

The Influence of Low-Intensity Millimeter-Wave Radiation on the Growth of Cress Roots

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Low-Intensity Microwave Effect, Root Growth in Plants

An inhibition of growth in cress roots (*Lepidium sativum L.*) by irradiation with low-intensity millimeter-waves was found using a computer controlled optical system which is capable of measuring nearly continuously the length of the roots to an accuracy of $\pm 2 \mu\text{m}$. The effect is reversible and, for a power density of 6 mWcm^{-2} , results in completely halting the root growth. It occurs within about 100 s after the onset of irradiation. The microwave-induced temperature increase at the surface of the root tip was found to be less than 0.3°C at this power density. The effect did not show a sharp frequency dependence however it depended strongly on the polarization of the microwaves with respect to the root orientation.

The sensitivity of the root growth to the ambient temperature was examined. Only a weak temperature dependence was found which could not explain the observed effects. However simulating the microwave-induced temperature increase at the surface of the root tip with (incoherent) far-infrared light ($\lambda \geq 20 \mu\text{m}$) resulted in similar effects as with microwaves. Hence one can conclude that the observed effects are primarily caused by the small local irradiation induced thermal gradients across the surface of the root tip.

Introduction

The influence of low-intensity millimeter-wave radiation on biological systems is a topic of considerable importance not only for the understanding of the mechanisms of interaction between electromagnetic radiation and living systems [1–2] but also for the establishment of microwave safety standards. Several biological effects of low-intensity millimeter-wave radiation have been reported [3–7]. In the search for an influence of this radiation on living systems we chose the growing roots of cress seedlings which are widely used as a model system for rapidly proliferating cells and for the investigation of geotropism [8–10]. Two different processes contribute to the growth of cress roots: growth by elongation and growth by cell division. The latter is localized in the meristematic zone of the root tip and contributes to approximately 10% of the total elongation of the root [9]. The fact that the seedlings grow well in an atmosphere of high relative humidity makes them especially suitable for irradiation experiments with millimeter waves which would have a penetration depth of only about 0.5 mm in an aqueous medium solution [11].

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Experimental

The cress seeds, commercially available “Garten-Kresse”, were submerged in distilled water for 1 h at $24 \pm 0.5^\circ\text{C}$. The seedlings were then placed on a moist filter paper in the sample chamber (Figs. 1a/b) and incubated for 20 h at $24 \pm 0.5^\circ\text{C}$ before irradiation (root length at that time: about 5 mm). The sample chamber was a sealed plexiglas container with windows of polyethylene foil (area: $14 \times 6 \text{ cm}^2$, thickness: $100 \mu\text{m}$) which is almost transparent to millimeter-waves. Inside the chamber the atmosphere was saturated with humidity using a water bath at the bottom on which a piece of folded filter paper was laid in order to increase the evaporating surface area. The whole chamber and the optical system were kept in a dark, temperature controlled ($24 \pm 0.5^\circ\text{C}$) wooden box. To determine the growth rates of individual roots, a computer controlled optical system (Figs. 1a/b) was employed. It included a microscope with twofold magnification, 4 LED's ($\lambda \approx 1 \mu\text{m}$) for weak illumination of the seedlings and a linear array of 128 photodetectors (CCD-array: area of each detector element was $26 \times 26 \mu\text{m}^2$). The whole optical system was mounted on a stepper motor controlled platform that could be moved in three dimensions. Use of image processing techniques in the computer (HP 9826) allowed quasi-continuous measurements of the positions of one or



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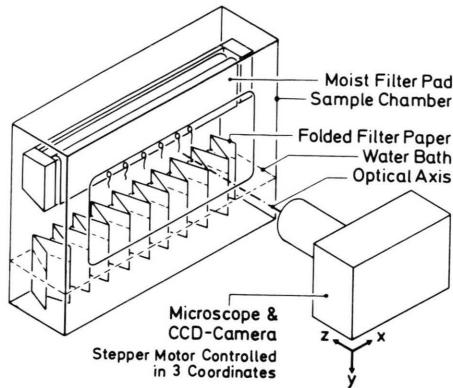
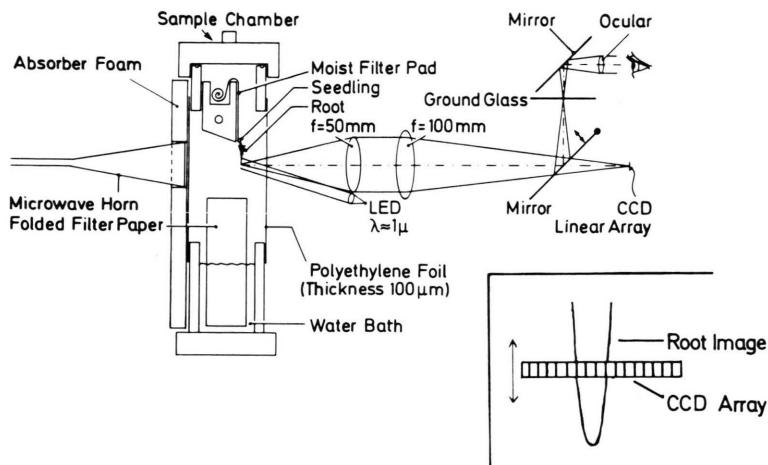


Fig. 1. a. Schematic description of the sample chamber and the microscope.

b. Scheme of the optical path of the microscope and the millimeter-wave horn antenna (not to scale; inset: image of the root on the CCD-array).



more root tips with an accuracy of $\pm 2 \mu\text{m}$ in three dimensions. This was achieved by automatically focusing the image of the root on the CCD-array as part of the image processing. To measure the position of an individual root tip a time of about 50 s was necessary. Usually 4 roots were observed, two as controls, two for irradiation. To demonstrate that the measurement system including the illumination with light of $\lambda \approx 1 \mu\text{m}$ had no effect on the root growth, tests were carried out in which a group of roots whose growth rate was measured with the optical system was compared with a control group in the same container. No significant differences in the root lengths of these two groups were found.

The millimeter-wave irradiation system, schematically shown in Fig. 2, includes a backward-wave oscillator (Siemens RWO 60), an isolator, a precision attenuator, a frequency meter and a calibrated ther-

mistor mount (Hughes) in connection with a power meter (HP 432 A). The frequencies were arbitrarily chosen between 42 GHz and 58 GHz and measured to an accuracy of $\pm 25 \text{ MHz}$. The forward power in the output waveguide ranged between 0.7 mW and 21 mW corresponding to a power density of 0.2 to 6 mW cm^{-2} respectively (horn area: 3.6 cm^2 , gain of the horn: 20 dB). To protect the control roots in the sample chamber from the millimeter-wave radiation, a mask made from absorber foam (Eccosorb AN 72) was placed across the front of the sample chamber (s. Fig. 1b). The microwave-induced temperature increase was measured by two different methods: i) by use of an IR-radiometric system and ii), with a microminiature thermal probe. The former contained a KRS5 lens, a black polyethylene filter (thickness: 0.1 mm), a germanium lens and a metal mesh (spacing: 0.4 mm) and allowed the root tip to be focused

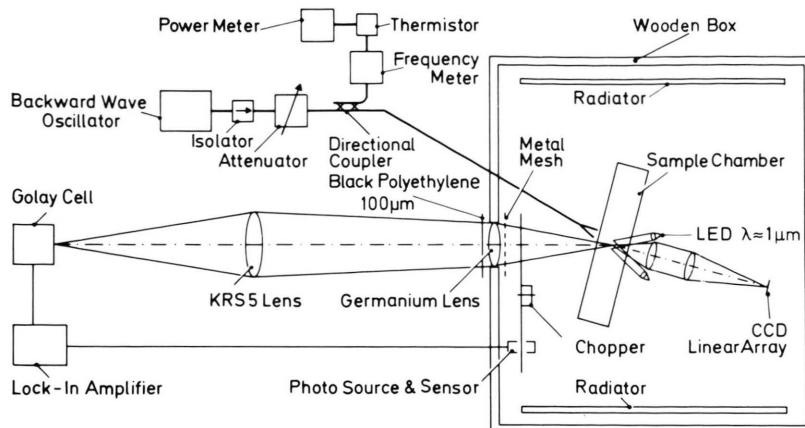


Fig. 2. Scheme of the irradiation system, the IR-radiometric system for the measurement of the microwave-induced temperature increase, the sample chamber and the optical system.

on a Golay cell and thus its thermal emission could be measured (s. Fig. 2). For calibration a free standing, temperature controlled water jet was used.

In the second method the microwave-induced temperature increase was measured with a micro-miniature thermocouple (Omega Engineering, Inc.). This thermocouple was brought into contact with the root tip on the side opposite to the millimeter-wave horn. It was aligned perpendicular to both the electrical and the magnetic millimeter-wave field. Thus the perturbation of the field was about the same for both polarizations. Microwave-induced temperature increases measured with both methods were equal within limits of experimental accuracy (± 0.1 °C for the IR-radiometric system and ± 0.01 °C for the micro-miniature thermal probe).

Results

Temperature dependence of the root growth rate

The temperature dependence of the growth rate of cress seedlings under our experimental conditions is shown in Fig. 3. It shows a large experimental variability in the growth rate of individual seedlings. This shows the necessity to study a possible microwave effect with single roots of which the growth can be measured quasi-continuously with and without irradiation. At around 25 °C a maximum in the growth rate is found. Hence in the experiments an ambient temperature of 24 °C (± 0.5 °C) was chosen to make sure that an overall irradiation-induced temperature rise of even 0.1 °C could not cause a decline in the growth rate.

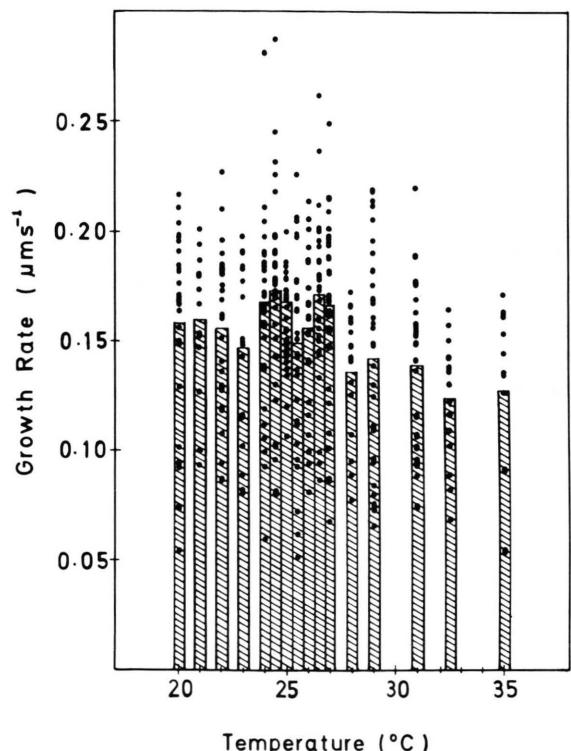


Fig. 3. Temperature dependence of the growth rate determined by measuring the length of a root after about 45 hrs of growth. Each dot represents the growth rate of a single root kept for 45 h in a chamber of corresponding temperature. The shaded bars indicate the mean values of the growth rate.

Millimeter-wave irradiation effect

A typical irradiation experiment is shown in Fig. 4. The growth rate was observed with the computer

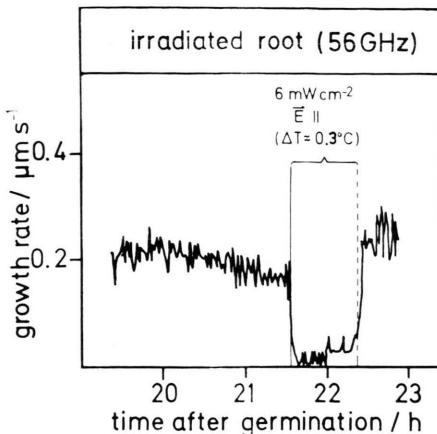


Fig. 4. Growth rate vs. time after germination of a root irradiated with 56 ± 0.025 GHz microwaves. The irradiation started (solid line) at 21.5 h and stopped (broken line) at 22.4 h after germination. The power density was 6 mWcm^{-2} , the E-Vector of the microwaves was parallel to the central axis of the root. The microwave-induced temperature increase of the root was measured to be 0.3 ± 0.1 °C.

controlled optical system for several hours and a value of about 0.2 μm s^{-1} was measured. Irradiating with millimeter-waves at a frequency of 56 GHz and a power density of 6 mWcm^{-2} with the E-vector polarized parallel to the central root axis resulted in a strong reduction of the growth. After switching off the irradiation the root recovered and reached a level of growth comparable to the value before the irradiation. On measuring the time constant for the occurrence of the irradiation effect one finds a value of $100 \text{ s} \pm 50 \text{ s}$. This indicates that the irradiation primarily influences the growth by elongation rather than the growth by cell division. The microwave-induced temperature rise of the root tip was measured to be 0.3 °C ± 0.1 °C.

Localisation of the millimeter-wave irradiation effect

To study whether the microwave effect is localised in the root tip or in another part of the root, irradiation experiments were carried out in which, by use of an aluminum mask, different parts of the roots were shielded. The power deposition (measured as the microwave-induced temperature increase) for both experiments was found to be equal within experimental accuracy. Irradiation to the root hair zone did not show an effect on the growth rate thus proving that the microwave-sensitive process is located in the root tip. For further experiments an aluminum mask

was always used which shielded the root so that the last two millimeters of the root tip were preferentially irradiated.

Power dependence of the millimeter-wave irradiation effect

The power dependence of the microwave effect was studied by irradiating a single root sequentially with power densities between 0.2 mWcm^{-2} and 6 mWcm^{-2} (Fig. 5a/b). Fig. 5a shows that even with a power density of 2 mWcm^{-2} a clear decrease of the growth rate resulted. For the dose-effect relationship a sigmoid function is found (Fig. 5b).

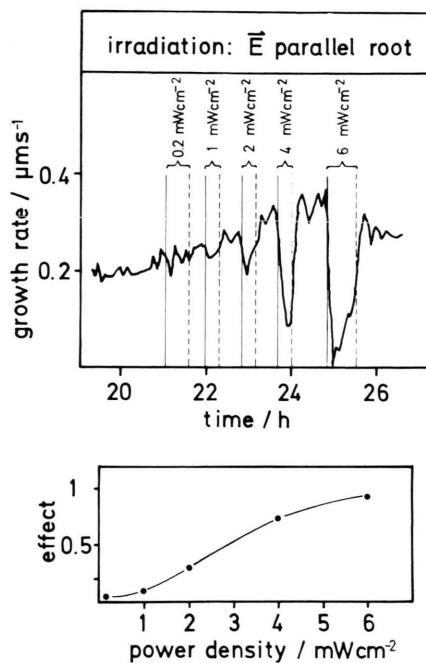


Fig. 5. a. Power dependence of the effect. The root was successively irradiated with power densities between 0.2 mWcm^{-2} and 6 mWcm^{-2} . The frequency was 56 GHz. The E-vector was parallel to the central axis of the root. b. "Dose-effect-relationship" as determined from Fig. 6a. The value of an effect is given as the ratio between the growth rate before the irradiation with a certain power density started and the difference between this value and the growth rate during irradiation with this power density.

The dependence of the irradiation effect on the polarization of the millimeter-waves

After proving that the microwave-sensitive processes are located in the root tip it seemed to be of

special interest to study the microwave effect for different polarizations of the radiation, *i.e.* parallel and perpendicular to the central axis of the root. For this, the local field behind the aluminum mask along the root axis was measured using a small piece (2 mm^3) of microwave absorber (Eccosorb ZN) which was mounted on a micro-miniature thermal probe. The latter could be moved with a stepper motor along the axis of the root and the microwave induced temperature increase of the absorber piece was measured at certain positions along the root axis for both polarizations (Fig. 6). The local power densities turned out to be very similar for both experimental conditions.

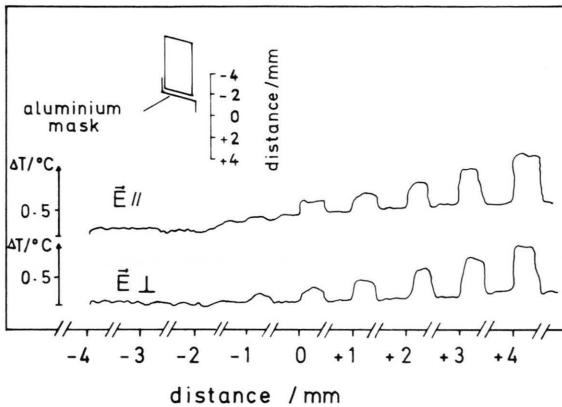


Fig. 6. Local power density of the microwave field for both polarizations ($E \parallel$ and $E \perp$) in positions along the vertical axis in the plane of the roots (s. inset) as determined by switching the radiation on and off and measuring the microwave-induced temperature rise ΔT in the small absorber piece. Distance 0 mm means the position at the lower edge of the aluminum mask, negative numbers correspond to higher, positive to a lower position as indicated in the inset.

However, to obtain a comparable reduction of the growth rate for both polarizations, a fourfold higher power density was necessary in the case of perpendicular polarization (20 mWcm^{-2}) than for parallel polarization (5 mWcm^{-2}), as shown in Fig. 7a. This difference in the required power density is caused by the fact that the absorption cross-section of the root in the microwave field is different, depending on the polarization of the E -vector. For perpendicular polarization the E -field in the root is weakened due to depolarization, which is not the case for parallel polarization.

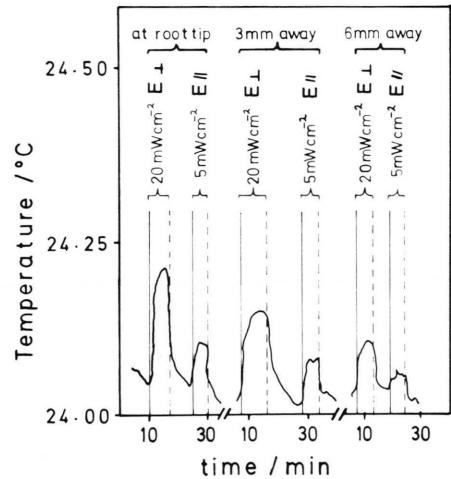
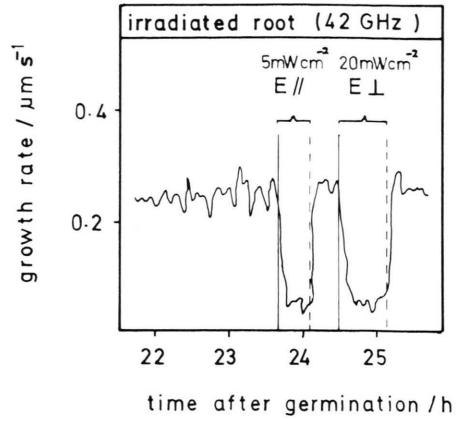


Fig. 7. a. Polarisation dependence of the irradiation effect on the growth rate. A frequency of $42.000 \pm 0.025 \text{ GHz}$ was used. For parallel polarization of the microwaves with respect to the central axis of the root ($E \parallel$) a power density of 5 mWcm^{-2} and for perpendicular polarization power density of 20 mWcm^{-2} were used. Both irradiation conditions resulted in an growth reduction of similar magnitude. b. Temperature conditions of the experiment reported in Fig. 7a. The microwave-induced temperature increase was measured with a micro-miniature thermocouple ($\varnothing 25 \mu\text{m}$) for both irradiation conditions i) at the root tip, ii) 3 mm away from the root tip and iii) 6 mm away from the root tip.

Measuring the temperature changes involved in the different experimental conditions (Fig. 7b) one finds a more than three times higher temperature rise at the root tip in the case of perpendicular polarization (20 mWcm^{-2}) compared to the parallel polarization case (5 mWcm^{-2}). At first sight the fact, that the irradiation effect on the root growth is similar, while the temperature rises are strongly different may indi-

cate towards a primarily non-thermal interaction of the millimeter-waves with the biological system. But, on measuring the temperature rise in the neighbourhood of the root tip (3 mm and 6 mm away) a polarization dependent temperature increase was observed as well. Thus the temperature gradients across the surface of the root tips (between the root tip and a position 6 mm away) were 0.09 °C for perpendicular polarization and 0.05 °C for parallel polarization, which are of similar magnitude.

Frequency dependence of the millimeter-wave irradiation effect

To study a possible frequency dependence of the irradiation effect frequencies of 42 GHz, 55.8 GHz,

56 GHz; 56.2 GHz, 57.8 GHz and 58 GHz were arbitrarily chosen. In all cases a similar effect was found. A typical example is shown in Fig. 8, in which one root was irradiated sequentially with 56 GHz, 58 GHz, 56 GHz and 58 GHz millimeter-waves. The power density was 3 mWcm^{-2} . This experiment might indicate towards a frequency dependence of the irradiation effect. But on measuring the microwave-induced temperature increase one finds, for the 58 GHz irradiation, a slightly smaller temperature rise than for the 56 GHz irradiation. This small difference might be attributed to a frequency dependence of the local field in front of the horn antenna. Comparisons of the effect at other frequencies (e.g. $57.8 \pm 0.025 \text{ GHz}$ and $55.8 \pm 0.025 \text{ GHz}$) did not show a sharp frequency dependence as well.

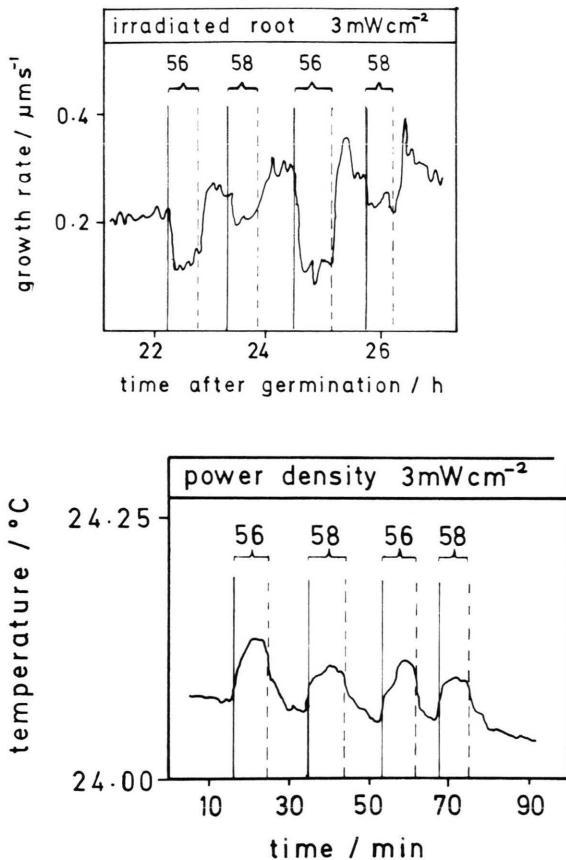


Fig. 8. a. Growth rate vs. time after germination for irradiation of the roots with $56.000 \pm 0.025 \text{ GHz}$ and $58.000 \pm 0.025 \text{ GHz}$ millimeter-waves in sequence. The power density was 3 mWcm^{-2} .

b. Microwave-induced temperature increase of the root tip for the irradiation described in Fig. 8a.

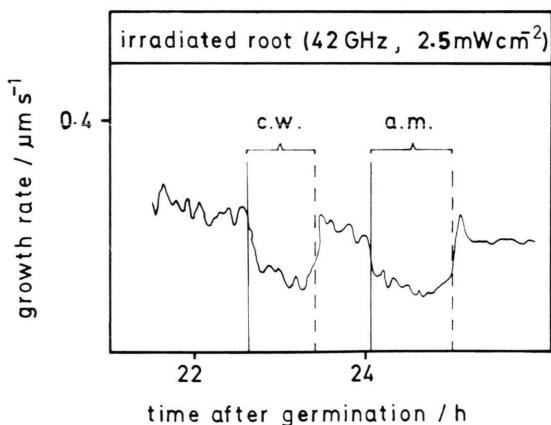


Fig. 9. Growth rate vs. time after germination for irradiation of the root with continuous waves and (1 kHz) amplitude modulated waves. The average power density was 2.5 mWcm^{-2} .

Difference between continuous wave and amplitude modulated irradiation?

For the study of the interaction mechanism between millimeter-waves and the biological system it could be of interest to determine whether the biological system would react to continuous wave (c.w.) irradiation in a different way as to an amplitude modulated irradiation (a.m.). The amplitude modulation had a modulation frequency of 1 kHz. The power density was in both cases (c.w. and a.m.) adjusted to 2.5 mWcm^{-2} . As shown in Fig. 9 no difference is observable for both irradiation conditions.

Simulation of the microwave-induced temperature rise with incoherent infrared radiation

The question arises if the small microwave-induced temperature changes in the root tip exert an influence on the growth rate. In order to study this, the microwave-induced temperature rise at the location of the root tip was simulated with incoherent far-infrared light ($\lambda \geq 20 \mu\text{m}$) of a thermal source. For that the Golay cell in Fig. 2 was replaced by a globar and additionally a PTFE-filter (thickness 0.5 mm) was mounted in front of the germanium lens, so that only radiation of $\lambda \geq 20 \mu\text{m}$ could reach the sample. Thus infrared light, which might influence the cytochrome C in the root tip [12] was filtered out. The far-infrared radiation was focused on the root tip and the effect was observed with the computer controlled

system. A typical result is shown in Fig. 10. The temperature conditions for the experiment are described in Fig. 10b. It shows that for temperature changes comparable to those observed in the millimeter-wave irradiation experiment similar effects are found.

Conclusions

Experiments are reported showing an influence of low-intensity millimeter-wave and far-infrared radiation on the root growth of cress seedlings. The growth rate was only weakly influenced by the ambient temperature. However, the small irradiation induced temperature gradients across the surface of the root tip proved to be of high importance. In this sense the observed irradiation effects are of thermal origin.

Acknowledgements

In the course of these experiments more than 5000 roots were examined. This was possible only due to the technical assistance of A. Schäfer, Chr. Finking, A. Cardona. Their help is gratefully acknowledged. Furthermore we thank Prof. M. H. Weisenseel (University of Karlsruhe) and Dr. H. Behrens (University of Bonn) for helpful discussions. Especially, we would like to thank our engineers D. Böhme and W. Schlieter for their excellent and engaged support in constructing the computer-controlled optical system.

Note added in proof:

H. Fröhlich has pointed out to us that in his model on coherent excitations, supply of energy to the system at a rate s leads to this excitation provided s exceeds a critical s_0 , $s > s_0$. The system is then found in a state which in some respect is far from thermal equilibrium. External heating (e.g. through microwaves or infrared radiation) may then impose temperature differences, resulting in a further flow of energy at a rate s_T . If this is negative, i.e. if the temperature of the relevant region is above that of its surroundings then the critical rate s_0 is no longer reached, provided $|s_T| > s - s_0$. The biological activity arising from the original activation is then cancelled. Clearly this holds for any other excitation arising from a continuous energy supply at a rate s larger than a critical s_0 . The reverse may also hold if the original s was below s_0 and thermal energy flows into the system.

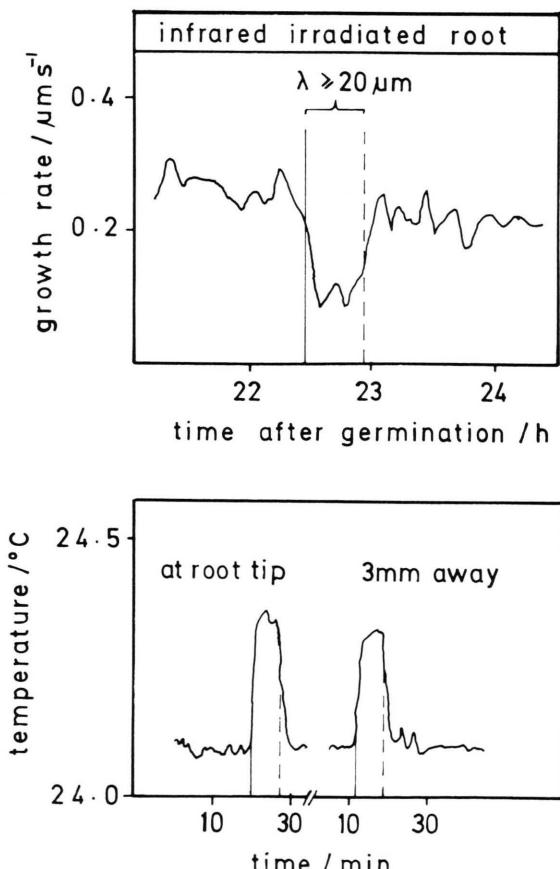


Fig. 10. a. Growth rate vs. time after germination for a root irradiated with incoherent far-infrared light of a thermal source (globar). Wavelength $\lambda \geq 20 \mu\text{m}$. b. Irradiation-induced temperature increase at the root tip and 3 mm away for the experiment described in Fig. 10a.

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