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Response of *Pinus sylvestris L.* needles to electromagnetic fields. Cytological and ultrastructural aspects

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Abstract

The effects of pulsed radio frequency electromagnetic field (RF EMF) on plant growth and development are still unclear and contradictory. The aim of this work is to study the impact of RF EMF generated from the Skrunda Radio Location Station, Latvia, on growth and development of pine trees. Pine needles and cones were collected in 1993 from the tops of 50–60-year-old pine trees in four locations near the Skrunda RLS: A — no EMF exposure, B — low EMF exposure, C and D — high EMF exposure. The chloroplasts of mesophyll of second-year needles are ultrastructurally characterised by dense stroma, a compact granal membrane system and variable amounts of starch granules and plastoglobules. Low and especially high EMF exposure from the Skrunda RLS increased the amount of plastoglobules. The structure and export products of Golgi apparatus were changed and in different types of cells, large osmiophilic globules appeared. Evidently, EMF induces modification of Golgi apparatus and switches its functions from synthesis of predecessors of cell walls (lignins) to formation and export of resin predecessors. The stress due to RF EMF generated from Skrunda RLS causes an unspecific response — accelerated resin production and promoted senescence of pine trees.

Keywords: *Pinus sylvestris L.*; Chloroplasts; Golgi apparatus; Osmiophilic globules

1. Introduction

The claimed range of biological implications of RF EM radiation near the Skrunda Radio Location Station (RLS), in operation since 1967, is very wide. It has been demonstrated that RF EMF decreases tree ring increment width [2], remarkably decreases the viability of pine trees and changes the structure of wood [12]. Neverthe-

less, the possible mechanism of action of this radiation has not yet been proposed. According to our previous investigations, the ultrastructure of mesophyll cells of leaves reflects the character of metabolism of the whole plant and may serve for understanding of the universal rules of stress and adaptation [10,23].

In the present work, the germination of seeds, formation of seedlobes, and the anatomy and ultrastructure of needles were studied as mediators of external response of pine trees to EMF. Particular attention was given to the state of

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Golgi apparatus and content of chloroplasts. The basic ultrastructural mechanisms of plant adaptation to various unbalanced conditions are changes of secretion in plants, and the ability of chloroplasts to form temporary deposits, which are osmotically inactive and vary in chemical character and degree of polymerisation [22,23].

2. Material and methods

Pine needles and cones were collected in dominant pine forests from the tops of 50–60-year-old pine trees in four sample plots near the Skrunda RLS: A — no EMF exposure, B — low EMF exposure, C and D — high EMF exposure. The wave frequency of the pulse EM radiation around the Skrunda radar ranges from 154 to 162 MHz, and the pulse level can have intensities of 375 $\mu\text{W}/\text{cm}^2$ or more. The field measurements of EM radiation intensities in sample plots were the following [11]: A — 0.04 mV/m, B — 9.5 mV/m, C — 79.4 mV/m, D — 250 mV/m. Needles were collected at the end of May 1993, and seeds in February 1994.

The seeds were germinated on filter paper moistened with tap water in the dark for 10 days at an air temperature of 22°C. Seedlings were planted in peat, moistened with tap water, and grown in a glasshouse. Replication of this procedure was carried out four times.

For anatomy and electron microscopy, immediately after collection, 2-mm pieces of the middle part of second-year needles were double fixed with 2% glutaraldehyde in sodium cacodylate buffer (24 h in the dark at 5°C), rinsed in the buffer (3 × 40 min), fixed in 2% osmium tetroxide in the same buffer (15 h in the dark at 5°C) and rinsed in the buffer (3 × 40 min). Samples were dehydrated in ethanol, 20–96% (30 min in each concentration), contrasted with 1% phosphowolfram acid and embedded in a medley of Epon epoxy resin (Epons: 812, MNA, HY 964 DY064(DMP 30)). For anatomy, semi-thin sections were cut with an ultramicrotome LKB 8800. For electron microscopy, ultrathin sections were cut with an ultramicrotome LKB 8800, contrasted

with lead citrate [6] and analysed with an electron microscope TESLA BS 500.

3. Results and discussion

The influence of the environment during plant ontogenesis is summarised in the seed. Compared to the germination success of the control unexposed seeds (85%), the germination of low exposure seeds was enhanced (100%) but decreased for high exposure collections, 55% and 27% at locations C and D, respectively. Similar growth stimulation in the early stages of development, and later promoted reproduction and senescence, were found to be effects of UV irradiation and the chemical retardant Ethephon [23]. However, for the Skrunda material, the influence of past exposure to further growth after germination requires testing.

The great variability of pine trees complicates silviculture and variable morphobiological processes are apparent already in the early stages of ontogenesis of pine trees. The number of seed lobes fluctuated from three to eight in all groups of examined pine seedlings (Fig. 1). This suggests great heterogeneity of *Pinus sylvestris* within each population, which can provide a forest stand with the ability of selective response to environmental conditions.

The basic anatomic structures are similar between pine needles [4,17,30]. The morphometric parameters of needles, cells and cell groups specific for *Pinus sylvestris* L. (Fig. 2) were examined in the Skrunda material, including: (1) the epidermal cells which consist mainly of thick secondary wall and narrow central cavity; (2) stomata along both adaxial and abaxial sides of the needle with large guard cells; (3) compact layer of hypodermic cells with large vacuoles which contain tannin; (4) mesophyll cells containing large tanniferous vacuoles and cytoplasm with its constituents — nucleus, chloroplasts, mitochondria, Golgi apparatus, endoplasmic reticulum, etc.; (5) 8–10 resin ducts embedded in the mesophyll tissues linked with an interior layer of epithelium cells and enclosed in a fiber sheath continuous with the hypodermis at one side of the duct; (6) thick-walled endodermis consisting of a more or

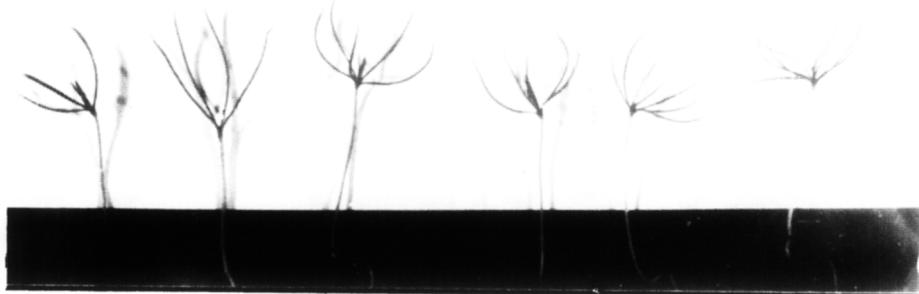


Fig. 1. Seedlings of *Pinus sylvestris* L. with different number of seed lobes: a, 3; b, 4; c, 5; d, 6; e, 7; f, 8.

less continuous layer of cells containing large chloroplasts; (7) separate thick-walled tracheids and small circular parenchyma cells which sometimes contain large lipid bodies; (8) two vascular bundles in which xylem points towards the adaxial side and phloem towards the abaxial side. The structural pathways of *in vivo* biosynthesis of complicated, highly polymerised products of pine needle metabolism were studied.

We observed that resin ducts of needles from non-irradiated pine trees had wide central tubes and flat light epithelium cells (Fig. 3a). The absence of any electron-microscopically distinguishable product in the central cavity of the resin ducts of second-year needles of all investigated pine trees confirms earlier observations [4,14,17,30]. However, in the central space of young resin ducts, a material interpreted as resin

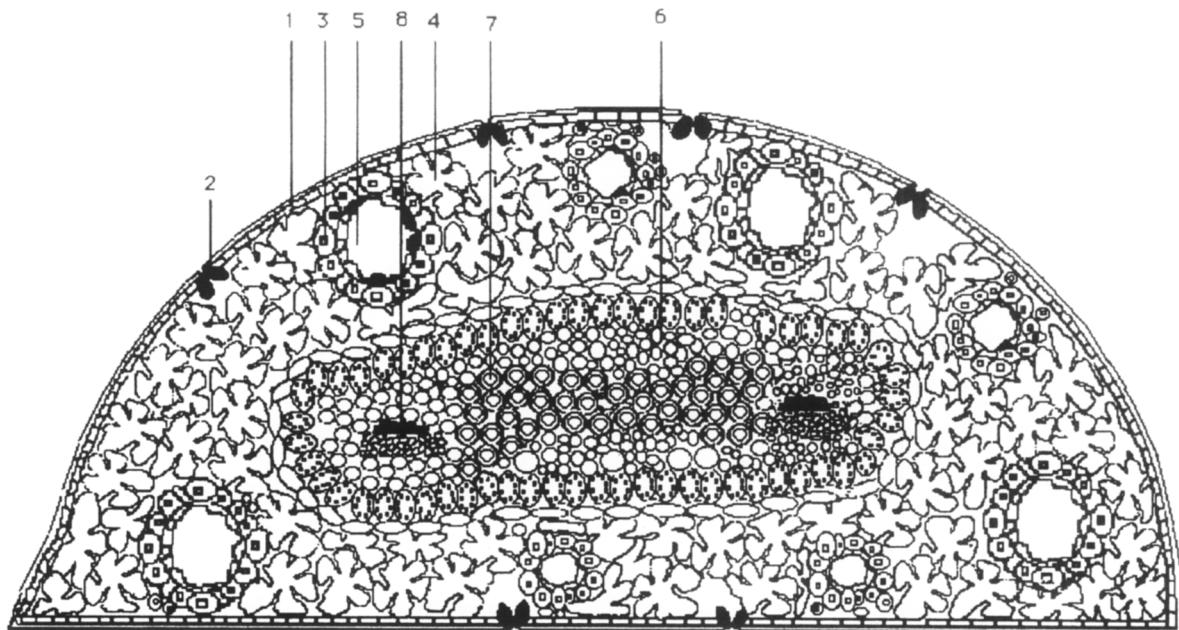


Fig. 2. Cross-section of *Pinus sylvestris* L. second-year needle: 1, epidermal cells; 2, stomata; 3, hypodermis; 4, mesophyll cells; 5, resin ducts; 6, endodermis; 7, tracheids and parenchyma cells; 8, vascular bundles.

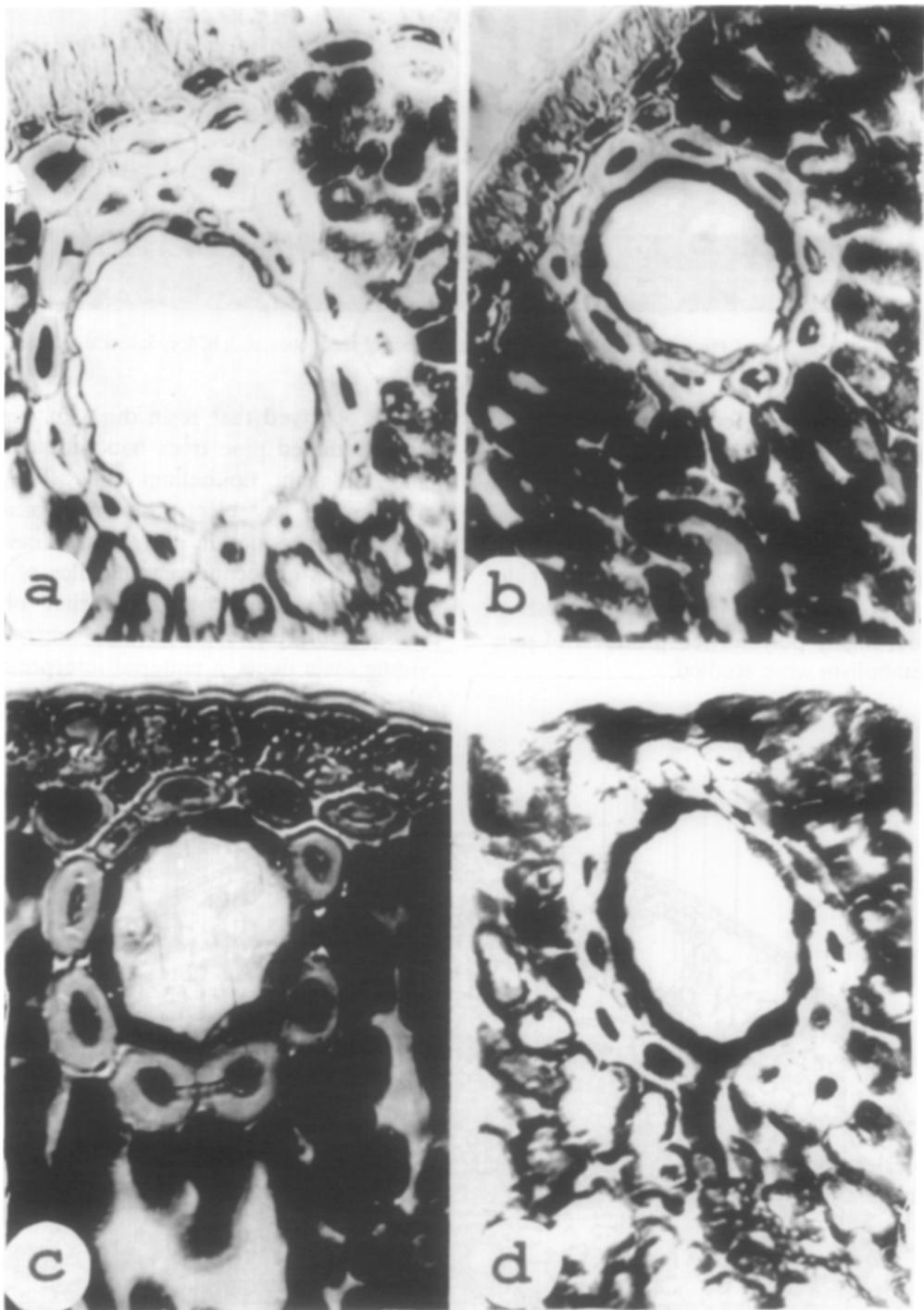


Fig. 3. The resin ducts in *Pinus sylvestris* L. second-year needles: (a) no EM exposure; (b) low EM exposure; (c) and (d) high EM exposure. Magnification: $\times 500$.

has been found [3]. Under the influence of low exposure to the Skrunda RLS, selective filling of some epithelium cells of the resin ducts of second-year needles with variable contents was typical. (Fig. 3b). High exposure to the Skrunda RLS caused abundant accumulation of an electron microscopically dense substance in the secretory epithelium and surrounding fiber sheath cells of the resin ducts (Fig. 3c,d). Evidently, this substance could be considered to be resin or a mixture of its predecessors. The resin is a complicated medley of fatty acids, noncyclic and cyclic terpenes, alcohols, phenol compounds and others. The basic content of the resin is controlled by the genetic system but environmental factors can affect cyclization, condensation or mutual transformation of constituents [18].

Resin is an accessory product of metabolism in epithelial cells of pine trees [4]. On the other hand, resin formation and secretion are metabolically active responses of pine trees to injury for self-protection against sap-rot and insect pests, since the resin hardens by evaporation of turpentine and covers wounded areas. It is well known that an increase in the ethylene level in plant tissues is a universal response to wounding and other stress effects [28]. We further propose that resin formation can be an additional process influenced by ethylene. Accumulation of dense substances in the epithelium and in the surrounding tissues of resin ducts of unwounded pine trees was observed after γ -radiation and was considered to be promoted by secretion of resin [14].

The ultrastructural features of mesophyll chloroplasts of second-year needles are as follows: dense stroma, a compact granal membrane system and a changeable amount of the starch granules and plastoglobules. Low and high exposure of EMF caused an increase in the amount of plastoglobules (Fig. 4a–d). Increases in number and area of plastoglobules in chloroplasts are unspecific stress responses due to different stress factors [8,16,22,23]. Osmiophilic globules were located near the chloroplasts in cytoplasm and a frequent sequence of emergence of the plastoglobules from chloroplasts was observed. Plastoglobules accumulate plastoquinone, α -tocopherol, α -tocochinon and other lipid-like substances as

newly developed lipid deposits or as chloroplast membrane decay products, which both can be exported from chloroplasts by exocytosis [9,15,21,26,27]. We propose two testable explanations for the appearance of high amounts of plastoglobules in chloroplasts of pine needles exposed to high EMF. The formation of temporary deposits of lipids in chloroplasts and their direct export into the cytoplasm can be the beginning of a complicated chain of resin production. On the other hand, lipids of both plastoglobules and osmiophilic globules of the cytoplasm can participate in the binding of hydrogen in electron transport and in the endogenous formation of the water, as in the metabolism of lipids in camels during lasting aridity [29]. Such a metabolism is typical in anoxic conditions [7] and in senescent plant tissues [25]. Changes in lipids of *Pinus sylvestris L.* needles have been reported to be caused by industrial air pollution [1].

The well developed Golgi apparatus, variable in position, size and state of physiologic activity, in the mesophyll cells of all investigated pine trees indicates high and varied secretory activities. This is consistent with our earlier proposition of several activities for Golgi apparatus: increased maturing and transport of proteins, polysaccharides and possibly conjugates of ethylene, both to the periphery of cytoplasm and in the secretory pathway; participation in the endogenous regulation of plants; building of plant structure; and in detoxification in plants [24]. In second-year needles of pine trees living in areas without increased levels of RF EMF, as well as in low exposure pine trees, the Golgi stacks are composed of a few short cisternae with considerably widened ends containing electron microscopic light substances (Fig. 5a,b). These are features of highly active Golgi complexes that actively transport different enzymes and substrate (predecessor of lignin and cellulose) to the periphery of cytoplasm. This reflects highly active cell wall thickening and wood formation [19].

With high RF EMF irradiation, the Golgi stacks are usually associated with large osmophilic globules formed at the maturing side of the Golgi complex (Fig. 5c,d). Similar globules have been identified as spherosomes, lysosomes or secretory

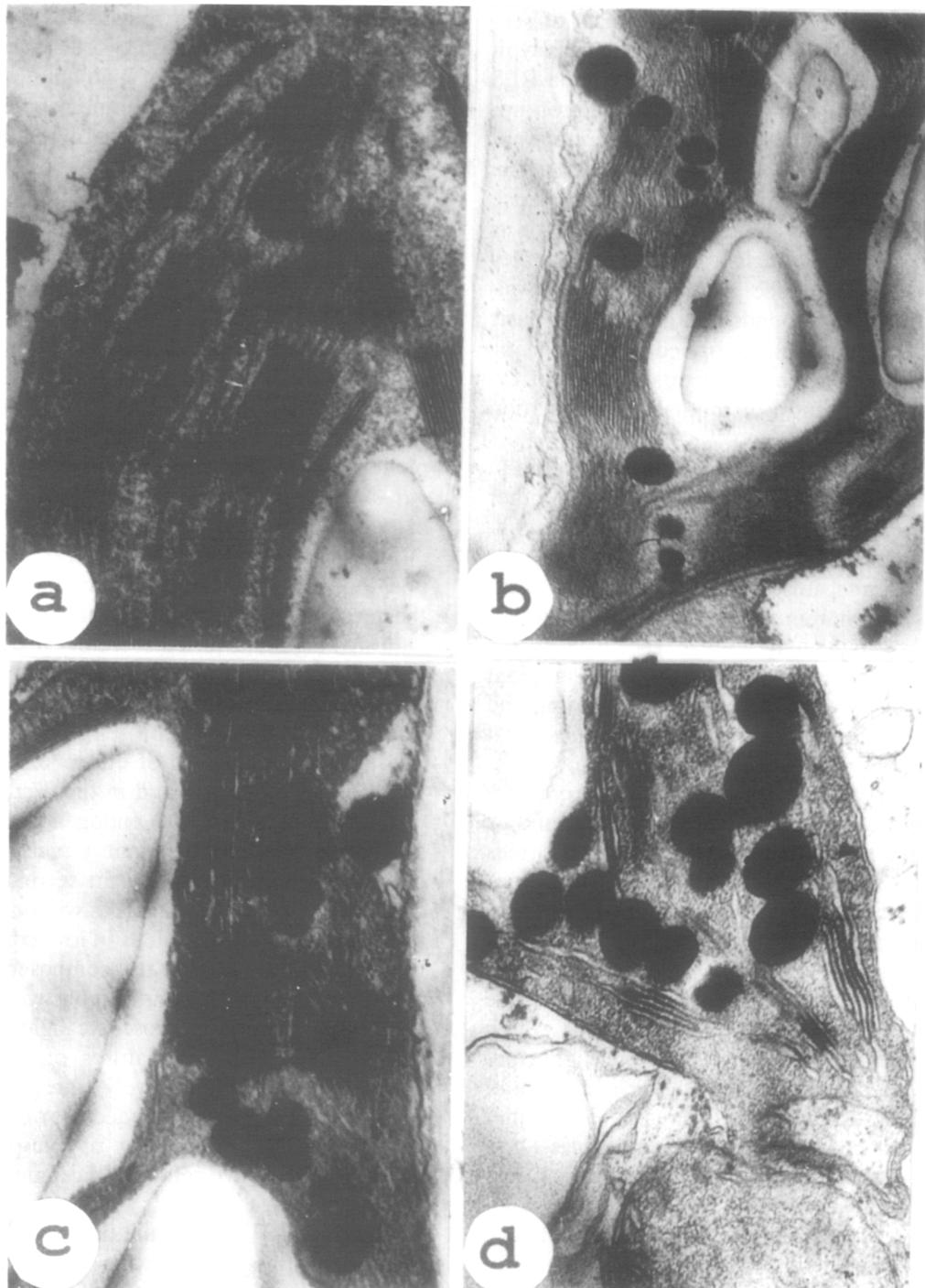


Fig. 4. The chloroplasts of mesophyll cells of second-year needles of *Pinus sylvestris* L. (a) no EM exposure; (b) low EM exposure; (c) and (d) high EM exposure; G, grana; S, starch; P, plastoglobules; O, osmiophilic globule. Magnification: $\times 20\,000$.

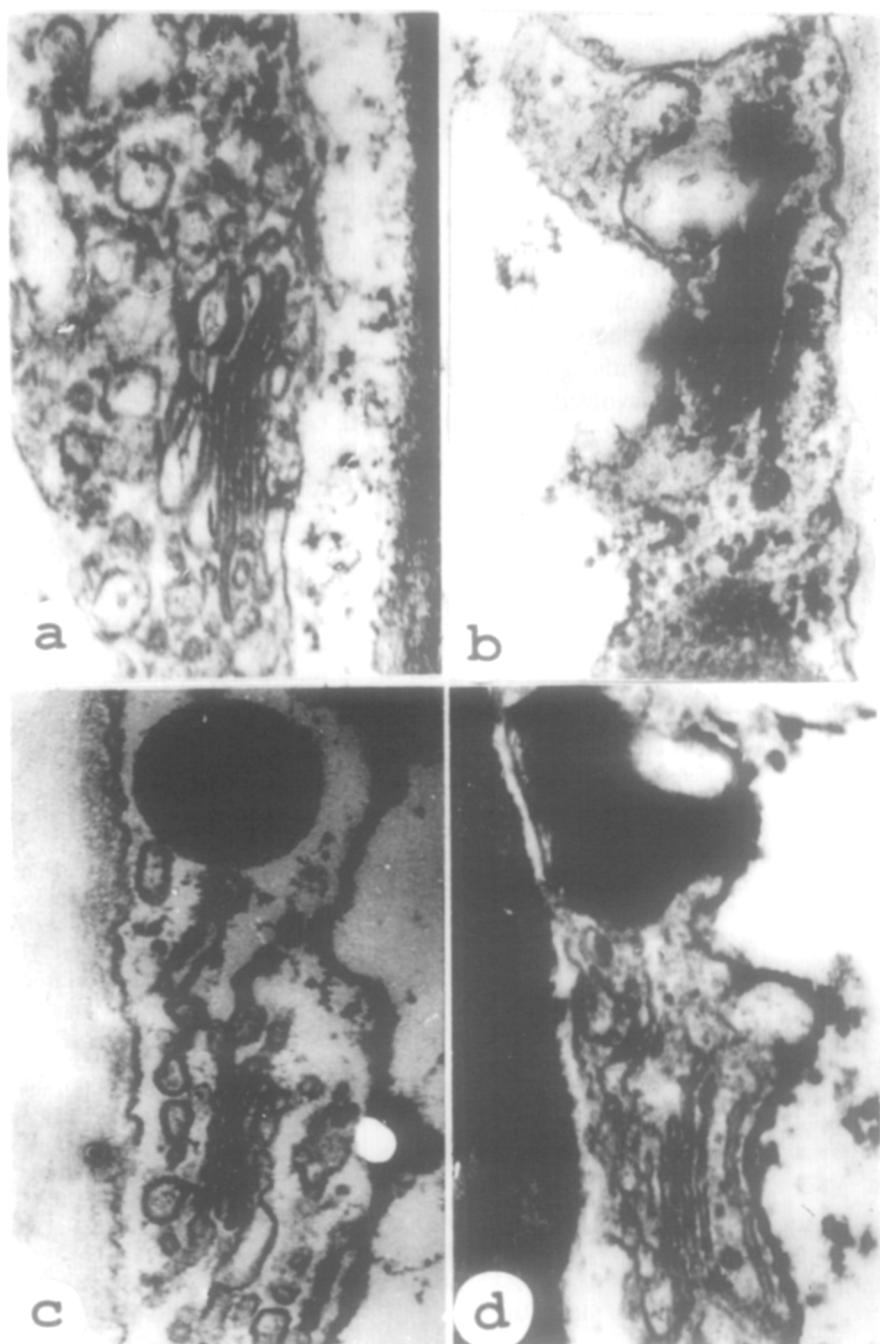


Fig. 5. The Golgi apparatus in the mesophyll cells of second-year needles of *Pinus sylvestris* L. (a) unirradiated tree; (b) indirectly irradiated tree; (c) and (d) directly irradiated tree. Magnification: $\times 60\,000$.

granules and they contain phospholipids and neutral lipids [5,16]. We observed that osmophilic globules are located near the plasmatic membrane, at the ends of short cisternae of endoplasmic reticulum connected with the concave, maturing face of Golgi apparatus. This Golgi-associated endoplasmic reticulum was termed GERL [20]. The functions of this structural chain in the secretory pathway remain unclear. Under the stress caused by RF EMF generated from the Skrunda RLS, the function of this chain could be involved in the secretion of resin precursors.

It can be assumed that osmophilic globules near the plasmatic membrane are involved in the synthesis of phenols. Phenol-type substances have been stated to be promoters of plant development and senescence [13]. Phenol-induced senescence of pine trees can explain the decrease in tree ring increment width and viability of pine forests caused by direct pulsed RF EMF irradiation.

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