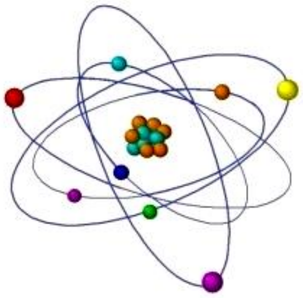
GIGANTISM OF PHOTOSYNTHESIZING ORGANS IN DECIDUOUS AND CONIFEROUS TREE SPECIES UNDER CONDITIONS OF ELEVATED BACKGROUND OF IONIZING RADIATION



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ABSTRACT: *The research results on the effects of indirect action of ionizing radiation on tree species according to morphological parameters are presented. The phenomenon of gigantism in the needles of Pinus silvestris and Picea excelsa was observed at absorbed doses in the range of 2-10 Gy. In this case, the shoots carrying numerous large buds were distinguished by very large needles, the length of which was 1.5-2.5 times greater than the control values. The phenomenon of gigantism in the leaf apparatus, particularly in needles, served as a basis for considering the possible role of integral reactions to irradiation, which could be expressed in the radiation modification of physiologically normal correlative interactions between different parts and organs of the plant. The assumption that radiomorphoses in the form of giant leaf blades are caused by the reproductive death of cells of individual apical meristems, resulting in disrupted trophic interactions in the irradiated plant, is justified.*

Key words: ionizing radiation, integral reactions, radiomorphoses, radiation chimeras.

After the Chernobyl Nuclear Power Plant accident, a unique situation emerged in its exclusion zone, allowing for the study of not only purely radioecological problems but also general problems of plant biology, particularly those related to the morphogenesis of plant organs. It is clear that the Chernobyl disaster could not go “unnoticed” by radiobiology and radioecology. The interest generated by this event led to a surge of publications, suggesting the emergence of a fundamentally new field requiring additional research efforts. But was this really so? Did the Chernobyl accident indeed lead to any truly unique radiobiological and radioecological phenomena and processes, differentiating them from previously known phenomena? From our point of view, the peculiarity of the accident can be discussed only in terms of its “technical” characteristics, mainly concerning the nature of radionuclide contamination of natural and man-made objects (the patchiness of radionuclide contamination of landscapes, the peculiarities of radionuclide fractionation in radioactive precipitation, etc.). The uneven nature of the distribution of radionuclides (R.n.) in the territories adjacent to the accident zone, the presence of vertical heterogeneity in the distribution of R.n. in the soil and the presence of “hot” fuel and aerosol (condensation) particles in the habitat of plants and animals led to the fact that living organisms in the zone of radionuclide contamination began to be unevenly influenced by sources of ionizing radiation of very heterogeneous composition and spatial distribution. However, considering that the actual radiobiological stage of interaction of the radiation factor with the biosystem begins with the penetration of R.n. into the organism or with the onset of significant external exposure from radionuclides concentrated in the environment, the “prehistory” of R.n. migration at this stage is of no significant importance for assessing the radiobiological effect, only reflecting on the kinetics of the external exposure or the dose and power characteristics of radiation from R.n. incorporation into the organism. While the power of radiation impact and the nature of R.n. distribution in tissues may change, this does not create, practically, new situations of interaction between radiation and biological objects, and therefore does not entail a qualitatively new radiobiological phenomenology. Thus, it can be assumed that at this stage, existing observations, experimental facts, and theoretical positions in radiobiology should be sufficient to explain the radiobiological phenomena ultimately caused (directly or indirectly) by the Chernobyl accident.

Nevertheless, the Chernobyl accident led to significant changes in radiobiology and radioecology. If the totality of radiobiological problems is represented as a problem spectrum with its qualitative (a multitude of problem areas) and quantitative (human, material, and time resources spent on solving a specific problem) characteristics, it can be stated that in the post-accident period it underwent only quantitative changes, and the shift in research emphasis occurred in the area of previously little studied and researched problems, such as problems of radioadaptation, radiation aging, carcinogenesis, etc. In addition, radiobiologists began to pay more attention to radiobiological and radioecological effects caused by irradiation with ionizing radiation in the range of doses and powers close to the corresponding values of the natural radiation background (NRF).

One of the radiobiological directions whose problems have gained particular relevance is the study of integral (distant, mediated, indirect) reactions of biological systems to irradiation. The complexity, multi-level nature of biological systems, and the correlated functioning of their components and structures inevitably reflect in their radiobiological reactions. Depending on the range of applied or studied doses/powers, the balance of direct and mediated reactions of biological objects to irradiation changes. In general, radiobiology faces the task of obtaining a statistical or analog model of the reaction of any biological system to irradiation, taking into account the reactions of all its constituent elements. Since biological systems are multi-level, in fact, it is necessary to build an entire hierarchy of such models, i.e., mechanisms.

The search for mechanisms of a specific observed radiobiological phenomenon for a specific structure must always be conducted with regard to the interconnectedness of the studied structure with other structures of the same level of organization that may also participate in forming its reaction to irradiation. For example, the well-known phenomenon of gigantism in spruce needles, observed in plants growing in some areas of the 30-km zone of the Chernobyl Nuclear Power Plant with an elevated level of radionuclide contamination and, accordingly, with increased (several orders of magnitude exceeding the NRF levels) doses of ionizing radiation, could be explained by considering it as a direct result of irradiation on the needles [1]. However, the strict adherence to the principles of a systemic approach, and in particular, the requirement to describe the mechanism of the phenomenon at a specific structural and functional level, involving information about the behavior or reaction of elements directly forming this level, forces us to look for another explanation for the phenomenon of needle gigantism.

In 1986, after the Chernobyl accident, there was a significant reduction in the growth of apical shoots in common pine (*Pinus silvestris* L.) in almost all areas of the Chernobyl Exclusion Zone with absorbed doses from 3 to 20 Gr, and the shoots themselves had a curved form. In common spruce (*Picea excelsa* (Lam.) Link.), as a rule, shoots with shortened growth retained linear growth but were distinguished by a strong thickening of needles and acquired the form of a “lamp brush” [2-4]. Irradiation in 1986 and 1987 led to the increase in the quantitative characteristics of the leaf apparatus in coniferous and deciduous tree species in some cases (Fig.1).

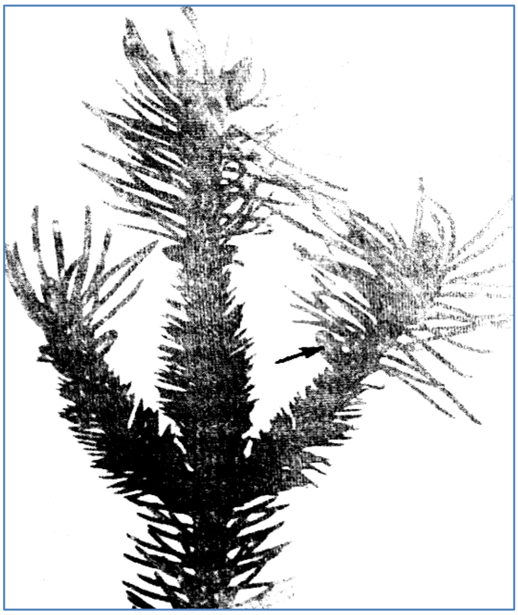

Figure.1. A lateral shoot from the top of the crown of a 25-year-old spruce with giant needles formed in 1987 under radiation exposure at a dose of 4-5 Gr. Lateral buds at the base are shown with an arrow [4]

Gigantism was also observed in the needles of *Pinus silvestris* and *Picea excelsa* at absorbed doses in the range of 2-10 Grays. Typically, shoots carrying numerous large buds were characterized by very large needles, with lengths 1.5-2.5 times, and cross-sectional areas 2.6-5.6 times bigger than the control values. The increase in needle size was generally noted at the apical part of the shoots in *Picea excelsa* and at the base of the shoots in *Pinus silvestris*. In deciduous species (*Guercus rubra* L. and *Guercus rubra* L., red and common oak, *Betula verrucosa* Ehrh. weeping birch, *Sorbus aucuparia* L. common rowan, *Tilia codata* Mill. small-leaved lime, *Robinia pseudacacia* L. white acacia, etc.), leaf gigantism was mainly observed in 1987. It was most clearly observed in *B. verrucosa* at doses of 40-60 Grays and higher, and in *G. rubra* at 8-10 Grays. The volume of needles in *Picea excelsa* in 1987 in some areas was 10-15 times bigger than that of the needles in 1986. Such giant needles were formed mainly on P. excelsa that received an elevated dose of ionizing radiation. The noted tendency towards gigantism persisted in 1988. The phenomenon of radiation-induced gigantism of the leaf apparatus and, in particular, the needles, served as a basis for us to consider the possible role of integral reactions to irradiation, which could be expressed in radiation modification of physiologically normal correlative interactions between different parts and organs of the plant. From our point of view, irradiation at comparatively low (subthreshold) doses for differentiated cells (forming the bulk of the photosynthesizing tissue), but lethal for meristematic cells (primarily, cells of apical stem meristems), led to the mass but not continuous death of stem growth points. It can be assumed that a small number of surviving apices received an increased amount of assimilation products (primarily sugars), synthesized by a photosynthetic apparatus that has a significantly higher radio resistance [1,5], and which, thus, provided an increased amount of nutrients substances to a limited number of stem apices ("waiting" meristems) and needles embedded in their structures. Thus, needle gigantism is probably a consequence of the disruption of correlative relationships in the irradiated plant. In any case, such an assumption should be considered first when studying the mechanism of the described phenomenon. The validity of such an approach is confirmed by data from N.I. Goltsova [6]. It turned out that the complete or partial death of terminal and lateral buds of irradiated shoots of *Pinus Silvestris L.* led to an increase in the lifespan of the old, pre-accident needles of 1984-1985. This may indicate that leaf aging is the result of complex correlative influences, in which the competition of plant organs for phytohormones (cytokinins, auxins, and gibberellins), assimilates, and water play an important role. Apparently, with the death of individual apical buds, such competition decreased, i.e., reduced or completely disappeared apical dominance, regulated by auxins and cytokinins, as a result of which cytokinins, transported upwards from the root in conditions of the death of apical buds, stimulated the growth of remaining adventitious and dormant buds. Another confirmation of the made assumption is the results of research by G.M. Kozubov and A.I. Taskaev [2], in particular, the indication that the partial volumes of tissues in the giant needles were close to the norm, i.e., the usual ratio of quantitative parameters of needle tissues was not violated. Moreover, according to these researchers, giant needles were formed practically on all trees with partially affected crowns, with the needles on pines with a higher (but not 100%) degree of radiation damage being the largest. It is also evident that the gigantism of needles could not significantly manifest in the first year after the disaster, as in the zones of sublethal radiation action, its mass influence on growth processes was not yet observed. And, indeed, gigantism was observed mainly in needles formed in 1987, i.e. in needles formed after the Chernobyl accident.

If the gigantism of the needles were a direct result of ionizing radiation, then massive changes at the cytological and histological levels would be observed. Such changes occured [7], but they were not total. Most mesophyll cells were close to normal size, and no reliable correlation was found between changes in their parameters and specific volume and the absorbed dose of ionizing radiation.

What was the reason for the increase in the size of the needles? In the giant needles of the pine, the diameter of the resin channels increased, and the cross-sectional area of the epidermis and hypodermis, as well as the cross-sectional area of the conducting cylinder, increased by 1.5-2 times. Overall, the increase in the cross-section of individual elements was parallel to the increase in the total cross-sectional area of the needles. A similar pattern was observed in the needles of spruce. In 1987, the majority of trees saw a sharp increase in all needle parameters, and vegetative shoots formed large, straight, and highly curved, thickened needles. Along with the general increase in spruce needle parameters in 1987, there was an increase in the diameters of cells in the epidermis, hypodermis, and mesophyll, with the most intense enlargement of histological elements observed in needles with the maximum cross-sectional areas (which exceeded even the pre-accident 1985 values, i.e., the absolute values were generally proportional to the overall morphometric indicators). The relative (partial) volumes of tissues remained close to those of normal needles. An exception was the relative volume of the conducting cylinder, which reached its greatest values in the severely shortened needles in 1986 and in the giant needles in 1987. The diameters of the resin channels also increased significantly [7]. It is not by chance that we dwelled in such detail on the cytological characteristics of the giant needles. It is known that the difference in size of identical and same-aged plant organs is determined not by the number of cells but by their size [8]. The results mentioned above perfectly illustrated this point. This is most true for spruce needles, in which practically all types of cells uniformly reacted by increasing their size. The mesophyll cells of giant pine needles were practically indistinguishable from analogous control cells. Considering that hormesis (the positively stimulating effect of ionizing radiation, regardless of whether it affects the object directly or indirectly) can reveal the phenotypic (morpho-functional) potential for the growth and development of a biological object, it can be concluded that the morpho-functional potential of mesophyll cells of pine needles was more fully realized. Thus, the radiation factor can act as a factor testing the expression of gene activity at the epigenetic level of regulation [9].

Critical to the inhibitory effect of ionizing radiation on woody plants are their forming tissues - apical (top) and lateral (side) meristems. It is their reaction that determines the morphofunctional character of the remote consequences of radiation [10]. In the Chernobyl disaster zone, coniferous and deciduous trees were subjected to dose loads during active growth and formative processes, determined by a high level of proliferative activity of meristematic cells. In this state, they have increased radiosensitivity and are easily damaged by relatively low doses [11]. Acute and subsequently powerful chronic irradiation in the spring of 1986, on the one hand, affected the buds of shoots, leaves, needles and reproductive organs that were formed during the previous year, and, on the other hand, affected the primordia that were formed in the year of the disaster. What types of cellular reactions might underlie the morpho-functional disturbances induced by radiation? Firstly, they should be divided into lethal and non-lethal. The former, in its turn, are observed in the form of interphase (metabolic and apoptotic) and reproductive (proliferative) death. Metabolic death of plant cells is not associated with the genetic effect of ionizing radiation, but is caused mainly by multiple damage of membrane structures. Most likely, this type of death of differentiated cells caused the death of needles in the zone of lethal damage (lethal doses).

Another possible form of death is apoptosis, which normally is an integral part of plant development mechanisms and represents a genetically determined cell death program (programmed cell death - PCD). The implementation of this program in ontogenesis is accompanied by specific changes in cell morphology, nuclear and cytoplasmic structure, activation of caspases, nucleases, domain, and internucleosomal fragmentation of nuclear DNA. This process is observed in various plant tissues and, like cell differentiation, may be controlled by phytohormones. The apoptotic type of plant cell death can be triggered by many factors, including unfavorable ones (oxidative stress, UV, and gamma radiation, etc.). The key role in the initiation of certain types of PCD belongs to mitochondria: as in animals, the induced exit of cytochrome and other protein factors from the mitochondria triggers apoptosis in plant cells. Reactive oxygen species (ROS) can serve as trigger molecules of apoptosis, and antioxidants suppress apoptosis in plants [12].

The reason for reproductive death of cells is lethal mutations associated with the irreversible disruption of chromosomal structure, leading to the cell's loss of proliferative ability. A cell that has died by the mechanism of reproductive or interphase death loses the ability to give rise to a cell line (stream), a necessary element of the morphogenetic process, the consequence of which can be various morphological anomalies - radiomorphoses. Does the interphase type of cell death (ID) play any role in the formation of radiomorphoses? It is known that the ID of plant cells is observed at doses exceeding those that induce reproductive death by two orders of magnitude - several hundred and several Grays respectively [13]. ID is characterized by a threshold, mass manifestation, i.e., practically 100% of irradiated cells die by this mechanism when certain absorbed doses are reached. Probably, ID of cells due to its massiveness can lead to a complete stop of growth and morphogenetic processes. Thus, if we are talking about morphological abnormalities induced by radiation, then to explain them it is enough to assume the activation of mechanisms of reproductive cell death.

Based on the above and using the results of observations of needle death and morphological anomalies, it is possible to hope that in the future a method of biological retrodosimetry can be developed based on the mentioned facts and patterns. Thus, irradiation at doses of 10-20 Grays led to the complete death of the above-ground organs of pine and spruce during the vegetation period in 1986, which indicates the metabolic type of death of the forming cells. Even if we did not know the corresponding doses, they could be restored by the nature of cell death, for which, however, additional research would be required to obtain calibration curves. Unlike interphase death, reproductive death is not a mandatory total (mass) effect when a cell population or tissue is irradiated. Cells that have lost their reproductive ability differentiate rapidly, age, but do not die immediately by the metabolic mechanism. The surrounding cells do not lose their reproductive ability and give rise to normal cell lines.

Thus, radiomorphoses are most likely caused by the reproductive death of individual cells (the result of lethal mutations) scattered randomly through the formative tissue and/or are the result of non-lethal somatic mutations affecting the physiological and biochemical parameters of the mutated cell and the surrounding cells. In the latter case, chimeric tissues consisting of genetically heterogeneous somatic cells are formed [14-15].

The real spectrum of morphological anomalies of plant organs of woody species, identified during the survey of forests in the zones of sublethal and medium damage, in the autumn of 1986 and spring of 1987, was very broad. Can the goal be set to create a semblance of the periodic system of chemical elements based on radiation-induced anomalies and find out to what extent all possible variants of anomalies were fully realized in real conditions, i.e., how fully the cells of such a “periodic system” were filled? Creating such a system would have enormous heuristic value for the theory of biological morphogenesis, as well as for the theory of radiation-induced anomalies (theory of radiomorphoses). Moreover, assuming a dependence of the qualitative and quantitative characteristics of the spectrum of induced morphoses on the qualitative and quantitative characteristics of the radiation factor, one could apply the method of generalized parameters and correspond a certain gradation of dose load to a certain expression of the characteristics of morphological anomalies. In this way, a calibration dependence would be obtained, which could become the basis for the method of biomorphological retrodosimetry. It is obvious that such a task setting is also possible concerning functional parameters.

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