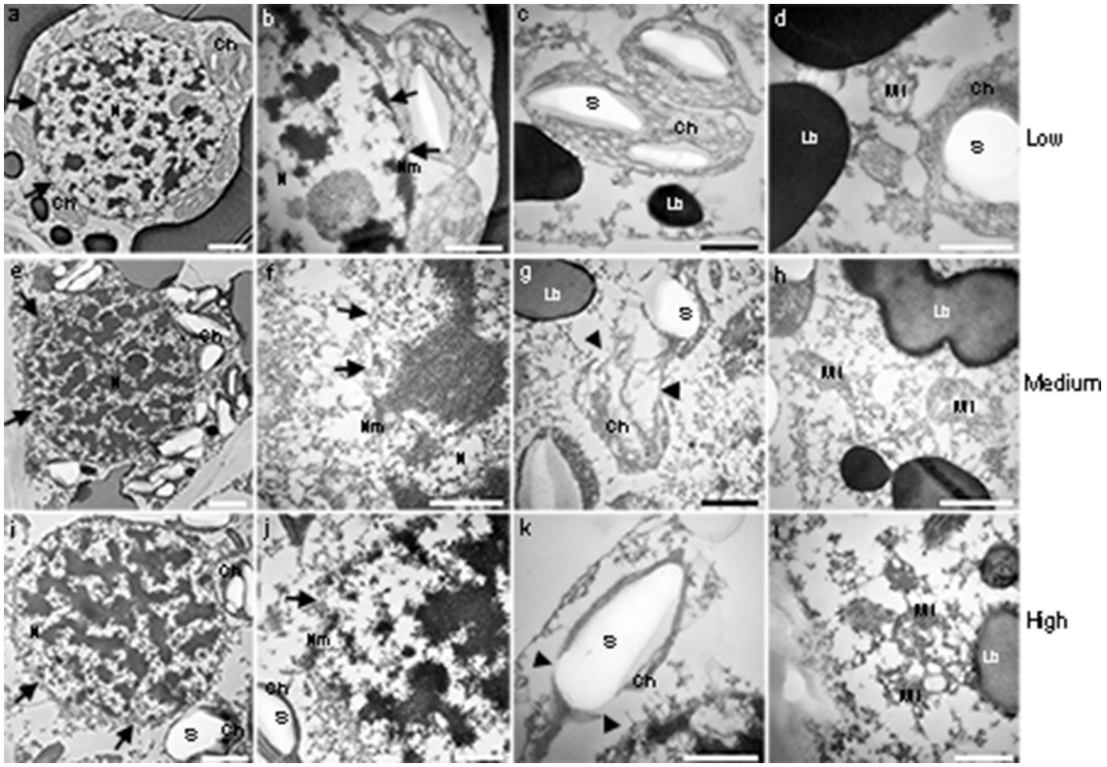

Fig. 5. Transmission electron micrographs of samples from the base of young needles of Scots pine from a–d) Low, e–h) Medium and i–l) High radiation sites in the Chernobyl Exclusion Zone. a, e, i with higher magnifications in b, f, j: cell nuclei, c, g, k: chloroplasts with starch grains and d, h, I: mitochondria. Arrows: nuclear membrane. Arrowheads: disruption of chloroplast membranes. Lb: lipid body, Ch: chloroplast, Mt: mitochondria, N: nucleus, Nm: nuclear membrane, S: starch grain. Scale bars: a, e and I: 2 μm. b–d, f–h and j–l: 1 μm.

3.1. Low molecular mass phenolic compounds

We identified 19 individual low molecular mass phenolic compounds in the pine needles. The level varied between trees within each site, which resulted in only three compounds differing significantly between sites (Supplementary Table 1). The content of acetophenone was higher in needles from the High and Medium radiation sites compared with those from the Low site, while only one epicatechin derivative was higher in the High site compared with the other two sites (Fig. 6). One dihydromyricetin derivative, on the other hand, was higher in needles from the Low site compared with the other sites. The total concentration of low molecular phenolic compounds was not significantly different between the sites (Fig. 6, Supplementary Table 1).

3.2. Condensed tannins

The concentration of the MeOH-soluble fraction of condensed tannins was significantly higher in needles from the High radiation site, compared with the other two sites. (Fig. 7). The MeOH-insoluble fraction of condensed tannins did not differ between the sites.

3.3. Terpenes

We identified and quantified 12 individual monoterpenes and two sesquiterpenes in the pine needles (Supplementary Table 2). The total monoterpene concentration (ng g^{-1}) was >100 % higher in samples from the High radiation site compared with the two other sites, while there were no significant differences in sesquiterpene contents (Fig. 8). None of the sesquiterpenes differed between sites (Supplementary Table 2).

The concentrations of monoterpenes camphene, beta-pinene, beta-myrcene, beta-phellandrene and sabinene were significantly higher in samples from the High radiation site compared with those from the Medium and Low site, while 3-carene was only significantly different between the High and the Medium site (Fig. 9).

4. Discussion

Morphological defects and biochemical changes in response to ionising radiation have been observed in Scots pine trees following the ChNPP accident in 1986 (Caplin and Willey, 2018). However, information about the consequences of long-term elevated ionising radiation at the physiological, cellular and organelle levels as well as on DNA integrity in conifers is limited (Geras'kin et al., 2021). Furthermore, the current understanding of the impact of ionising radiation on the chemical defence system of conifer species is rudimentary, particularly with respect to the level of terpenes and phenolics, which are known to act as stress-protective antioxidants (Ahmad et al., 2010). Here we provide evidence of induction of stress-protective antioxidants and adverse effects in Scots pine growing within the ChEZ >30 years after the ChNPP accident.

The dosimetry measurements (Table 1) showed that both the High and Medium sites were within the ICRP benchmark screening levels (4–40 μGy h^{-1}) for Scots pine (ICRP, 2008). Furthermore, the internal doses from Cs^{137} and Sr^{90} are the main contributors to the total dose. In the High and Medium sites, internal dose rates (179 μGy h^{-1} and 6.2 μGy h^{-1} respectively) comprised 82 % and 56 % of the total exposure, respectively. These results are in line with previous studies and emphasize the importance of taking internal dose into account for reliable assessment of radiation exposure in the field (Beresford et al., 2020a, 2020b).

The impaired growth and reduced apical dominance in young Scots pine trees from the High radiation site (Fig. 1) is consistent with previously reported loss of apical dominance in Scots pine trees in the Red

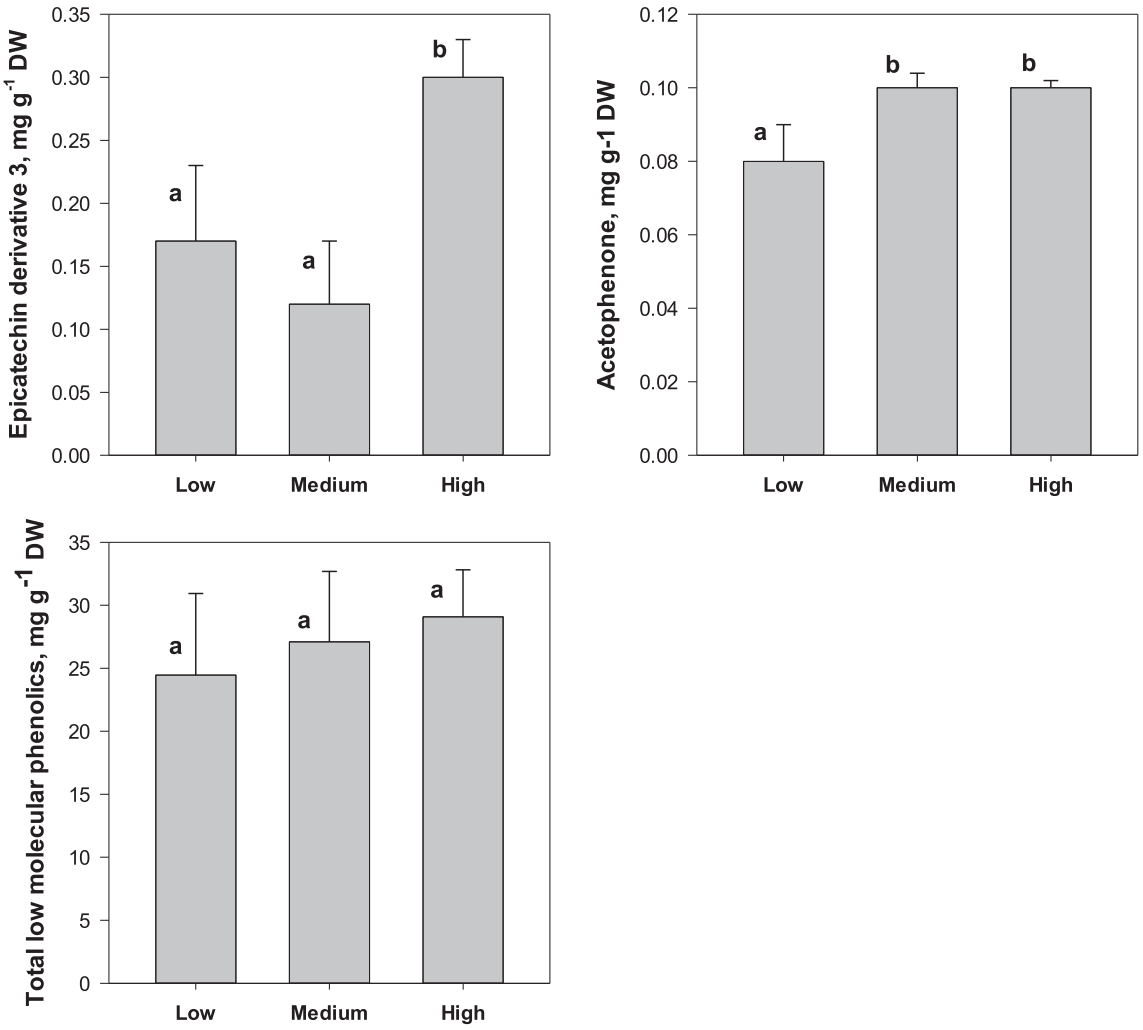

Fig. 6. Mean content (mg g^{-1}) ± S.E. of an epicatechin derivative, an acetophenone and the total low molecular weight phenolics in needles of Scots pine ($n = 5$) from Low, Medium and High radiation sites in the Chernobyl Exclusion Zone. Different letters within a subfigure indicate significant difference between groups ($p \leq 0.05$) based on one-way ANOVA and an All Pairwise Multiple Comparison Procedure after the Holm-Sidak method.

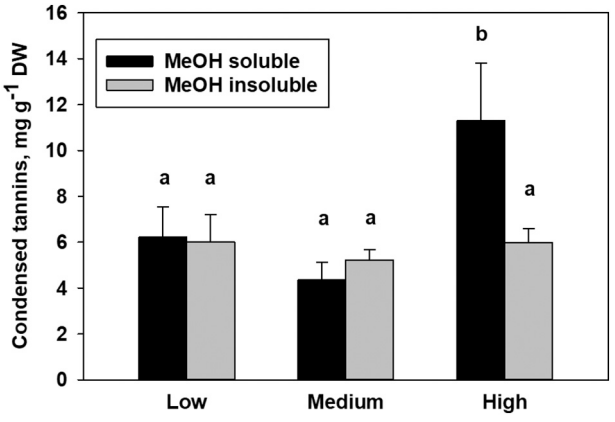

Fig. 7. Mean content (mg g^{-1}) ± S.E. of condensed tannins in needles of young Scots pine trees (n = 5) from Low, Medium and High radiation sites in the Chernobyl Exclusion Zone. Different letters indicate significant difference between groups ($p \leq 0.05$) based on one-way ANOVA and an All Pairwise Multiple Comparison Procedure after the Holm-Sidak method.

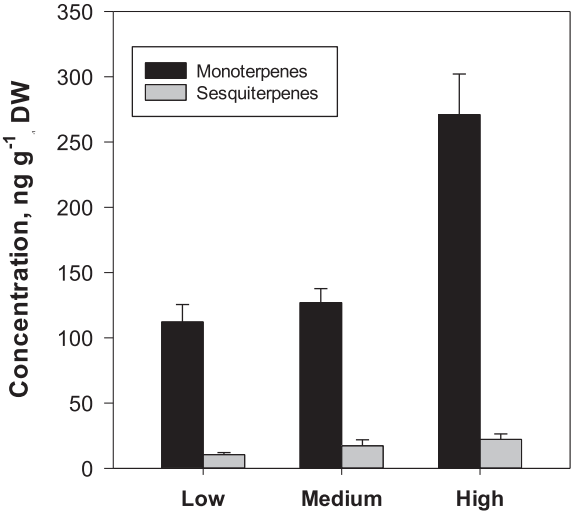

Fig. 8. Mean concentration (ng g^{-1}) ± S.E. of terpenes in needles of young Scots pine trees (n = 5) from Low, Medium and High radiation sites in the Chernobyl Exclusion Zone. Different letters indicate significant difference between groups ($p \leq 0.05$) based on one-way ANOVA and an All Pairwise Multiple Comparison Procedure after the Holm-Sidak method.

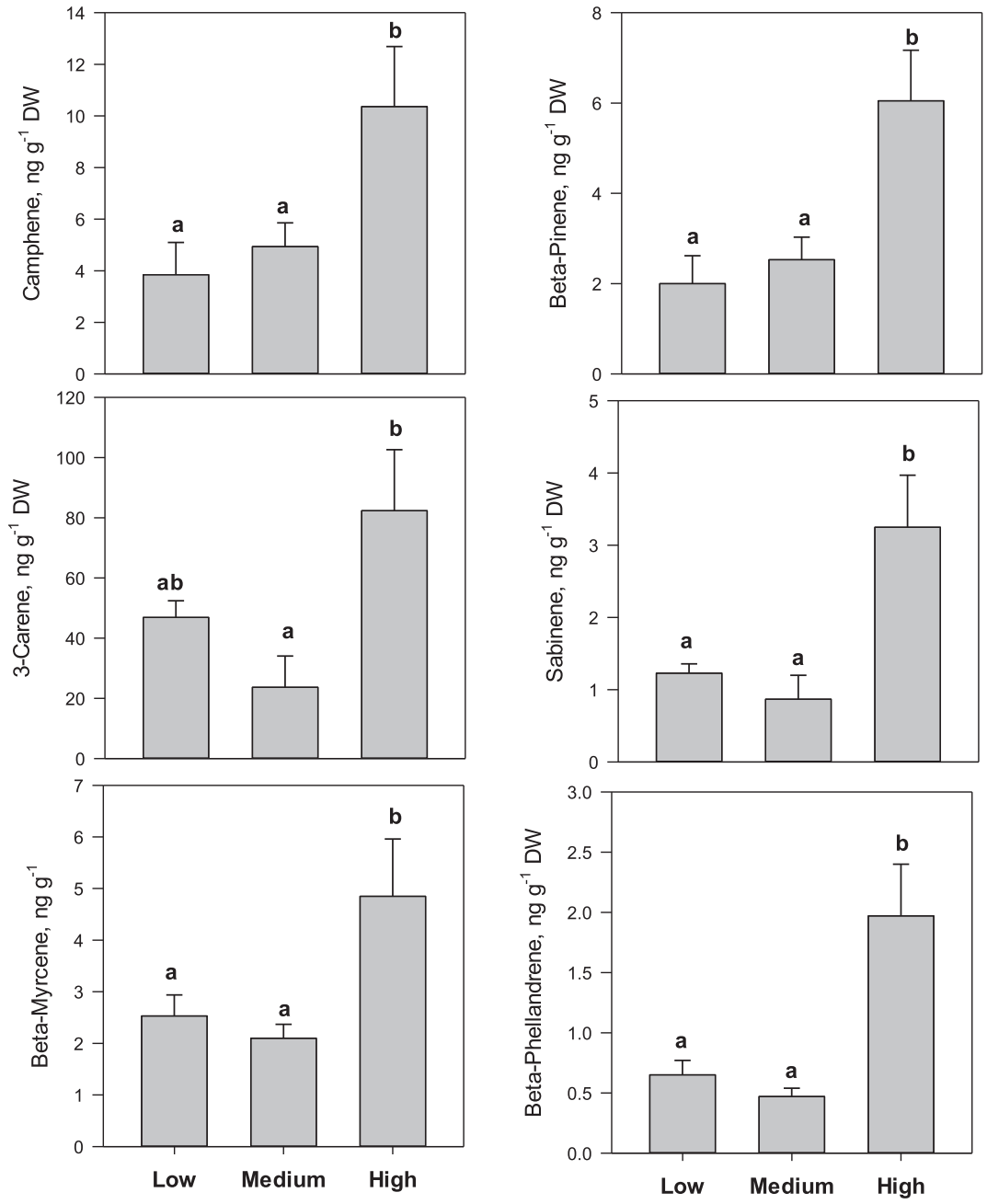

Fig. 9. Mean concentration (mg g^{-1}) ± S.E. of the monoterpenes camphene, beta-pinene, 3-carene, sabinene, beta-myrcene and beta-phellandrene in needles of Scots pine (n = 5) from Low, Medium and High radiation site in the Chernobyl Exclusion Zone. Different letters within a subfigure indicate significant difference between groups ($p \leq 0.05$) based on one-way ANOVA and an All Pairwise Multiple Comparison Procedure after the Holm-Sidak method.

Forest at a total dose rate of ≥40 μG h^{-1} (Yoschenko et al., 2011). Similar morphological effects have been observed in Japanese fir (*Abies firma*) and red pine (*Pinus densiflora*) at comparable dose rates after the accident at the Fukushima Dai-ichi Nuclear Power Plant in 2011 (Watanabe et al., 2015; Yoschenko et al., 2016). The growth impairment and the high concentrations of ^{137}Cs and ^{90}Sr in the shoot tissues from the Red Forest indicate that meristems and multiple cellular functions in needles and shoots might have been affected.

The negative effect of the High radiation level on the tree morphology in the Red forest site only, was consistent with the higher DNA damage in the shoot tips from this site compared with those from the Low and Medium sites (Fig. 2). Although there was slightly elevated DNA damage also in the shoot tips from the Medium radiation site, the lack of morphological effects in this case indicates tolerance of the cells of the shoot apical meristem (SAM) to some DNA damage. Nevertheless, proliferating cells of the SAM are particularly susceptible to damage from high levels of ionising radiation (Pritchard et al., 1999). In shoot tips of saplings and older trees, cell division is a prerequisite for continued growth but also for the formation of leaf primordia within the terminal buds after growth cessation (Owens and Molder, 1976). By contrast, cell division does no longer occur in fully developed needles, possibly explaining the lower level of DNA damage in needles than shoot tips from the High radiation site.

Among plant organelles, the mitochondria and chloroplasts are particularly vulnerable to ionising radiation (Wi et al., 2007). The occurrence of some compromised nuclei, chloroplasts and mitochondria in Scots pine plants from the sites with elevated radiation in the current study (Figs. 3 and 4), imply that the altered phenotype in the High

radiation site may to a certain extent be ascribed to compromised photosynthetic capacity and energy metabolism. However, damage was only observed in a proportion of the organelles, which implies that the combination of repair and protective mechanisms such as autophagy and antioxidant defences, were able to prevent more extensive damage.

It is well known that conifer species produce traumatic resin ducts in response to environmental stress stimuli, such as climatic factors, herbivore browsing, or when subjected to methyl jasmonate treatment (Krokene and Nagy, 2012; Lombardero et al., 2002; Martin et al., 2002; McKay et al., 2003; Jankowski et al., 2017; Vázquez-González et al., 2019). The higher number of resin ducts especially in the needles from the High radiation site (Fig. 4) where growth was impaired, may indicate that resins play a role in protection against stress due to ionising radiation and that this occurs at the expense of growth. This is supported by the significant increase in monoterpenes in the needles from the highly contaminated site (Fig. 5), since terpenes are the main ingredients in conifer oleoresin, i.e., the viscous fluid stored in resin ducts (Martin et al., 2002). Isoprenes, the building blocks for terpene biosynthesis, have been shown to reduce the accumulation of ROS during heat stress either by directly quenching ROS or by stabilizing thylakoids (Velikova et al., 2011).

We found only two individual phenolic compounds that were present in higher concentrations in samples exposed to High compared to Low ionising radiation and there were no significant differences in the total low molecular weight phenolic compound contents in pine needles from our study sites (Fig. 6). This is consistent with previous results from Scots pine seedlings exposed to gamma radiation under controlled environmental conditions (Blagojevic et al., 2019b). Although flavonoids (one group of low molecular phenolics) are typically induced by other stressors, e.g., ultraviolet radiation and low temperature (Julkunen-Tiitto et al., 2015), these results may suggest that the metabolic pathways producing these compounds are not activated by ionising radiation. However, the soluble fraction of high molecular phenolics, the condensed tannins, was doubled in needles from our High radiation site compared with the Medium and Low sites. Condensed tannins are polymers built up of flavonoid units and are as such (end) products of the same metabolic pathway (Rauf et al., 2019). Although best known for their herbivore-deterrent function, condensed tannins have strong antioxidant capacity (Barbehenn and Constabel, 2011; Hagerman et al., 1998), are induced by high light, UV-B irradiation, pathogen infection and drought (Mellway et al., 2009; Popovic et al., 2016) and have recently been shown to protect poplar against methyl viologen-induced oxidative stress (Gourlay and Constabel, 2019). The elevated levels of phenolic compounds in Scots pine needles from the High radiation site in the Red Forest may as such be a response to ROS-induced stress caused by the chronic exposure to ionising radiation. However, we cannot rule out that the increased concentration is a result of the observed reduced growth, meaning that the concentration increases when the content is the same in a lower volume of tissue.

In conclusion, more than three decades after the ChNPP accident, the elevated levels of ionising radiation of $\geq$11 μGy h^{-1} (annual dose $\geq$ 98 mGy) cause substantial DNA damage and (sub)cellular effects in young Scots pine trees. At the high-level Red Forest contaminated sites Scots pine needles receive an annual dose of $\geq$1900 mGy. In addition to the impaired growth and adverse cellular effects, the radiation in the Red Forest area induces production of stress-protective substances possessing antioxidant activity, possibly protecting against more extensive damage. This demonstrates that the radiation levels in the Red Forest still significantly impact the plant community and will continue to do so for generations. Furthermore, the fact that cellular and DNA integrity was affected at 11 μGy h^{-1} is important when considering protection of plant communities in radionuclide-contaminated ecosystems.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166844.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Agati, G., Azzarello, E., Pollastri, S., Tattini, M., 2012. Flavonoids as antioxidants in plants: location and functional significance. Plant Sci. 196, 67–76.
Ahmad, P., Jaleel, C.A., Salem, M.A., Nabi, G., Sharma, S., 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Crit. Rev. Biotechnol. 30, 161–175.
Alscher, R.G., Hess, J.L., 2017. Antioxidants in Higher Plants. CRC press.
Amiro, B.D., Sheppard, S.C., 1994. Effects of ionizing radiation on the boreal forest: Canada's FIG experiment, with implications for radionuclides. Sci. Total Environ. 157, 371–382.
Azzam, E.I., Jay-Gerin, J.P., Pain, D., 2012. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett. 327, 48–60.
Baetcke, K.P., Sparrow, A.H., Nauman, C.H., Schwemmer, S.S., 1967. The relationship of DNA content to nuclear and chromosome volumes and to radiosensitivity (LD50). Proc. Natl. Acad. Sci. U. S. A. 58, 533–540.
Barbehenn, R.V., Constabel, C.P., 2011. Tannins in plant–herbivore interactions. Phytochemistry 72 (13), 1551–1565.
Beresford, N.A., Barnett, C.L., Gashchak, S., Maksimenko, A., Guliaichenko, E., Wood, M. D., Izquierdo, M., 2020a. Radionuclide transfer to wildlife at a 'reference site' in the Chernobyl Exclusion Zone and resultant radiation exposures. J. Environ. Radioact. 211, 105661 https://doi.org/10.1016/j.jenvrad.2018.02.007.
Beresford, N.A., Scott, E.M., Copplestone, D., 2020b. Field effects studies in the Chernobyl Exclusion Zone: lessons to be learnt. J. Environ. Radioact. 211, 105893 https://doi.org/10.1016/j.jenvrad.2019.01.005.
Blagojevic, D., Lee, Y., Brede, D.A., Lind, O.C., Yakovlev, I., Solhaug, K.A., et al., 2019a. Comparative sensitivity to gamma radiation at the organismal, cell and DNA level in young plants of Norway spruce, Scots pine and *Arabidopsis thaliana*. Planta 250, 1567–1590. https://doi.org/10.1007/s00425-019-03250-y.
Blagojevic, D., Lee, Y.K., Xie, L., Brede, D.A., Nybakken, L., Lind, O.C., Tollefsen, K.E., Salbu, B., Solhaug, K.A., Olsen, J.E., 2019b. No evidence of a protective or cumulative negative effect of UV-B on growth inhibition induced by gamma radiation in Scots pine (*Pinus sylvestris*) seedlings. Photochem. Photobiol. Sci. 8, 1945–1962.
Bowen, H.J.M., 1961. Radiosensitivity of higher plants, and correlations with cell weight and DNA content. Radiat. Bot. 1, 223–228.
Caplin, N., Willey, N., 2018. Ionizing radiation, higher plants, and radioprotection: from acute high doses to chronic low doses. Front. Plant Sci. 9, 847. https://doi.org/10.3389/fpls.2018.00847.
Celedon, J.M., Bohlmann, J., 2019. Oleoresin defenses in conifers: chemical diversity, terpene synthases and limitations of oleoresin defense under climate change. New Phytol. 224, 1444–1463.
Esnault, M.A., Legue, F., Chenal, C., 2010. Ionizing radiation: advances in plant response. Environ. Exp. Bot. 68, 231–237.
Fesenko, S., Spridonov, S., Geras'kin, S., 2022. Radiation effects in the forest ecosystems: acute irradiation. J. Environ. Radioact. 250, 106908 https://doi.org/10.1016/j.jenvrad.2022.106908.
Geras'kin, S.A., Fesenko, S.V., Alexakhin, R.M., 2008. Effects of non-human species irradiation after the Chernobyl NPP accident. Environ. Int. 34, 880–897.
Geras'kin, S., Evseeva, T., Oudalova, A., 2013. Effects of long-term chronic exposure to radionuclides in plant populations. J. Environ. Radioact. 121, 22–32.
Geras'kin, S., Yoschenko, V., Bitarishvili, S., Makarenko, E., Vasiliev, D., Prazyan, A., Lychenkova, M., Nanba, K., 2021. Multifaceted effects of chronic radiation exposure in Japanese red pines from Fukushima prefecture. Sci. Total Environ. 763.
Gichner, T., Patková, Z., Kim, J.K., 2003. DNA damage measured by the comet assay in eight agronomic plants. Biol. Plant. 47 (2), 185–188. https://doi.org/10.1023/B:BIOP.0000022249.86426.2a.
Gourlay, G., Constabel, C.P., 2019. Condensed tannins are inducible antioxidants and protect hybrid poplar against oxidative stress. Tree Physiol. https://doi.org/10.1093/treephys/tpy143.
Hagerman, A.E., 2002. The Tannin Handbook. Miami University, Oxford.

Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., Riechel, T.L., 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46, 1887–1892.
Holiaka, D., Fesenko, S., Kashparov, V., Protsak, V., Levchuk, S., Holiaka, M., 2020. Effects of radiation on radial growth of Scots pine in areas highly affected by the Chernobyl accident. J. Environ. Radioact. 222.
Holst, R.W., Nagel, D.J., 1997. Radiation Effects On Plants. CRC Press, Boca Raton, Florida, pp. 38–79.
ICRP (International Commission on Radiation Protection). Environmental Protection: The Concept and Use of Reference Animals and Plants Ann. ICRP 108 available from. http://www.icrp.org/annalslist.asp.
Jan, S., Parween, T., Siddiqi, T.O., 2012. Effect of gamma radiation on morphological, biochemical, and physiological aspects of plants and plant products. Environ. Rev. 20, 17–39.
Jankowski, A., Wyka, T.P., Żytkowiak, R., Nihlgård, B., Reich, P.B., Oleksyn, J., 2017. Cold adaptation drives variability in needle structure and anatomy in *Pinus sylvestris* L. along a 1,900 km temperate–boreal transect. Funct. Ecol. 31, 2212–2223.
Julkunen-Tiitto, R., Sorsa, S., 2001. Testing the effects of drying methods on willow flavonoids, tannins, and salicylates. J. Chem. Ecol. 27, 779–789. https://doi.org/10.1023/A:1010358120482.
Julkunen-Tiitto, R., Nybakken, L., Randriamanana, T., Virjamo, V., 2015. Boreal woody species resistance affected by climate change. In: Björkman, C., Niemelä, P. (Eds.), Climate Change and Insect Pests, CABI Climate Change Series. CABI, Wallingford, UK.
Kashparov, V., Yoschenko, V., Levchuk, S., Bugai, D., VanMeir, N., Simonucci, C., Martin-Garin, A., 2012. Radionuclide migration in the experimental polygon of the Red Forest waste site in the Chernobyl zone – part 1: characterization of the waste trench, fuel particle transformation processes in soils, biogenic fluxes and effects on biota. Appl. Geochem. 27, 1348–1358. https://doi.org/10.1016/j.apgeochem.2011.11.004.
Kashparov, V., Levchuk, S., Zhurba, M., Protsak, V., Khomutinin, Yu, Beresford, N.A., Chaplow, J.S., 2018. Spatial datasets of radionuclide contamination in the Ukrainian Chernobyl Exclusion Zone. Earth Syst. Sci. Data (ESSD) 10, 339–353.
Kashparov, V., Levchuk, S., Zhurba, M., Protsak, V., Beresford, N.A., Chaplow, J.S., 2020. Spatial radionuclide deposition data from the 60 km radial area around the Chernobyl Nuclear Power Plant: results from a sampling survey in 1987. Earth Syst. Sci. Data (ESSD) 12, 1861–1875.
Kashparova, E., Levchuk, S., Morozova, V., Kashparov, V., 2020. A dose rate causes no fluctuating asymmetry indexes changes in silver birch (*Betula pendula* (L.) Roth.) leaves and Scots pine (*Pinus sylvestris* L.) needles in the Chernobyl Exclusion Zone. J. Environ. Radioact. 211, 105731 https://doi.org/10.1016/j.jenvrad.2018.05.015.
Konoplev, A., Kato, K., Kalmykov, S.N., 2020. Behaviour of Radionuclides in the Environment II Chernobyl. Springer. https://doi.org/10.1007/978-981-15-3568-0, 443 pp. ISBN 978-981-15-3567-3.
Krokene, P., Nagy, N.E., 2012. Anatomical aspects of resin-based defences in pine. In: Pine Resin: Biology, Chemistry and Applications, pp. 67–86.
Krokene, P., Nagy, N.E., Krekling, T., 2008. Traumatic resin ducts and polyphenolic parenchyma cells in conifers. In: Schaller, A. (Ed.), Induced Plant Resistance to Herbivory. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-8182-8_7.
Lee, Y., Karunakaran, C., Lahlali, R., Liu, X., Tanino, K.K., Olsen, J.E., 2017. Photoperiodic regulation of growth-dormancy cycling through induction of multiple bud–shoot barriers preventing water transport into the winter buds of Norway spruce. Front. Plant Sci. 8, 2109.
Lomax, M.E., Folkes, L.K., O'Neill P., 2013. Biological consequences of radiation-induced DNA damage: relevance to radiotherapy. Clin. Oncol. 25, 578–585.
Lombardero, M.J., Ayres, M.P., Lorio Jr., P.L., Ruel, J.J., 2002. Environmental effects on constitutive and inducible resin defences of Pinus taeda. Ecol. Lett. 3 (4), 329–339.
Martin, D., Tholl, D., Gershenzon, J., Bohlmann, J., 2002. Methyl jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. Plant Physiol. 129, 1003–1018. https://doi.org/10.1104/pp.011001.
McKay, S.A.B., Hunter, W.L., Godard, K.A., Wang, S.X., Martin, D.M., Bohlmann, J., Plant, A.L., 2003. Insect attack and wounding induce traumatic resin duct development and gene expression of (—)-pinene synthase in Sitka spruce. Plant Physiol. 133, 368–378.
Mellway, R.D., Tran, L.T., Prouse, M.B., Campbell, M.M., Constabel, C.P., 2009. The wound-, pathogen-, and ultraviolet B-responsive MY134 gene encodes an R2R3 MYB transcription factor that regulates proanthocyanidin synthesis in poplar. Plant Physiol. 150, 924–941.
Nybakken, L., Lie, M.H., Julkunen-Tiitto, R., Asplund, J., Ohlson, M., 2018. Fertilization changes chemical defense in needles of mature Norway spruce (*Picea abies*). Front. Plant Sci. 9, 770. https://doi.org/10.3389/fpls.2018.00770.
Owens, J.N., Molder, M., 1976. Bud development in Sitka spruce. I. Annual growth cycle of vegetative buds and shoots. Can. J. Bot. 54, 313–325. https://doi.org/10.1146/annurev.arplant.54.072402.115741.
Popovic, B.M., Stajner, D., Zdero-Pavlovic, R., Tumbas-Saponjac, V., Canadanovic-Brunet, J., Orlovic, S., 2016. Water stress induces changes in polyphenol profile and antioxidant capacity in poplar plants (*Populus* spp.). Plant Physiol. Biochem. 105, 242–250.
Pritchard, S.G., Rogers, H.H., Prior, S.A., Peterson, C.M., 1999. Elevated CO2 and plant structure: a review. Glob. Chang. Biol. 5, 807–837. https://doi.org/10.1046/j.1365-2486.1999.00268.x.
Rauf, A., Imran, M., Abu-Izneid, T., Patel, S., Pan, X., Naz, S., Suleria, H.A.R., 2019. Proanthocyanidins: a comprehensive review. Biomed. Pharmacother. 116, 108999.
Roldan-Arjona, T., Ariza, R.R., 2009. Repair and tolerance of oxidative DNA damage in plants. Mutat. Res. 681, 169–179.
Salminen, R., De Vos, W., Tarvainen, T., 2006. Geochemical Atlas of Europe. Geological survey of Finland, Espoo, Finland.
Steinhauser, G., Brandl, A., Johnson, T.E., 2014. Comparison of the Chernobyl and Fukushima nuclear accidents: a review of the environmental impacts. Sci. Total Environ. 470–471, 800–817.
Tikhomirov, F.A., Shcheglov, A.I., 1994. Main investigation results on the forest radioecology in the Kyshtym and Chernobyl accident zones. Sci. Total Environ. 157, 45–57.
United Nations Scientific Committee on the Effects of Atomic Radiation, 1996. Sources and effects of ionizing radiation. In: UNSCEAR 1996 Report to the General Assembly, With Scientific Annex.
Van Hoeck, A., Horemans, N., Nauts, R., Van Hees, M., Vandenhove, H., Blust, R., 2017. *Lemna minor* plants chronically exposed to ionising radiation: RNA-seq analysis indicates a dose rate dependent shift from acclimation to survival strategies. Plant Sci. 257, 84–95. https://doi.org/10.1016/j.plantsci.2017.01.010.
Vandenhove, H., Vanhoudt, N., Wannijn, J., Van Hees, M., Cuypers, A., 2009. Effect of low-dose chronic gamma exposure on growth and oxidative stress related responses in *Arabidopsis thaliana*. Radioprotection 44, 487–491.
Vázquez-González, C., López-Goldar, X., Zas, R., Sampedro, L., 2019. Neutral and climate-driven adaptive processes contribute to explain population variation in resin duct traits in a Mediterranean pine species. Front. Plant Sci. 10, 1613.
Velikova, V., Várkonyi, Z., Szabó, M., Maslenkova, L., Nogues, I., Kovács, L., Loreto, F., 2011. Increased thermostability of thylakoid membranes in isoprene-emitting leaves probed with three biophysical techniques. Plant Physiol. 157, 905–916.
Vickers, C.E., Gershenzon, J., Lerdau, M.T., Loreto, F., 2009. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nat. Chem. Biol. 5 (5), 283–291.
Volkova, P.Y., Geras' kin, S.A., Kazakova, E.A., 2017. Radiation exposure in the remote period after the Chernobyl accident caused oxidative stress and genetic effects in Scots pine populations. Sci. Rep. 7 (1), 43009.
Volkova, P.Y., Geras' Kin, S.A., Horemans, N., Makarenko, E.S., Saenen, E., Duarte, G.T., Kudin, M., 2018. Chronic radiation exposure as an ecological factor: hypermethylation and genetic differentiation in irradiated Scots pine populations. Environ. Pollut. 232, 105–112.
Watanabe, Y., Ichikawa, S.E., Kubota, M., Hoshino, J., Kubota, Y., Maruyama, K., Yoshida, S., 2015. Morphological defects in native Japanese fir trees around the Fukushima Daiichi Nuclear Power Plant. Sci. Rep. 5 (1), 13232.
Wi, S.G., Chung, B., Kim, J.S., Kim, J.H., Baek, M.H., Lee, J.W., et al., 2007. Effects of gamma irradiation on morphological changes and biological responses in plants. Micron 38, 553–564. https://doi.org/10.1016/j.micron.2006.11.002.
Woodwell, G.M., Rebuck, A.L., 1967. Effects of chronic gamma radiation on the structure and diversity of an oak-pine forest. Ecol. Monogr. 37, 53–69.
Wu, H., Hu, Z., 1997. Comparative anatomy of resin ducts of the Pinaceae. Trees 11, 135–143.
Yoschenko, V., Kashparov, V., Melnychuk, M., Levchuk, S., Yu, B., Lazarev, M., Yoschenko, M., Farfan, E.B., Jannik, G.T., 2011. Chronic irradiation of Scots pine trees (*Pinus sylvestris*) in Chernobyl exclusion zone: dosimetry and radiobiological effects. Health Physiol. 101, 393–408.
Yoschenko, V.I., Nanba, K., Yoshida, S., Watanabe, Y., Takase, T., Sato, N., Keitoku, K., 2016. Morphological abnormalities in Japanese red pine (*Pinus densiflora*) at the territories contaminated as a result of the accident at Fukushima Dai-Ichi Nuclear Power Plant. J. Environ. Radioact. 165, 60–67.
Yoschenko, V., Ohkubo, T., Kashparov, V., 2018. Radioactive contaminated forest in Fukushima and Chernobyl. J. For. Res. 23, 1–14. https://doi.org/10.1080/13416979.2017.1356681.
You, J., Chan, Z., 2015. ROS regulation during abiotic stress responses in crop plants. Front. Plant Sci. 6, 1092. https://doi.org/10.3389/fpls.2015.01092.
Zelena, L., Sorochinsky, B., Von Arnold, S., Van Zyl, L., Clapham, D.H., 2005. Indications of limited altered gene expression in Pinus sylvestris trees from the Chernobyl region. J. Environ. Radioact. 84 (3), 363–373.