E. Czerska et al. continued

and subdivided into identical aliquots in T25 T-flasks. Flasks were placed in the sham and exposure Crawford cells inside a CO2 incubator. After the 24-hour exposure, cells were examined for DNA content by flow cytometry in the cell cycle analysis program. The percentage of cells in G1, S and G2+M phase in the exposed vs. sham exposed tissue cultures was compared. Statistically significant increases in cell proliferation were observed at both exposure levels. The increase also appeared to be dose dependent. Recordings of temperature probes showed minimal heating at SAR 1.6 W/kg, and 1oC at SAR 4.767 W/kg. The 1oC increase may be responsible for part of the observed increase in cell proliferation at the higher SAR. However, a conventional heating temperature increase of 1oC did not stimulate cell proliferation to the same extent.

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NONTHERMAL EFFECTS OF MICROWAVE RADIATION ON MAMMALIAN CELLS: EXPERIMENTAL AND THEORETICAL RESULTS.

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Richmond, Virginia 23298

ABSTRACT

The precise degree of experimental control afforded by in vitro exposure systems has permitted investigation of direct cell physiological responses to microwave radiation under nonthermal exposure conditions. A variety of cellular alterations have been attributed to nonthermal microwave interactions. Such effects include: 1) altered cation transport and binding; 2) ion channel kinetic changes; 3) biochemical alterations; 4) effects on the mitotic cycle; and 5) modulation of cell proliferation and transformation. Whereas such effects are generally consistent with microwave-induced alteration of signal transductive pathways at the membrane surface detailed mechanistic has been elusive. Recent theoretical studies indicate spatially nonuniform microwave energy absorption on cell membrane surfaces due to field coupling with cell membrane bound water molecules. Predictions based on this interaction model will be described together with supporting experimental data derived from studies of microwave and radiofrequency radiation effects on cytikine/ receptor binding.

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Dropping the 'H' in WHO

Air Force Microwave–Cancer Study Shrouded in Mystery

Fifteen years ago, Dr. James Toler of the Georgia Institute of Technology sent the U.S. Air Force (USAF) a proposal for a study on the effects of long-term, low-level microwaves. The animal exposures began in 1989 and were completed the following year. But today, more than six years later, the results have yet to be released officially.

In June 1995, Toler was expected to present his findings at a special workshop organized by the USAF, which was held in Boston the day before the start of the Bioelectromagnetics Society's (BEMS) annual meeting. But he declined to do so.

"I went to the meeting expecting to see some conclusive data and I am still waiting," Dr. Asher Sheppard, a consultant based in Redlands, CA, said in a recent interview.

In his Boston talk, Toler said that at one point in 1990 he had observed more mice with tumors among the exposed group than among the controls—42 versus 35—but that by the end of the experiment there were no differences in the numbers of tumors or survival rates between the two groups. He would not be more specific, promising instead to present the data during the BEMS meeting. Later that day, Toler went home to Atlanta and the results remained a mystery.

In an interview a month after the BEMS meeting, Toler still would not talk about the tumor counts, but he remained firm about the study's findings: "We saw no effects and the biostatistician saw no effects."

James Merritt, Toler's project officer at the USAF's Armstrong Laboratory

(continued on p.13)

Physicists and Biologists Butt Heads at First NIEHS EMF Risk Workshop

Once again, it was the biologists versus the physicists. This time they met at a symposium on cellular studies convened by the National Institute of Environmental Health Sciences (NIEHS) to help the agency assess health risks from exposures to electromagnetic fields (EMFs).

At the opening session on March 24 in Durham, NC, Dr. James Weaver, a physicist at the Massachusetts Institute of Technology (MIT) in Cambridge, MA, presented a model showing that the thresholds for EMF interactions have to be above 1 G. "I have a hard time understanding the experimental data for levels below that," he told *Microwave News*.

But the biologists were not buying it. "There appears to be wide acceptance of the existence of *in vitro* biological effects down to 10-20 mG," countered Dr. Martin Blank of Columbia University's medical school in New York City, who spoke for many of those at the meeting.

(continued on p.7)

Adey Finds No Effect, Detrimental or Protective, Among Rats Exposed to Analog Wireless Phone Radiation

Reprinted below is the abstract of a paper, "Brain Tumor Incidence in Rats Chronically Exposed to Frequency-Modulated (FM) Cellular Phone Fields," which will be presented by Dr. Ross Adey on June 9 at the 2nd World Congress for Electricity and Magnetism in Biology and Medicine *in Bologna, Italy (see p.18).*

Adey, who is with the VA Hospital in Loma Linda, CA, reported last summer that a similar experiment designed to mimic the signals from a TDMA digital mobile phone appeared to show a protective effect. That is, the animals exposed to pulsed radiation had fewer tumors than did controls (see MWN, *M/J96 and J/A96).*

Adey's more recent experiment used FM signals patterned after the radiation from an analog cellular phone. Both the FM and TDMA animal studies were sponsored by Motorola Corp.

One observer, who was aware of Adey's new results, commented that, "It's the low-frequency modulation that makes all the difference," referring to the 50 Hz modulation associated with the TDMA signals.

Frequency-modulated mobile phone systems have been the dominant technology for many years. Their continuing use worldwide appears certain in many applications, with typical RF carrier frequencies in the range from 0.1-1.5 GHz. Body tissues, specifically in the head and hand of the user, absorb up to 40% of the radiated signal. Portable FM phones, with average output powers of 0.6 W in the 800 MHz frequency band, induce field strengths in the most exposed tissues equivalent to 1 W/Kg±6db, depending on the device's position and design. Although occupational exposures of certain microwave workers to radar and other pulsed fields have been reported to carry an increased risk of brain tumors (e.g., Thomas et al., 1987), no comparable data have been reported for FM mobile phone users.

OBJECTIVE: Previous universal use of FM technology in mobile communication systems, as well as its continuing availability, suggest [the] need for assessment of possible human brain tumor risks in a suitable animal model; and to compare findings with our previous study in rats exposed to North American Digital Cellular (NADC) signals (Adey et al., *Proceedings of the Bioelectromagnetics Society,* 18th Annual Meeting, 1996). We have sought evidence for perturbation by FM phone fields of spontaneous brain tumor incidence, and in rats exposed to single doses of the short-lived carcinogen ENU *in utero,* and thereafter exposed intermittently to FM phone fields for 24 months (mean life span 26 months). Low ENU dosage was selected to give maximum sensitivity to possible tumor modulation by FM phone fields over the lifetime of the animal[s].

METHODS: We tested a frequency-modulated 836.55 MHz signal, with ±12.5 kHz maximum deviation. Modulation was by a recorded pattern of "balanced speech" that generated all major speech components in a 2-minute epoch that recycled continuously. Pregnant Fischer 344 rats were randomly assigned to 6 groups. They received either a single tail-vein injection of the carcinogen ethylnitrosourea (ENU, 4 mg/Kg) or inert buffer solution on gestational day 18. Far-field exposures (horn radiator, 836 MHz, circularly polarized) began on day 19 and continued after parturition until weaning at age 23 days. Offspring (n=540) of the 6 maternal groups then became treatment cohorts: Sham/Control (SC), n=90, 45M, 45F; ENU/Control (EC), n=90, 45M, 45F; Sham/Sham (SS), n=90, 45M, 45F; Sham/Field (SF), n=90, 45M, 45F; ENU/Sham (ES), n=90, 45M, 45F; ENU/Field (EF), n=90, 38M, 52F. Exposures simulating near-fields at a phone user's head began at 35 days, and continued for the next 23 months. Exposures were for 2 hours daily, antenna power 2.5 W, field-on 7.5 minutes, field-off 7.5 minutes. Far-field averaged SARs (modeled): pregnant dam (uterus) 1.0 W/Kg; fetus (brain) 0.9 W/Kg; isolated pup (brain) 0.1 W/Kg; young rat (brain) 0.4 W/Kg. Averaged near-field brain thermographic SARs: average males 2.3 W/Kg; average females 1.8 W/Kg. Survivors of the original 540 rats (n=372, 69%) were sacrificed at 730-733 days.

RESULTS: There were no effects on brain tumor incidence attributable to the FM fields in either the control or the ENU groups. There was the expected higher incidence of brain tumors in the ENU-exposed groups (EC, 15; ES, 17; EF, 15), in comparison with the low incidence of spontaneous brain tumors in the sham and control groups (SC, 2; SS, 1; SF, 3), $p < 0.001$. Comparing survival rates, lifetimes of ENU-exposed animals were significantly shorter than [those of] controls ($p < 0.0005$), but these differences were not influenced by FM field exposures.

DISCUSSION: The findings here are consistent with our previous study in rats exposed to NADC signals. The apparent "protective" effect in the NADC study, which did not gain statistical support, was not detected with FM fields. These effects have been modeled in the normal homeostatic balance between mechanisms regulating damage and repair in cell growth. They may indicate sensitivities to the 50/second pulsed characteristics of TDMA fields. Other packet frequencies in current use or proposed for cell phone use (GSM=217, iDEN=22, Iridium=11) may therefore merit specific study.

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In fact, Toler had sent the USAF his final report a full year before the BEMS meeting.

For close to a year after the 1995 meeting, the USAF refused to release the report to *Microwave News* despite repeated requests under the Freedom of Information Act. When the USAF finally conceded, the report was stamped "Do Not Cite or Quote."

Those within the military have also been denied access to the results. "I was trying to get the article from [Toler] for over two years before I gave up," said Dr. Edward Elson. At the time, Elson was working on radiofrequency and microwave (RF/MW) bioeffects at the Walter Reed Army Institute for Research in Washington. He is now studying Gulf War illnesses for the Army.

Even members of the Armstrong Lab have been denied access to the Toler data—as Dr. Richard Albanese of the Occupational and Environmental Health Directorate found out when he asked to see the report.

The Guy Study

In 1984, Dr. Bill Guy released results from his long-term, low-level exposure study which showed that rats exposed to pulsed microwave radiation had a statistically significant increase in malignant tumors. The five-year, $5 million project, which was sponsored by the USAF, was the first long-term RF/MW study ever done in the U.S. (see *MWN*, J/A 84 and Mar85).

Guy exposed 100 male rats to 0.48 mW/cm^2, 2,450 MHz pulsed microwaves (10 microsecond pulses, 800 pulses per second) 21 hours per day for up to 25 months. The specific absorption rate, or SAR, was 0.4 W/Kg or less. A second set of 100 unexposed rats were used as controls. All the rats were housed in a pathogen-free environment.

Guy found a total of 18 malignancies among the exposed group and 5 among the controls. In particular, there were 9 endocrine tumors in the exposed group and 2 in the control group.

Although the study did not turn up an excess of benign tumors, there were 6 pheochromocytomas—benign adrenal tumors—all in the exposed rats.

Results from the study were first presented at the 1984 BEMS meeting in Atlanta. They were published eight years later in a special issue of *Bioelectromagnetics*, commemorating Guy's retirement from the University of Washington, Seattle (see *MWN*, J/F93).

"It's unfortunate that the data have not been released sooner, especially since it is a chronic exposure study. We should have had it a long while ago," said Dr. Stephen Cleary of Virginia Commonwealth University in Richmond.

Toler's $600,000 study is widely seen as a small-scale follow-up to Dr. Bill Guy's $5 million, long-term exposure study—also sponsored by the USAF and thought by many to show an elevated risk of cancer (see box above). As Dr. Samuel Milham, now a consultant based in Olympia, WA, commented more than ten years ago: "The findings are so suggestive of potential microwave effects that the portions of Dr. Guy's study dealing with immune status and neoplasia should, at a minimum, be replicated" (see *MWN*, N/D86).

Guy himself agrees. "I thought it should be repeated from the beginning," he told *Microwave News* in mid-March.

Dr. C.K. Chou, Guy's collaborator on the long-term exposure study, also favors more research. "The biological significance of these effects needs to be explored," he said at the 1995 USAF workshop. After leaving Guy's lab, Chou joined the City of Hope National Medical Center in Duarte, CA.

Guy's results continue to be cited as showing a positive effect. In a March 13 letter to Wireless Technology Research (WTR), Dr. Elizabeth Jacobson of the U.S. Food and Drug Administration (FDA) recommended that WTR perform a "careful replication of the Chou and Guy study which suggests that chronic exposure of rats to microwaves is associated with an increase in tumors" (see p.11).

The USAF, however, maintains that Guy's results do not point to adverse health effects. "His findings were that there were no effects of radiation—I mean, that's well known," Merritt said in an interview.

Toler's study differed from Guy's in several key respects. First, although Toler had initially set out to expose rats as Guy had done, the USAF amended the study design in 1987, stipulating instead the use of breast cancer-prone mice.

Also, Toler exposed 200 mice to radiation at 435 MHz rather than 100 rats at 2.45 GHz as Guy had done (see box below). According to Merritt, the lower frequency was designed to mimic the signal of a USAF early-warning radar system, PAVE PAWS.

Blood chemistry indices were monitored by Guy and were part of Toler's original study design. But they were later rejected by the USAF. When asked why, Toler responded, "As I recall, we could have done a whole lot more analyses. The primary interest was in mammary tumors."

In the report he wrote for the USAF, Toler did not give the final, overall tumor counts, but stated, "There was no evidence to suggest differences in tumor rates between sham-exposed and exposed animals." He stated that the final survival rates were the same for both groups.

Toler looked at a variety of tissues for cancer but did not examine the mice for leukemia. An appendix to the report with results of tissue analyses conducted by Pathology Associates Inc. (PAI) in Frederick, MD, appears to show that 178 of the exposed mice had cancer, as compared to 158 of the sham-exposed. There were also more total malignancies among the exposed mice (297) than among the sham-exposed mice (261). Some animals had more than one tumor. (These totals were added up by *Microwave News* from the data in the report by PAI.)

Liver cancer was the most prevalent type of malignant tumor, with 104 cases among the exposed mice and 98 among the controls. Toler did find a statistically significant increase ($p=0.033$) in hemangioma, a cancer of the blood vessels. But he dismissed the result as not significant because of "the absence of evidence of a difference between exposed and sham-exposed animals with

The Toler Study

Beginning in March 1989, Dr. James Toler exposed 200 female, mammary tumor-prone mice to 1 mW/cm^2, 435 MHz pulsed microwaves. The pulses had a width of 1 microsecond and a repetition rate of 1 kHz. The specific absorption rate, or SAR, was 0.32 W/Kg.

A second group of 200 mice served as controls. And an additional 25 mice were included among both the exposed mice and the controls to serve as "sentinels"—to be sacrificed in the course of the study to monitor the health of the entire colony.

The mice were exposed starting when they were approximately five weeks old for an average of 20 hours a day, seven days a week for 21 months. Once a week the animals were palpated for tumors and weighed.

In December 1990, the mice that were still alive were killed. Samples of 22 different tissues from all the animals were analyzed by Pathology Associates Inc. in Frederick, MD.

respect to combined hemangioma or hemangiosarcoma."

As expected, cases of mammary gland adenocarcinomas were high, but the exposed mice had only three more tumors than did the controls, 85 compared to 82. The median tumor onset time for these adenocarcinomas was nine days earlier for the exposed mice, though this finding was not statistically significant at the 0.05 level, according to Toler's report.

A year after the experiment began in March 1989, Toler indicated a consistent trend of a higher tumor incidence among exposed animals. By the end of May 1990, he had observed 42 exposed mice with tumors and 34 controls with tumors, a slightly different figure from what he reported in Boston in 1995.

In a July 20, 1990, memo to Merritt, Toler suggested extending the experiment by three months into December to avoid accusations of not exposing the mice for an adequate length of time. Merritt agreed with the proposal, scribbling in response at the bottom of the memo: "This has been a very well run study so far. It will become a landmark study in time."

At the end of that July, Toler reported finding 76 exposed mice with tumors and 67 sham-exposed mice with tumors. The following month, Toler reported to the USAF that the total number of animals with tumors, including possible tumors, was 109 for the exposed group and 87 for the sham-exposed group.

That September, when the experiment had originally been scheduled to end, Toler stopped reporting tumor counts to the USAF. He explained that he had "difficulty in assuring that observed abnormalities [were] actually tumors when the examinations [were] based solely on palpation."

Nonetheless, the health status updates for each animal continued to note, on the basis of palpation, those that appeared to have tumors. Based on these figures, the total of exposed animals with tumors at the end of September rose to 115, with 88 tumors among the controls.

The USAF recently submitted Toler's paper to *Radiation Research*. In an effort to explain the delay in publication, Toler said that the USAF had sent his paper to other journals before, but was told that the paper would have to be shortened. "At any rate, it was a sizable effort to trim it down to get it published," he said.

Merritt said that he would not comment on the results until they are published and was reluctant to say whether such research was worthy of further investigation. "You never close the door on studies. You'll always get complaints—you didn't do this, you didn't do that," he said in a recent interview. "I don't know whether this should be continued."

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"MICROWAVE NEWS" FLASHBACK

Years 15 Ago

- Two-way radios attached to workers' suits interfere with gas monitors, causing an eight and a half hour alert at the Three Mile Island nuclear plant in Middletown, PA.
- The Navy announces a $49.8 million plan to upgrade its ELF submarine communications system, with most of the money to be spent in Michigan and Wisconsin, and says it will monitor the ecological effects of the plan in Michigan but not in Wisconsin.
- Two legal secretaries in Rhode Island reach an undisclosed settlement with Amana Refrigeration Co., makers of the Radarange microwave oven, after only one day of testimony in the U.S. District Court in Providence. The two claimed to have developed cataracts after being exposed to radiation from a faulty oven in their lunchroom.
- Occupational Safety and Health Administration officials decide to stop citing companies for violating the RF/MW exposure standard, saying that the limits are meant only as an "advisory" guideline.

Years 10 Ago

- A committee assembled by the World Health Organization and the International Radiation Protection Association recommends magnetic field safety limits based on short-term health effects, not cancer risks.
- Two British researchers publish a paper in *Clinical Ecology* showing that weak EMFs can cause allergic reactions such as migraines and convulsions.
- A joint EPA–FCC study finds RF "hot spots," as much as ten times above the ANSI exposure limits, in public areas near some FM broadcast towers.

Years 5 Ago

- A Finnish study concludes that women exposed to ELF magnetic fields of more than 3 mG (rms) from video display terminals (VDTs) are more likely to have miscarriages, but does not find a link between the number of hours spent using VDTs and miscarriages.
- The U.K.'s Coordinating Committee for Cancer Research launches a £6 million study of the possible causes of childhood leukemia, including the role of EMFs.
- Traffic radar units are blamed in seven testicular cancer cases among police officers in Grand Rapids, MI, three times more than would be expected, according to a study by the University of Washington.

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1996
SESSION LAWS
OF THE
STATE OF WASHINGTON

REGULAR SESSION
FIFTY-FOURTH LEGISLATURE
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Chapter 6, Laws of 1969.

DENNIS W. COOPER
Code Reviser

E172

Section 1 of Engrossed House Bill No. 2613 contains a provision that would allow school districts to deny an application for admission from a nonresident student if the student's disciplinary record indicates a history of behavior that has been disruptive to the educational process.

I understand that several school districts are seeking this authority because they have been unable, in the past, to deny admission to nonresident students with long histories of serious disciplinary problems. While I appreciate the frustration of these districts in such cases, I am concerned that this broadly-worded provision would authorize school districts across the state to deny admission to nonresident students with any kind of disciplinary record. Such authority is clearly inappropriate and inconsistent with the responsibility of our public school system to provide educational services for all our children. A more targeted approach is necessary to address the concerns raised by these school districts.

For these reasons, I have vetoed section 1 of Engrossed House Bill No. 2613.

With the exception of section 1, Engrossed House Bill No. 2613 is approved."

CHAPTER 322

[House Bill 2716]

WASTE DISCHARGE PERMITS—EXEMPTION FROM STATE ENVIRONMENTAL POLICY ACT PROVISION FOR EXISTING DISCHARGES

AN ACT Relating to review of renewal of waste discharge permits; and adding a new section to chapter 43.21C RCW.

Be it enacted by the Legislature of the State of Washington:

NEW SECTION. Sec. 1. A new section is added to chapter 43.21C RCW to read as follows:

The issuance, reissuance, or modification of a waste discharge permit that contains conditions no less stringent than federal effluent limitations and state rules is not subject to the requirements of RCW 43.21C.030(2)(c). This exemption applies to existing discharges only and does not apply to new source discharges.

Passed the House February 6, 1996.
Passed the Senate March 7, 1996.
Approved by the Governor March 30, 1996.
Filed in Office of Secretary of State March 30, 1996.

CHAPTER 323

[Engrossed Substitute House Bill 2828]

PERSONAL WIRELESS SERVICE FACILITIES

AN ACT Relating to personal wireless service facilities; adding a new section to chapter 43.21C RCW; adding a new section to chapter 80.36 RCW; adding a new section to chapter 19.27A RCW; adding a new section to chapter 70.92 RCW; adding new sections to chapter 43.70 RCW; and creating a new section.

Be it enacted by the Legislature of the State of Washington:

NEW SECTION. Sec. 1. The legislature finds that concerns have been raised over possible health effects from exposure to some wireless telecommunications facilities, and that exposures from these facilities should be kept as low as reasonably achievable while still allowing the operation of these networks.

The legislature further finds that the department of health should serve as the state agency that follows the issues and compiles information pertaining to potential health effects from wireless telecommunications facilities.

NEW SECTION. Sec. 2. A new section is added to chapter 43.21C RCW to read as follows:

(1) Decisions pertaining to applications to site personal wireless service facilities are not subject to the requirements of RCW 43.21C.030(2)(c), if those facilities meet the following requirements:

(a)(i) The facility to be sited is a microcell and is to be attached to an existing structure that is not a residence or school and does not contain a residence or a school; or (ii) the facility includes personal wireless service antennas, other than a microcell, and is to be attached to an existing structure (that may be an existing tower) that is not a residence or school and does not contain a residence or a school, and the existing structure to which it is to be attached is located in a commercial, industrial, manufacturing, forest, or agricultural zone; or (iii) the siting project involves constructing a personal wireless service tower less than sixty feet in height that is located in a commercial, industrial, manufacturing, forest, or agricultural zone; and

(b) The project is not in a designated environmentally sensitive area; and

(c) The project does not consist of a series of actions: (i) Some of which are not categorically exempt; or (ii) that together may have a probable significant adverse environmental impact.

(2) The department of ecology shall adopt rules to create a categorical exemption for microcells and other personal wireless service facilities that meet the conditions set forth in subsection (1) of this section.

(3) For the purposes of this section:

(a) "Personal wireless services" means commercial mobile services, unlicensed wireless services, and common carrier wireless exchange access services, as defined by federal laws and regulations.

(b) "Personal wireless service facilities" means facilities for the provision of personal wireless services.

(c) "Microcell" means a wireless communication facility consisting of an antenna that is either: (i) Four feet in height and with an area of not more than five hundred eighty square inches; or (ii) if a tubular antenna, no more than four inches in diameter and no more than six feet in length.

NEW SECTION. Sec. 3. A new section is added to chapter 80.36 RCW to read as follows:

(1) If a personal wireless service provider applies to site several microcells in a single geographical area:

(a) If one or more of the microcells are not exempt from the requirements of RCW 43.21C.030(2)(c), local governmental entities are encouraged: (i) To allow the applicant, at the applicant's discretion, to file a single set of documents required by chapter 43.21C RCW that will apply to all the microcells to be sited;

and (ii) to render decisions under chapter 43.21C RCW regarding all the microcells in a single administrative proceeding; and

(b) Local governmental entities are encouraged: (i) To allow the applicant, at the applicant's discretion, to file a single set of documents for land use permits that will apply to all the microcells to be sited; and (ii) to render decisions regarding land use permits for all the microcells in a single administrative proceeding.

(2) For the purposes of this section:

(a) "Personal wireless services" means commercial mobile services, unlicensed wireless services, and common carrier wireless exchange access services, as defined by federal laws and regulations.

(b) "Microcell" means a wireless communication facility consisting of an antenna that is either: (i) Four feet in height and with an area of not more than five hundred eighty square inches; or (ii) if a tubular antenna, no more than four inches in diameter and no more than six feet in length.

NEW SECTION. Sec. 4. A new section is added to chapter 19.27A RCW to read as follows:

(1) The state building code council shall exempt equipment shelters of personal wireless service facilities from building envelope insulation requirements.

(2) For the purposes of this section, "personal wireless service facilities" means facilities for the provision of personal wireless services.

NEW SECTION. Sec. 5. A new section is added to chapter 70.92 RCW to read as follows:

(1) The state building code council shall amend its rules under chapter 70.92 RCW, to the extent practicable while still maintaining the certification of those regulations under the federal Americans with disabilities act, to exempt personal wireless services equipment shelters, or the room or enclosure housing equipment for personal wireless service facilities, that meet the following conditions: (a) The shelter is not staffed, and (b) to conduct maintenance activities, employees who visit the shelter must be able to climb.

(2) For the purposes of this section, "personal wireless service facilities" means facilities for the provision of personal wireless services.

NEW SECTION. Sec. 6. A new section is added to chapter 43.70 RCW to read as follows:

When funds are appropriated for this purpose, the department shall conduct a survey of scientific literature regarding the possible health effects of human exposure to the radio frequency part of the electromagnetic spectrum (300Hz to 300GHz). The department shall submit the survey results to the legislature, prepare a summary of that survey, and make the summary available to the public. The department shall update the survey and summary periodically.

NEW SECTION. Sec. 7. A new section is added to chapter 43.70 RCW to read as follows:

Unless this section is preempted by applicable federal statutes, the department may require that in residential zones or areas, all providers of personal wireless services, as defined in section 1 of this act, provide random test results on power density analysis for the provider's licensed frequencies showing radio frequency levels before and after development of the personal wireless service antenna facilities, following national standards or protocols of the federal communications commission or other federal agencies. This section shall not apply to microcells as defined in section 3 of this act. The department may adopt rules to implement this section.

Passed the House March 6, 1996.
Passed the Senate March 7, 1996.
Approved by the Governor March 30, 1996.
Filed in Office of Secretary of State March 30, 1996.

CHAPTER 324

[Senate Bill 6171]

SPECIAL PURPOSE DISTRICT ELECTIONS—CERTAIN PRIMARIES ELIMINATED

AN ACT Relating to special purpose district elections; and amending RCW 29.21.015, 36.69.090, 68.52.140, and 68.52.155.

Be it enacted by the Legislature of the State of Washington:

Sec. 1. RCW 29.21.015 and 1990 c 59 s 90 are each amended to read as follows:

(1) No primary may be held for any single position in any city, town, or district, as required by RCW 29.21.010, if, after the last day allowed for candidates to withdraw, there are no more than two candidates filed for the position. The county auditor shall, as soon as possible, notify all the candidates so affected that the office for which they filed will not appear on the primary ballot.

(2) No primary may be held for the office of commissioner of a park and recreation district or for the office of cemetery district commissioner.

(3) Names of candidates ((~~so notified~~)) for offices that do not appear on the primary ballot shall be printed upon the general election ballot in the manner specified by RCW 29.30.025.

Sec. 2. RCW 36.69.090 and 1994 c 223 s 45 are each amended to read as follows:

A park and recreation district shall be governed by a board of five commissioners. Except for the initial commissioners, all commissioners shall be elected to staggered four-year terms of office and shall serve until their successors are elected and qualified and assume office in accordance with RCW 29.04.170. Candidates shall run for specific commissioner positions.

Elections for park and recreation district commissioners shall be held biennially in conjunction with the general election in each odd-numbered year. Elections shall be held in accordance with the provisions of Title 29 RCW

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Does the Skrunda Radio Location Station diminish the radial growth of pine trees?

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Abstract

The Skrunda Radio Location Station (RLS), which has operated continuously for more than 20 years, has created a unique area for the study of pulse radio-frequency electromagnetic field effects. Trees were chosen to assess these effects. Since 1990, permanent plots have been established in pine forest stands around the Skrunda RLS and in control areas. The dynamics of tree growth changes were analysed using retrospective tree ring data. There is a statistically significant ($P < 0.01$) negative correlation between the relative additional increment in tree growth and the intensity of the electric field. The radial growth of pine trees has diminished in all plots that received electromagnetic radiation. This decrease in growth began after 1970, which coincided with the start of operation of the Skrunda RLS, and was subsequently observed throughout the period of study. The effects of many other environmental and anthropogenic factors were evaluated, but no significant effects on tree growth were observed.

Keywords: Radio-frequency electromagnetic radiation; Radars; Tree rings; Dendroecology

1. Introduction

The question of whether weak radio-frequency (RF) electromagnetic fields pose risks continues to divide researchers into opposing, even antagonistic, camps. If any effects of RF radiation on living organisms exist, then they are hard to prove. Thus, the most suitable organisms for investigation must be chosen, and original methods are required.

In the present paper, trees were chosen to assess the effects of Skrunda Radio Location Station (RLS) electromagnetic radiation. Essential physiological processes, such as assimilation, cell division and growth, mineral nutrition and others, occur in every tree and are affected by the environment in a hundred square meter surrounding area. Therefore, trees are particularly sensitive and react to environmental changes. Tree growth is fixed in increments (tree rings) and thus the measured increments indicate positive or negative environmental conditions. A suitable tree ring chronological method has been developed in

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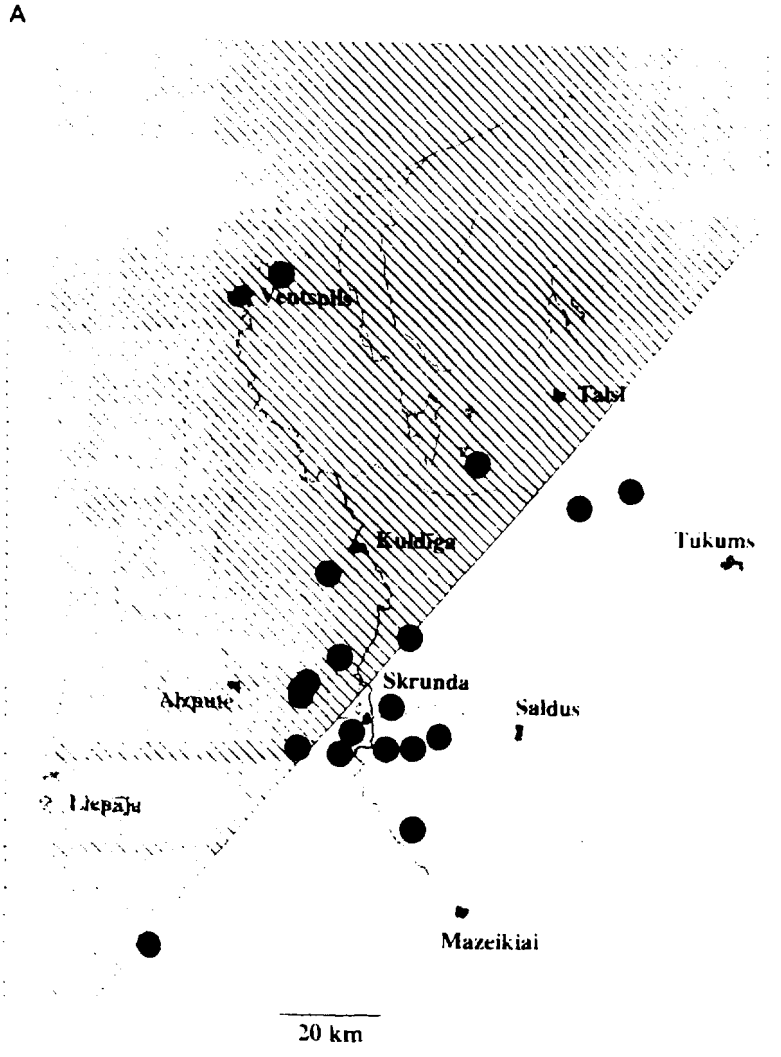
Latvia that allows the determination of the effect of various environmental factors [1]. Multidisciplinary research, based on tree ring analysis, has been conducted in forest ecosystems around Skrunda since 1990. Tree ring dynamics are described in the present paper for a 30-year period (1959–1988), and changes are discussed in relation to electromagnetic radiation as well as geomorphologic, hydrological, silvicultural, edaphic, chemical and radioactive factors.

2. Subjects and methods

2.1. Skrunda Radio Location Station

The impulse type Skrunda RLS for space control, located in the Venta River Valley, has operated continuously since 1971. It is composed of two sectors, each with receiving-transmitting apparatus that ensure the RLS function in four independent sectors. The transmission parameters of the Skrunda RLS are: (1) pulse power of



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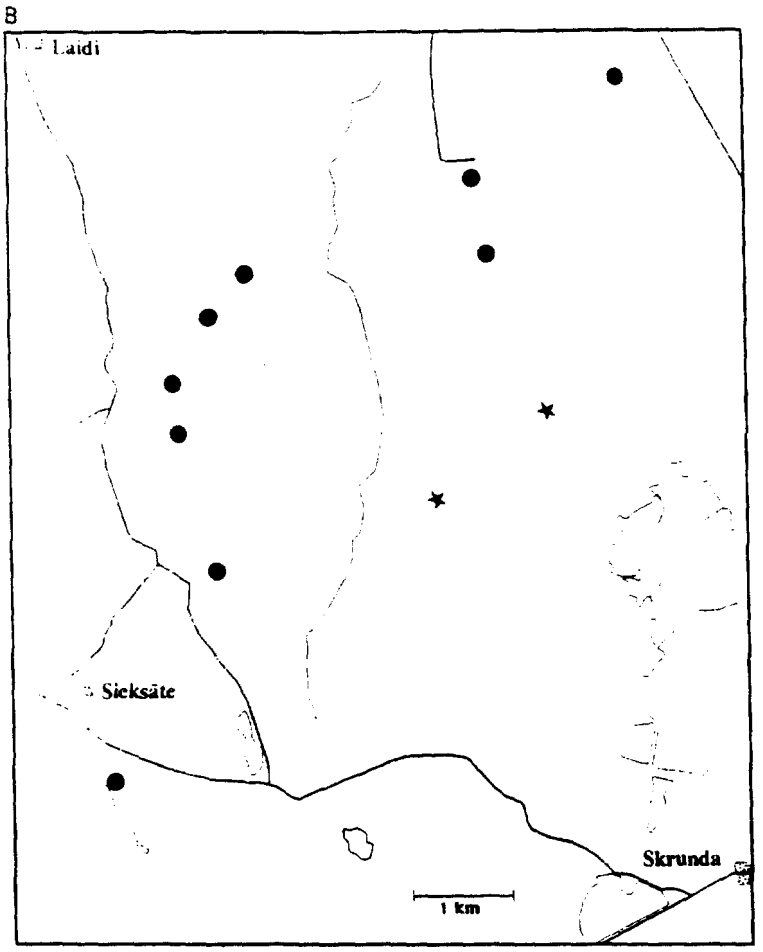

Fig. 1. Sampling plots (●) in Western Latvia (A) and the Skrunda region (B). The location of the Skrunda radar system (★) and its emission zone are shown.

each of four transmitters — 1.25 MW; (2) pulse duration — 0.8 ms; (3) duty cycle — 50; (4) frequency range — 154–162 MHz; (5) polarisation of transmitting signal — horizontal. The radar operating parameters and the method of radiation intensity measurement are given in this issue [2].

2.2. Sampling locations

In the Skrunda area, 29 permanent sampling locations were established (Fig. 1) at various distances from the source. Plots were set up in dominant Scots pine (*Pinus sylvestris* L.) stands with spruce as an understorey. Tree age was 50–90 years. Plot size was at least 400 m^2 and each plot contained a minimum of 20 overstorey pine stems per plot. Pines were sampled with a Pressler borer and their diameters and heights were measured. A wealth of other variables were measured, including metal concentrations, ionising radiation, etc., but since these data are only briefly mentioned in this paper, the methods used (all standard methods) are not described here.

2.3. Absolute values of the tree ring widths

The radial annual growth increments $w(i,t,l)$; i = individual, t = year, l = plot, were measured at a precision of ± 0.01 mm. For each tree, the geometric mean width $\overline{w}_c = (i,\Delta t_c,l)$ of the control time period $\Delta t_c = t_1 ... t_2$, where t_1 the year at

the beginning of the control period, and t_2, the year at the end of the control period, is given by:

$$\bar{w}_c(i,\Delta t_c,l) = \sqrt[n_c]{\prod_{t=t_1}^{t_2} \bar{w}(i,t,l)} \quad (1)$$

where $n_c = t_2 - t_1 + 1$ is the number of annual increments in the control period.

2.4. Relative additional annual increment values

The mean control increment width $\bar{w}_c = (i,\Delta t_c,l)$ was used to calculate the relative additional annual increment value $d(i,t,l|\Delta t_c)$ for every tree i and year t during the period of the investigation:

$$d\left(i,t,l\,\middle|\,\Delta t_c\right) = \ln\left[\frac{w(i,t,l) - \bar{w}_c(i,\Delta t_c,l)}{\bar{w}_c(i,\Delta t_c,l)}\right] = \ln\frac{w(i,t,l)}{\bar{w}_c(i,\Delta t_c,l)} \quad (2)$$

The value $d()$ eliminates increment width variability between individuals that is due to genetic and relatively constant environmental factors (e.g. edaphic, hydrological, etc.). The logarithmic transformation is used since cambial growth is an exponential process. During the control period, $d()$ values have a normal distribution ($P < 0.05$). Each individual $d()$ value identifies the deviation of each increment from the mean or normal value for the tree. If $d() = 0$, then the increment is equal to the mean. If $d() > 0$, then the increment width is larger than mean. If $d() < 0$, then the increment width is less than the mean.

The individual relative additional annual increment values were used to calculate the mean relative additional annual increment, for each year t of every plot l:

$$\bar{d}\left(t,l\,\middle|\,\Delta t_c\right) = \frac{\sum_{i=1}^{N} d\left(i,t,l\,\middle|\,\Delta t_c\right)}{N} \quad (3)$$

where N is the number of sampled trees.

The overall means $\bar{d}_c(\Delta t_c,l)$ for each plot l during the control period Δt_c are given by:

$$\bar{d}_c(\Delta t_c,l) = \sum_{i=1}^{N}\sum_{t=t_1}^{t_2} d\left(i,t,l\,\middle|\,\Delta t_c\right) = 0 \quad (4)$$

Based on Eq. 4 and the normal distribution of $d(i,t,l|\Delta t_c)$ values, the null hypothesis

$$H_O: \bar{d}\left(t,l\,\middle|\,\Delta t_c\right) = \bar{d}_c(\Delta t_c,l) = 0 \quad (5)$$

was tested parametrically. Thus, it was determined if the additional increment for plot l and year t significantly differs from the mean and if the difference is positive or negative.

2.5. Cumulative relative additional annual increment

The cumulative relative additional annual increment reflects the changes in increment width in a retrospective time period $\Delta t_r = t_3 ... t_4$, where t_3 is the beginning, and t_4 the end of the period of the retrospection. The cumulative relative additional annual increment value $D_r(i,\Delta t_r,l|\ \Delta t_c)$ is calculated for every individual i:

$$D_r\left(i,\Delta t_r,l\,\middle|\,\Delta t_c\right) = \frac{\sum_{t=t_3}^{t_4} d\left(i,t,l\,\middle|\,\Delta t_c\right)}{n_r} \quad (6)$$

where $n_r = t_4 - t_3 + 1$ is the number of years in the retrospective period;

The cumulative relative additional annual increment values are used to calculate the respective plot mean $\bar{D}_r(\Delta t_r,l|\ \Delta t_c)$ values as follows:

$$\bar{D}_r\left(\Delta t_r,l\,\middle|\,\Delta t_c\right) = \frac{\sum_{i=1}^{N} D_r\left(i,\Delta t_r,l\,\middle|\,\Delta t_c\right)}{N} \quad (7)$$

where N is the number of sample trees in plot l.

2.6. Atmospheric deposition in the Skrunda area

The concentrations of the elements in forest soils and moss in the Skrunda area have been studied from 1991 to 1993. At sampling locations, soil samples by horizon and moss (*Hylocomium splendens* and/or *Pleurozium schreberi*) were collected for determination of elements to give some

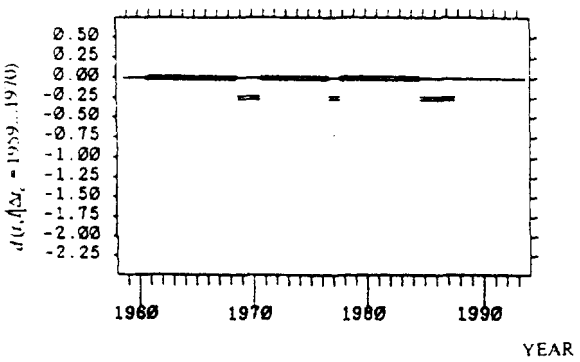

Fig. 2. The mean relative additional increment $d()$ of pine trees during 1960–1987 for a control plot located 66 km from the Skrunda RLS.

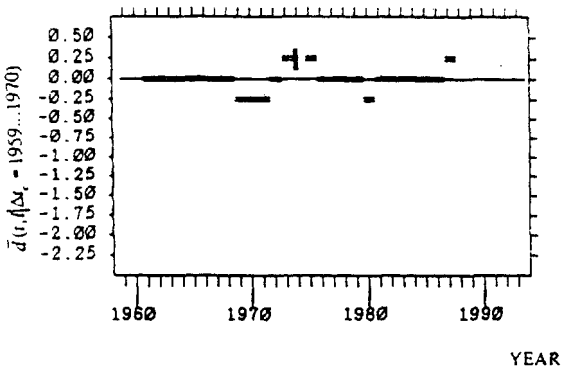

Fig. 3. The mean relative additional increment $d()$ for a control plot located 10 km behind the Skrunda RLS. The solid bars depict significant ($P < 0.05$) deviations from normal growth.

idea of the potential sources of deposition that could affect the forests around the Skrunda radar. Initially, the two most obvious candidates as major polluters were the metal smelting industry at Liepaja and the oil refinery in Mazeikiai, Lithuania. Total Cu, Zn, Fe, Pb, Ca and Cd concentrations in moss were measured with AAS, but V by colorimetry. Soils were analysed for Cu, Pb, Sn, Cr, Zn, Ni, Co, Mn, B, Ti, La, Y, P, V, Sr, Ga, Zr, and Ba concentrations with emission spectrophotometry and for Pb, Zn, Cu, Ni, Cd, and Cr (1 M HCl extract) with AAS. Analyses were checked with moss calibration standards used for the European Atmospheric Deposition Mapping Project [18].

3. Results

3.1. Mean relative additional annual increment dynamics

The mean relative additional annual increments $\bar{d}(t,l|\Delta t_c)$ values were calculated for every year t from 1960 to 1987 for each plot l. The control period was set at Δt_c = 1959–1970. Trees in plots located outside of the zone that receives electromagnetic radiation from the Skrunda RLS had no significantly different annual increments compared to the mean, i.e. $\bar{d}(t,l|\Delta t_c) \approx 0$ (Fig. 2), excepting isolated outliers with irregular departures from the norm (Fig. 3). Significantly diminished mean additional annual increments occurred after 1980 in plots close to the Mazeikiai oil refinery (Fig. 4), which coincides with the start of emissions of pollutants from the plant. All plots that receive electromagnetic radiation from the Skrunda RLS have significant ($P < 0.05$) negative $\bar{d}(t,l|\Delta t_c)$ values, compared to 0, which begin only after 1970 and are observed throughout the period of study (Fig. 5). The Skrunda radar began operation in 1971.

3.2. Mean cumulative relative additional annual increment and the electromagnetic radiation

The mean cumulative relative additional annual increment $\bar{D}_r(\Delta t_r,l|\Delta t_c)$ during the retrospective period of electromagnetic radiation Δt_r

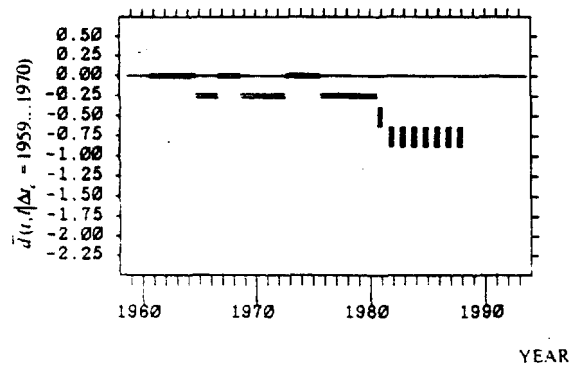

Fig. 4. The mean relative additional increment $d()$ of pine trees for a plot located 12 km north of the Mazeikiai oil refinery. The solid bars depict significant ($P < 0.05$) deviations from normal growth.

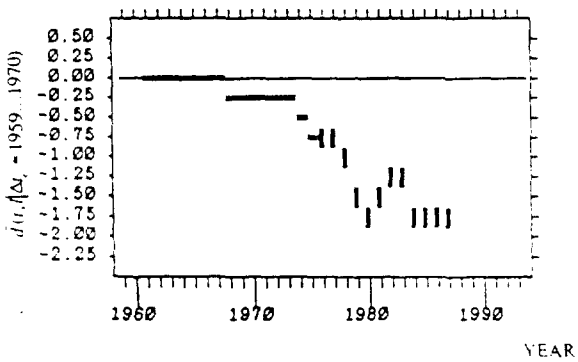

Fig. 5. The mean relative additional increment $d()$ of pine trees for a plot in front of the Skrunda RLS at 4 km distance. The solid bars depict significant deviations from normal growth.

= 1971–1988 was calculated relative to the control period before radiation, Δt_c = 1959–1970. The mean $\overline{D}_r(\Delta t_r, l|\ \Delta t_c)$ value of all plots in the radiation zone is −0.41 ± 0.07 compared to −0.13 ± 0.04 in plots not receiving radiation, significant at P = 0.001. The values of $\overline{D}_r(\Delta t_r, l|\ \Delta t_c)$ are negatively correlated with the measured intensity of electrical field in decibels (r = −0.55, P = 0.009). In the irradiated zone, $\overline{D}_r(\Delta t_r, l|\ \Delta t_c)$ is linearly correlated (r = +0.52, P = 0.016) with distance from the RLS. The distance correlation is expected since the electrical field intensity measurements are related to distance (r = −0.93). In the non-irradiated zone there is no significant correlation between $\overline{D}_r(\Delta t_r, l|\Delta t_c)$ and distance from RLS.

3.3. Mean cumulative relative additional annual increment and pollutants

It is well known that pollutants depress tree radial growth [3–7]. In the Skrunda area, the only major sources of pollution are the metal smelting industry at Liepaja (ca. 50 km from RLS) and the oil refinery in Mazeikiai (ca. 40 km from RLS). Heavy metal concentrations in moss and soil were well related to these pollution sources and could be used to identify the zones of deposition. The vanadium concentrations close to the Mazeikiai oil refinery reached 9.5 mg/kg in moss, three times higher than normal levels [8]. Elevated vanadium levels were found up to about 10–15 km from the Lithuania border radially from the Mazeikiai oil refinery, but levels around Skrunda were background (Fig. 6). This metal has high concentrations in oil and hence is a good indicator of oil combustion. Close to Liepaja, the Zn concentrations in moss were above 150 mg/kg, but only background levels of 40–50 mg/kg were found in a large area around Skrunda. No evidence was found of any elevated deposition levels of metals in the Skrunda area. In the irradiated zone, there were no significant correlations between $\overline{D}_r(\Delta t_r, l|\ \Delta t_c)$ and metal levels.

Despite the suggested lack of effect of the Mazeikiai oil refinery on the trees around Skrunda, cumulative growth $\overline{D}_r(\Delta t_r, l|\ \Delta t_c)$ during the time of operation of the factory Δt_r = 1981–1988 was assessed relative to the control period Δt_c = 1971–1980 before operation. For this retrospective period in the RLS radiation zone, again $\overline{D}_r(\Delta t_r, l|\ \Delta t_c)$ was linearly correlated with distance from the RLS (r = +0.51, P = 0.02). However, along a transect from Skrunda to Mazeikiai, there was a negative linear correlation (r = −0.78, P = 0.02) with distance from the RLS, i.e. less radial growth near Mazeikiai. The effect seen is not evident for a retrospective period that was set to begin with the operation of the Mazeikiai refinery and end with the start of the Skrunda RLS operation (Δt_r = 1971–1980),

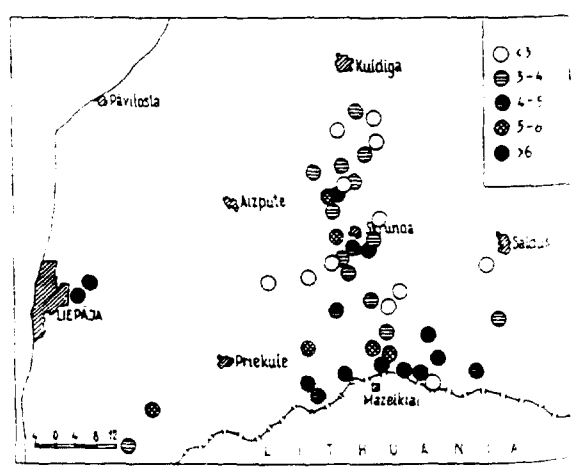

Fig. 6. V concentrations (mg/kg) in feathermoss in SW Latvia. From Nikodemus and Brumelis [8].

using a control period Δt_c = 1959–1970. In the radiation zone plots, the same pattern of decreased cumulative relative additional annual increment in the years 1971–1980, before the operation of the Mazeikiai refinery, was seen in 1981–1988, during operation of the Mazeikiai refinery.

3.4. Mean cumulative relative additional annual increment and other environmental factors

Besides the mentioned factors (electromagnetic radiation, distance to RLS, pollutants) the effect of other factors on the mean cumulative relative additional annual increment $\overline{D}_r(\Delta t_r, l|\Delta t_c)$ during the retrospective period Δt_r = 1971–1988 was assessed relative to the control period Δt_c = 1959–1970. The analysed factors were: height above sea level, topography, nutrient conditions, soil moisture and texture, litter and organic layer depth, soil pH, ground water level, ground water flow, ionising radiation, and silvicultural parameters. The effect of these factors were assessed by multiple regression and analysis of variance, but no significant effects on $\overline{D}_r(\Delta t_r$ = 1971–1988, $l|\Delta t_c$ = 1959–1970) values were observed.

4. Discussion

The results demonstrate that the mean relative additional annual increment $\overline{d}(t,l|\Delta t_c$ = 1959–1970) of pine has decreased in plots that receive electromagnetic radiation from the Skrunda RLS. This decrease becomes significant 3–5 years after the beginning of operation of the RLS and continues to the end of the period of study. Outside of the RLS radiation zone significant differences of $\overline{d}(t,l|\Delta t_c$ = 1959–1970) are isolated cases, which do not reflect a trend. An exception is the decreased values of $\overline{d}(t,l|\Delta t_c$ = 1959–1970) toward the Mazeikiai oil refinery after 1980, i.e. after the beginning of pollutant emissions.

The effect of the Mazeikiai refinery is clearly caused by pollutants and does not invoke controversy. However, the loss in additive growth increment in the RLS radiation zone in the retrospective studied period begins with the radar installation and no relations to chemical pollutants or other factors have been observed.

The values of mean cumulative relative additional annual increment are significantly negatively correlated to electrical field intensity during separate times of operation:

$$\overline{D}_r(\Delta t_r = 1971...1988, l|\Delta t_c = 1959...1970),$$

$$\overline{D}_r(\Delta t_r = 1971...1980, l|\Delta t_c = 1959...1970) \text{ and}$$

$$D_r(\Delta t_r = 1981...1988, l|\Delta t_c = 1971...1980).$$

The relation between $D_r(.)$ and distance to the RLS is positive.

Previous work does not provide conclusive evidence of the effect of weak electromagnetic fields on trees, since some work confirms an effect [9,10] and some denies its existence [11,12]. We cannot provide a mechanism for the observed suggested effect at Skrunda, from the many available hypotheses [13–15] of weak radio-frequency electromagnetic action. However, our results certainly lead one to strongly suppose that the Skrunda RLS radiation has affected pine radial growth. The confirmation of the effect has been further supported by evidence from other organisms [16,17]. Nevertheless, we respect the possibility that an unknown factor has caused the negative effect which by chance is associated with the observed temporal and spatial variations.

The Skrunda RLS area requires further work on the possible effects, particularly on the mechanisms of action. This area is unique for the study of radio-frequency electromagnetic radiation. The radars are located in a valley (and not on the highest possible site as usual) that is rich in ecosystems, and agricultural communities are found throughout the irradiated area. The results of a 20-year 'experiment' are waiting for observation and analysis. The RLS emits radiation in an insufficiently investigated frequency (154–162 MHz) in pulse regime, and the intensities of pulses exceed allowed limits for integrated doses by 50 times. However, pulse radiation types have no associated allowed limits.

Other work at Skrunda is in a preliminary phase, including experimental searches for mech-

anisms, and it is hoped that this work will provide an insight on the suggested effects. However, the most conclusive evidence for an effect (or no effect) may only be available when the Skrunda RLS terminates operation in 1998, as regulated by an international agreement between Russia and Latvia. Then it will be possible to determine if tree radial growth recovers. Since the RLS is expected to operate until 1998, the authors encourage all interested to further study the Skrunda RLS.

Acknowledgements

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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]. Nevertheless, 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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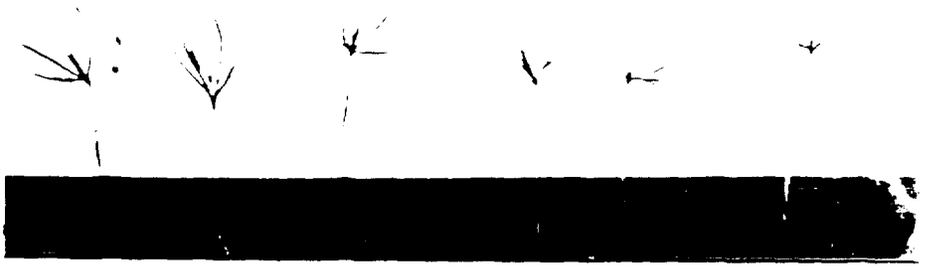

Fig. 1. Seedlings of *Pinus sylvestris* L. with different number of seed lobes: a, 3; b, 4; c, 5; d, 6; e, 7; f, 8.

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

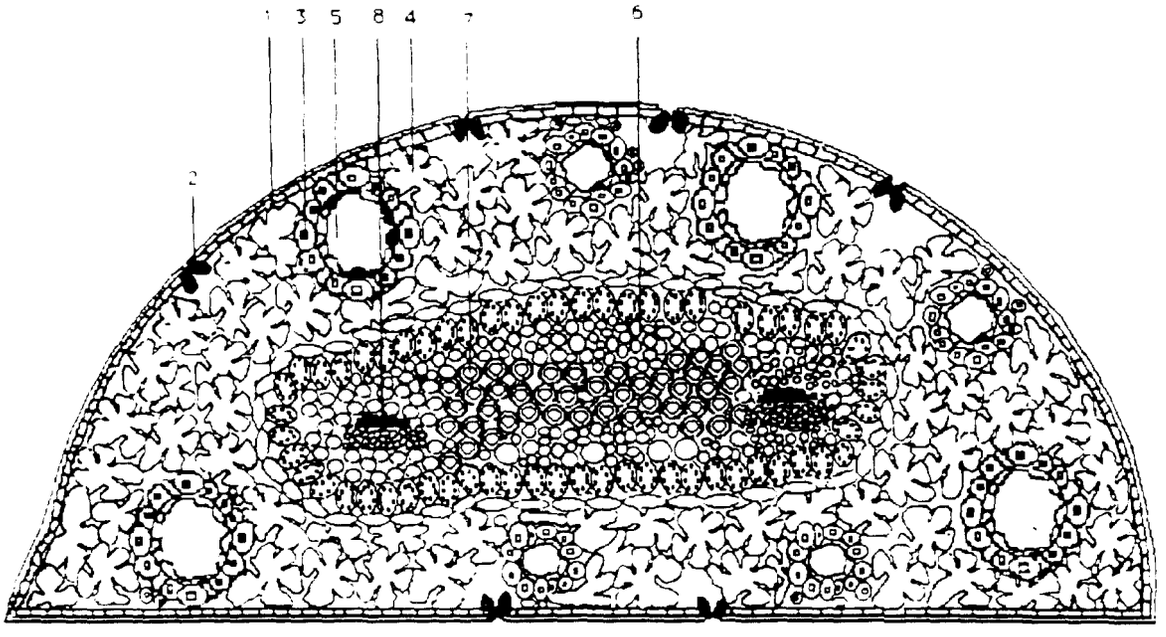

Fig. 2. Cross-section of *Pinus sylvestris* L. needle: 1, epidermal cells; 2, stomata; 3, hypodermis; 4, mesophyll cells; 5, resin ducts; 6, endodermis; 7, tracheids and parenchyma cells; 8, vascular bundles.

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 terpens, 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 an universal response to wounding and other str[illegible] 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]. Osmiophyle 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 osmiophylic 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 osmiophilic globules formed at the maturing side of the Golgi complex (Fig. 5c,d). Similar globules have been identified as spherosomes, lysosomes or secretory

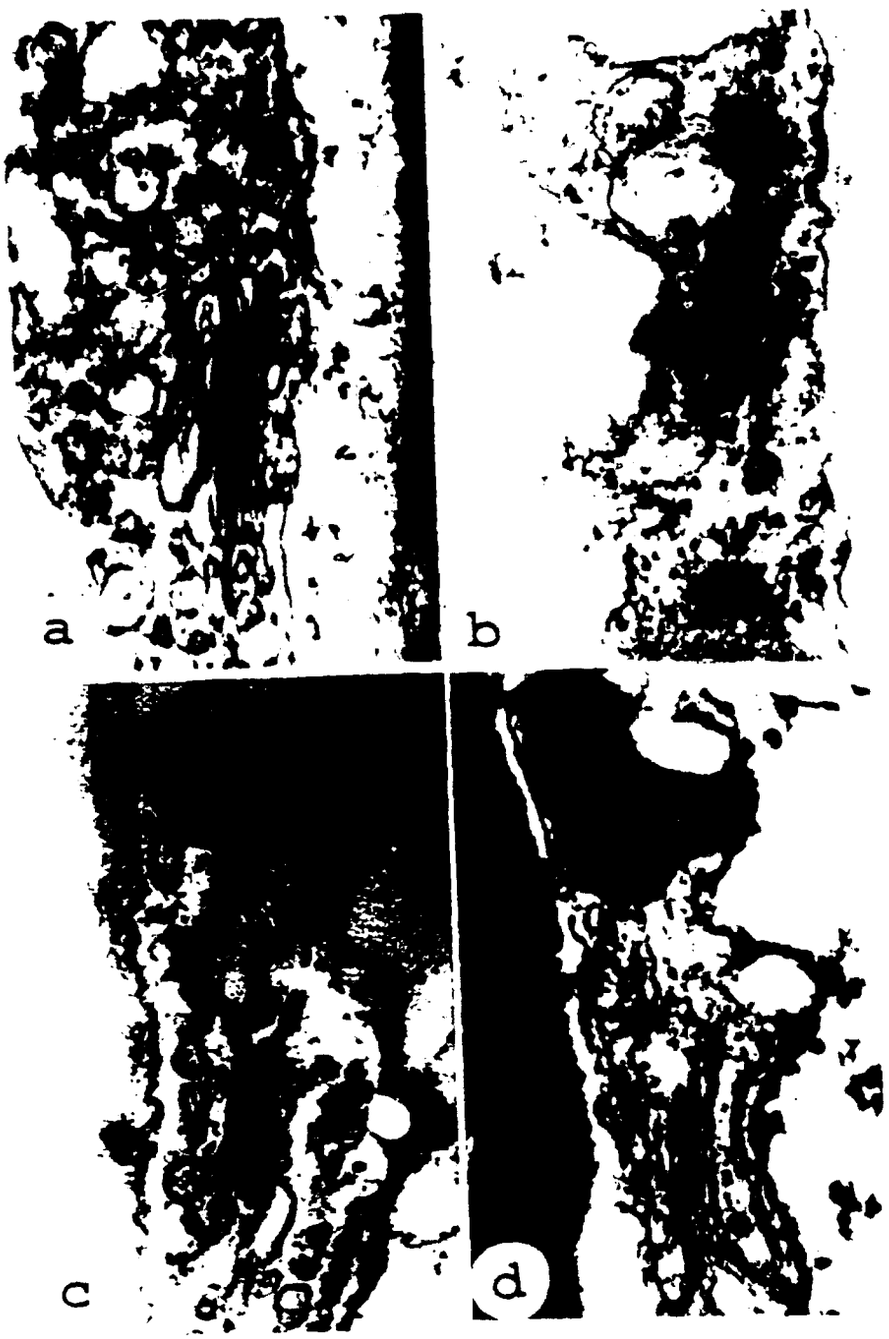

Fig. 5. The Golgi apparatus in the mesophyll cells of second-year needles of *Pinus sylvestris* L. (a) unirradiated tree, (b) indirectly irradiated tree, (c) and (d) directly irradiated tree. Magnification: ×60000.

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The effect of electromagnetic radiation from the Skrunda Radio Location Station on *Spirodela polyrhiza* (L.) Schleiden cultures

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Abstract

The effect of electromagnetic radiation from the Skrunda Radio Location Station was studied on the vegetative growth and morphology of the duckweed *Spirodela polyrhiza* (L.) Schleiden plant in the next generation. The impact of plant development stage and length of the exposure period were examined. The effect of short-term (5-day) exposures of *Spirodela* cultures depended on the stage of development at the time of exposure. Generally, the vegetative reproduction rate was accelerated in the first 20 days after the end of exposure. Exposure of plants just beginning formation lowered the vegetative growth rate. Eighty-eight-hour exposure caused the appearance of some abnormal individuals after 30 days of growth. At 55 days, various morphological and developmental abnormalities appeared in 6–10 daughter plants from 10 exposed mother plants, compared with 0.1 plants per 10 in the control condition. Plants developed completely to daughter fronds under exposure from the electromagnetic field had a shorter life-span (67 days compared to 87 days in the control) and fewer subsequent daughters (total eight compared to 10 in the control group).

Keywords: Radio frequency electromagnetic radiation; Radar; *Spirodela polyrhiza* L.; Electromagnetic radiation, plant growth; Electromagnetic radiation, plant reproduction; Electromagnetic radiation, plant deformities

1. Introduction

The current understanding of the effects of radio frequency electromagnetic radiation on organisms has not reached a level at which effects and mechanisms can be claimed with confidence. Different sources in the literature are difficult to compare and are contradictory, since different researchers have used different experimental conditions, organisms, electromagnetic field parameters, exposure times, and methods of measurement of effect. At the same time, contacts with different intensity electromagnetic fields are increasing. Therefore, further studies of different intensity radio frequency electromagnetic effects on organisms are necessary. Before the basic principles of biological mechanisms of effect at different frequencies and intensities of electromagnetic are known, generalisations cannot be made.

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In the area of exposure to the Skrunda Radio Location Station in Latvia, many trees and shrubs with abnormal branching and apex deformations have been observed [1]. In many cases, new branch development occurred from buds during the entire vegetative period, suggesting an effect on cell proliferation and changes in regulation processes. These initial observations prompted us to begin other work in the Skrunda area, using a plant species that can be grown experimentally under controlled conditions.

In the present work, a sensitive clone of *Spirodela polyrhiza* (L.) Schleiden (family Lemnaceae) was used as a test organism to study the effects of the Skrunda Radio Location Station. Lemnaceae are the simplest and smallest flowering plants, and they can be grown in aseptic conditions under controlled conditions of temperature, light and nutrition. They have high vegetative reproduction rates, and genetically uniform clones can be used for experimentation. Thus, Lemnaceae have been widely used in morphogenetic, physiological and biochemical research, as well as for pollution studies [2–9].

2. Methods

2.1. *Spirodela polyrhiza (L.) Schleiden*

Spirodela mostly reproduces vegetatively, producing daughter fronds from mother fronds at the proximal end in two meristematic regions in pockets [8]. It has two morphological stages, an active metabolic frond and a dormant frond (turion). Germination of turions (formation of the first active metabolic frond) can be induced by kinetin to produce synchronous aged plants. Daughter fronds are produced alternatively from either pocket. Normally, clones from the same mother plant will consistently produce the first daughter at either the left or right pocket, termed left and right symmetry, respectively (Fig. 1A). Doubling time of frond number is 2–7 days, depending on culture conditions [8]. Practically any environmental change alters meristematic growth and further reproduction. The mean life span of the clone used (CR, 1970, Institute of Biology, Latvia) was 80–90 days, during which time about 10 daughter fronds are produced. A detailed description of the clone, including germination kinetics, is in preparation.

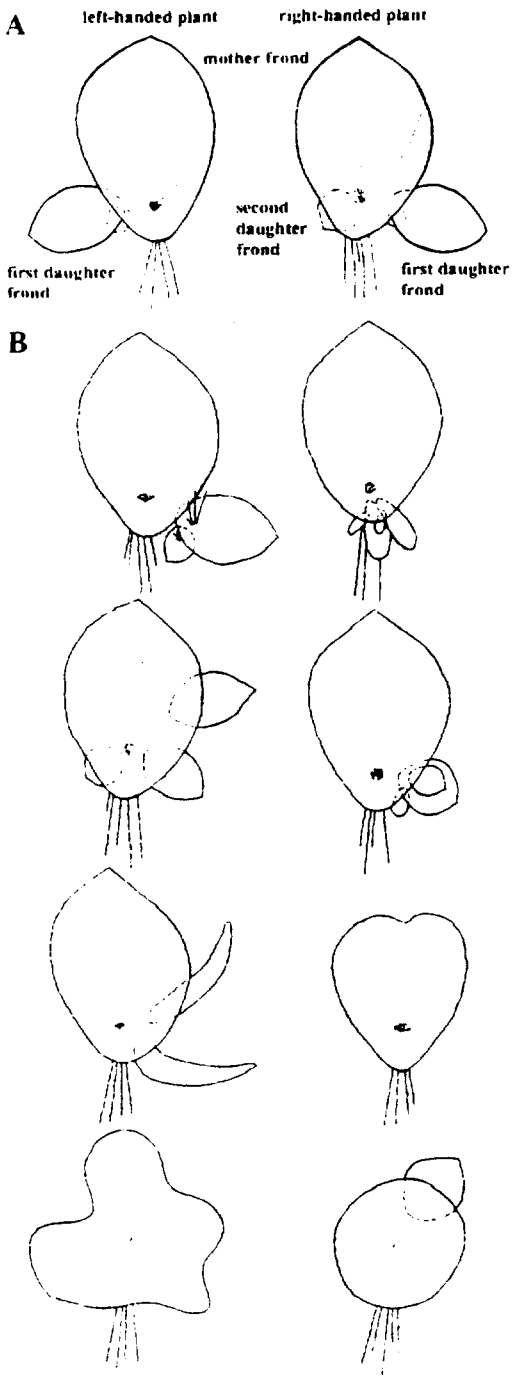

Fig. 1. *Spirodela polyrhiza* plants. (A) Normal individuals. (B) Abnormal individuals observed after exposure to the Skrunda Radio Location Station electromagnetic field.

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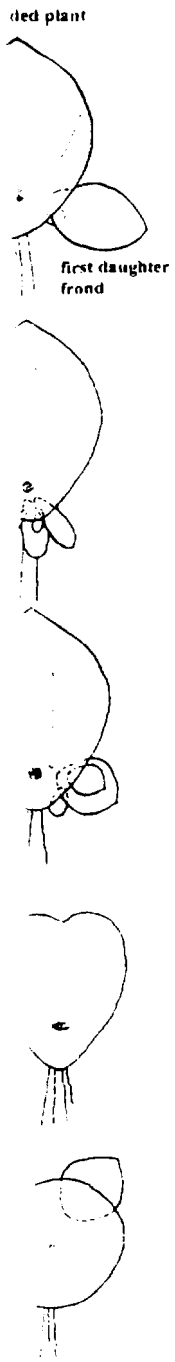

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2.2. Experimental

Experiments were conducted in the years 1992–1994. *Spirodela polyrhiza* plants were grown in sterile conditions in mineral nutrient medium [9] under continuous light (16 W/m^2 white light, wavelength 380–700 nm). The nutrient medium consisted of 100 mg/l KH_2PO_4, 200 mg/l, KNO_3, 250 mg/l $Ca(NO_3)_2 \cdot 2H_2O$, 150 mg/l $MgSO_4 \cdot 7H_2O$, 150 mg/l NaCl, 5 mg/l $FeSO_4 \cdot 7H_2O$, 1.4 mg/l H_3BO_3, 0.9 mg/l $MnCl_2 \cdot 9H_2O$, 0.1 mg/l $ZnSO_4 \cdot 7H_2O$, 0.075 mg/l Na_2NO_4 and 0.025 mg/l $CuSO_4 \cdot 5H_2O$. Turions were germinated in growth medium with 0.4 mg/l kinetin for 48 h to obtain even aged plants. Plants were exposed to electromagnetic radiation from the Skrunda Radio Location Station (2 km from the source) for 1–5 days in glass flasks during summer months. Measurements of radiation are given in [10]. Briefly, the radar operates in the 156–162 MHz frequency range in pulse mode. The measured integrated electric field intensities at the site of exposure in the years 1992–1993 ranged from about 0.6 V/m to 2.6 V/m, but the pulse intensities were up to 50 times higher.

Replication was using five flasks, each with 10–30 plants. Plant orientation was random. After exposure, plants were transported to the laboratory, transferred to Petri plates with fresh medium and cultivated at 28°C under continuous light for 10–120 days. Medium was changed at 2-day intervals. Control plants underwent identical treatment without exposure. Plants for exposure and control plants were transported to the Skrunda area from the laboratory in flasks enclosed in 1-cm thick aluminium capsules. Two types of control treatments were used: plants located beside the exposed plants but contained in aluminium capsules, and plants transported to a location behind the RLS. Exposed flasks were covered with black paper to maintain dark conditions similar to the control.

Observations were made on the vegetative reproduction rate and changes in growth and development. Plants of different age since turion germination were studied. To determine individual life span and total number of daughters produced, and to more accurately determine the number and types of morphological changes, plants were transferred to fresh medium plus 0.3% agar in Petri plates after exposure. The life of each awakened turion could then be monitored. In this case, replication of 12 plates with 10 individuals was used.

3. Results

Exposure of electromagnetic radiation from the Skrunda Radio Location Station for 88 h to *Spirodela* cultures aged 9, 28 and 58 h after turion germination, increased the vegetative reproduction rate to 105–195% of the control during the first 20 days after exposure, compared with the respective controls (Fig. 2). Similar patterns occurred for 24- and 48-h exposures (not shown). After the initial increase in reproductive rate for 20 days, this rate decreased. Free growing uneven aged cultures had increased vegetative reproduction rates up to 110% of the control in the first 10–12 days after 24-, 48- and 88-h exposures. However, when very young plants (50-min-old since turion dormant frond germination) were exposed, the future vegetative reproduction rate was lowered to 91% of the control, 10 days after a 48-h exposure.

After 30 days of growth after 88-h exposure, growth disturbances were observed, such as plants with upward growing roots and plants with left symmetry (Fig. 1A,B). After 55 days of growth of 9-, 28- and 52-h fronds and uneven aged plants in aqueous medium, the incidence of pathologies had further increased (Fig. 3). At 10 days, only the exposed 28-h fronds had developed pathological daughter fronds (20 per 100 mothers). This age corresponds to the initiation of development of the first daughter fronds.

Exposures of 120 h to free growing *Spirodela* cultures decreased frond numbers to 58.4% of the control after 80 days of subsequent growth (Fig. 4). Also, 50 deformities were observed per 100 mother fronds at 80 days. However, in these cultures, symmetry inversions and geotropic changes were not counted due to the large numbers of fronds produced. The exposure time of 120 h coincides with the time to complete development of the first daughter fronds from turions.

When EM exposure began with the breaking of dormancy turions with kinetin addition, the 120-h exposure caused a decrease in the life span of