G & BM
Tome IV
Iaşi 2003

ULTRA HIGH FREQUENCY WAVES EFFECT UPON ASSIMILATORY PIGMENTS IN OAK SEEDLINGS

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Key words: *Quercus robur L.*, ultra high frequency radiation, chlorophyll, carotene

Abstract: The influence of ultra high frequency radiation in forestry arbor seedlings was studied by spectral method. Chlorophyll pigment contents, very important for photosynthesis phenomena, were found to be diminished in pedunculate oak seedlings after daily exposure times of: 1;2; 3; 4; 6 and 8 hours, at ultra high frequency waves with 400 MHz frequency and 1 mW/cm2 power density. Non-thermal effect seems to underline the putative molecular and biochemical modifications that are supposed to be induced in the vegetal cell by electromagnetic daily stress.

INTRODUCTION

There is a significant amount of literature reports concerning the effects of static and variable magnetic fields on grassy plant species, especially regarding seed germination or initial growth stages (Carbonell et al, 2000, Namba et al, 1995, Pietruszewski, 1996, Dayal, and Shing, 1986, Ruzic, Jerman, 2002). Few studies were focused upon the germination of arbor species seeds after magnetic treatment (Celestino et al, 2000, 1998, Ruzic et al, 1992, 1998) or upon the photosynthetic activity changes induced in young plantlets exposed to electromagnetic waves (Lebedev et al, 1977, Phirke et al, 1996). It seems to be a significant lack of information regarding ultra high frequency (UHF) wave influence on arbor saplings.

GOAL OF THE INVESTIGATION

Forestry trees were chosen for the investigation since the superior biosphere level is mainly affected by cosmic electromagnetic radiation and its protective role for the rest of biosphere could be damaged by this type of atmosphere pollution. The experimental investigation was carried out on young seedlings since vegetal organisms in early ontogenetic stages are the most sensitive to external constraints.

MATERIAL AND METHOD

Biological material. The experimental research was carried out on *Quercus robur* L. (pedunculate oak) seedlings grown in laboratory. Selected seeds, with uniform genophond, (from the same natural stand, with

high biological parameters, situated in the hill region of Iasi city), were stored and let to germinate in adequate vessels containing natural soil from that very forest. After germination, vessels were exposed to light (12 hours light/12 hours darkness) and maintained to constant temperature (25 Celsius degree). Seedlings (Fig. 1) have grown this way during four months in quasi-natural conditions of soil, water and temperature. Then assimilatory pigments were evaluated in the seedling leaves, analyzing six replays for both control and exposed samples. **Exposure system.** The frequency of the exposure field was of about 400 MHz. The exposure field was non-modulated. The exposure system used for the electromagnetic treatment of the seedling samples consists in a transverse electromagnetic (TEM) cell fed by a UHF power generator connected to a cell end while, at the other end, the cell was terminated on a matched load. TEM sizes are large enough to allow simultaneous exposure of a sufficient number of seedlings. It is made by stainless steel and provided with small holes regularly distributed on all the walls, so that air and light can enter. Environmental conditions of constant temperature (24 Celsius degrees) and humidity were kept for exposed samples and controls. Inside the TEM cell oak seedlings were exposed to continuous travelling waves. **Spectrophotometric assay.** Seedling content of assimilatory pigments was measured, at about 24 hours after the last exposure was accomplished. Quantities of 0.02-0.03 g picked up from seedling leaves were crushed and acetone solution (90% in distilled water) was used for extraction according to standard procedure of Meyer-Bertenrath, modified by Stirban and Farcas (Stirban, 1986). Small aliquots of $CaCO_3$ and $MgCO_3$ were added during extraction in order to avoid chlorophyll transformation in pheophytine. Acetone extract was filtered through paper filter and quantitatively transferred to coated bottles of 25 ml. A Metrohm Herissau, spectrophotometer, type E-1009, with quartz cells of 1 cm width was used. Computation of chlorophyll **a** (Chl **a**), chlorophyll **b** (Chl **b**) and total carotenes pigment (t.c.) levels (mg pigment / 100 g green tissue) were performed by means of usual formulae: $Chla=(9.784E_{662}-0.99E_{644})100v/w$, $Chlb=(21.426E_{644}-4.65E_{662})100v/w$, $t.c.=[4.695E_{4405}-0.268(5.134E_{662}+20.436E_{644})]100v/w$, where:, $E\lambda$ - light extinction at the wavelength λ, w-green tissue mass (g) and v-acetone volume (ml).

RESULTS AND DISCUSSIONS

In Fig. 2 a the levels of assimilatory pigments in oak sapling leaves are represented. Averaged values obtained from measurements carried out in control and exposed samples, for every exposure time, are given. Statistic analysis was accomplished by t-test, two tailed, pair type, for comparison of control data series and every exposed sample series. Chlorophylls situation in analyzed also in Table I.

Fig. 2 a. Assimilatory pigment levels

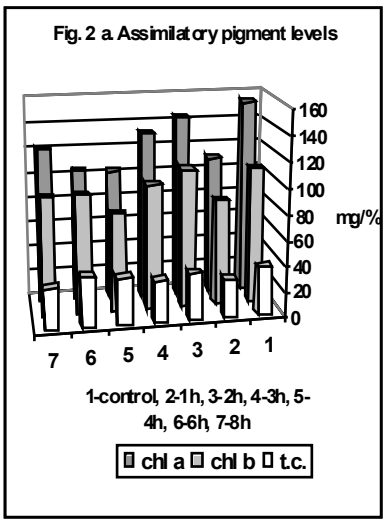
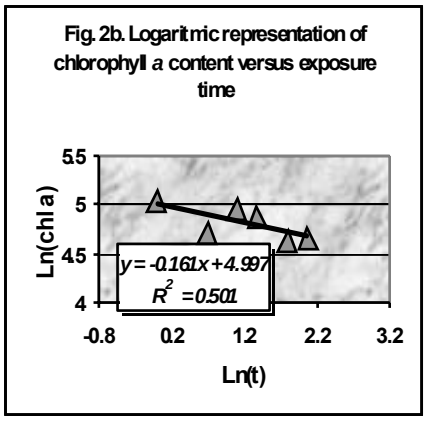
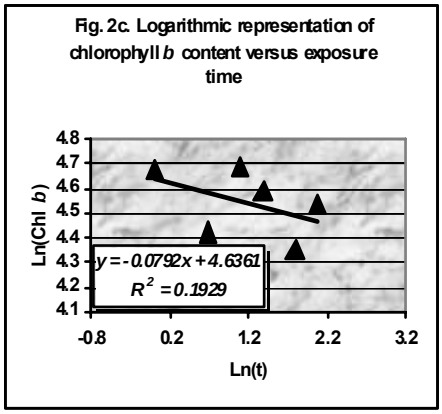
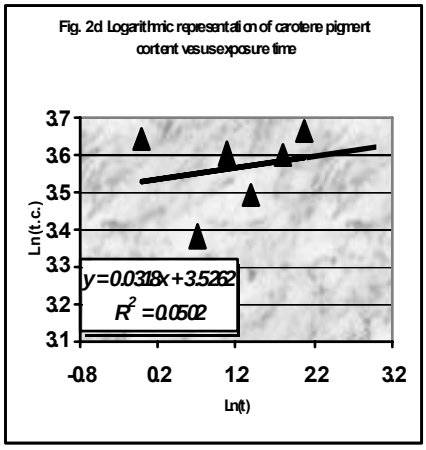


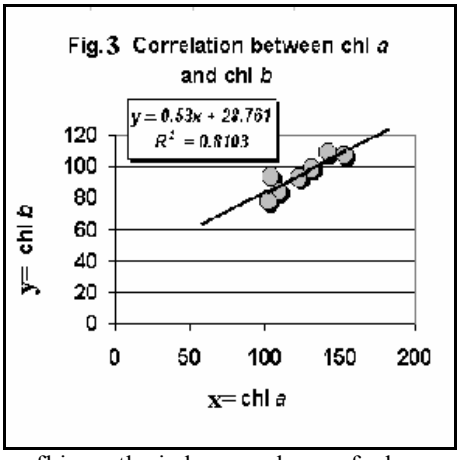
Fig. 1. Oak seedling grown in laboratory

It is visible the diminution of both chlorophyll **a** and chlorophyll **b** levels in exposed samples in comparison to the control for all exposure times tested inhere. In the range of small exposure times, statistical significant modifications were obtained for both chlorophylls (t-statistical parameter fulfills the criterion: $t<0.05$) except for the sample corresponding to exposure time equal to 2 hours, where the UHF influence is not clearly distinguishable in comparison to the control. Other statistical non-significant differences were recorded for higher exposure times: 6 h for chlorophyll **b** and 8 h for chlorophyll **a** (Table I). Chlorophyll **b** content modification in the sample corresponding to 3 h exposure is described by a t-value higher than the limit of 0.05 but rather close to it (0.06). The same situation appeared for carotene pigments in the sample corresponding to an exposure of 3 h. Carotene pigment content seems to be the less sensitive to electromagnetic field action, since only in two cases (for 1h and 8 h) we found statistical significant modifications. Logarithmic representation of every pigment content versus exposure time (Fig.2 b-d) shows linear negative regression for chlorophylls contents while for carotene pigments a positive correlation was found. Though correlation coefficients are rather poor in comparison to the unit (ideal theoretical case), these graphs suggested two distinct situations: inhibitory effect of UHF fields in chlorophylls and putative stimulatory effect in carotene pigments. In Figs. 3-5 we presented the correlations between pairs of pigments. In the case of chlorophyll **a** and chlorophyll **b** (Fig. 3) the linear regression is rather clear - correlation coefficient, R^2, is over 0.8, i.e. close to the unit (ideal case)meaning a high degree of experimental points grouping around the theoretical straight line. So, we may say that UHF treatment does not affect very much the chlorophyll ratio in oak sapling leaves. In Fig. 4 a-b the correlation between chlorophyll **a** and carotene pigment content is showed. Since the points corresponding to exposures of 6 h and 8 h were eliminated, the value of the correlation coefficient, much under the unit when all points are considered, is almost equal to the unit after the elimination of samples exposed for 6 and 8 hours. The elimination of these two points is consistent with the avoiding of the sample where the carotene pigment content is slightly enhanced in comparison to the control (for 6 h) as well as of one sample with a carotene content almost equal to that of the control (for 8 h). The remaining points exhibit the same general tendency of pigment content diminution after UHF treatment, i.e. the inhibitory effect on the photosynthesis. In Figs. 5a-b the same thing is revealed by the correlation between the chlorophyll **b** and carotene pigments: the elimination of the two samples mentioned above, "arrange" considerably better the points around the straight line theoretically calculated, sustaining the hypothesis of a dominant inhibitory effect in all pigments. Indeed, when the same two samples are avoided (i.e. 6 h and 8 h) the correlation coefficient is much closer to the unit ($R^2=0.9322$). We mention also that when the two samples are neglected the graph form Fig. 2d changes too, the slope sign being inverted - from the increasing tendency we got the decreasing of carotene content to the exposure time increasing. However, stimulatory influence of UHF waves should exist since thermal effect is characteristic to high frequency waves [18-19] absorption in the matter, and even in the case of a low power density, a slight warming of aqueous media from the vegetal tissues certainly occurred.



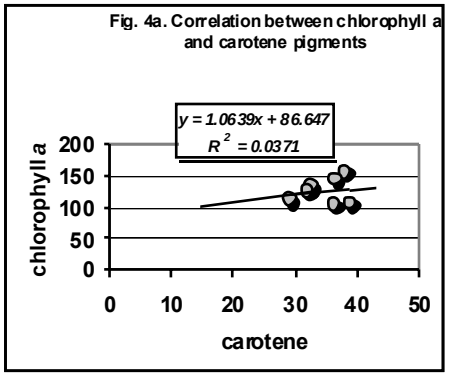


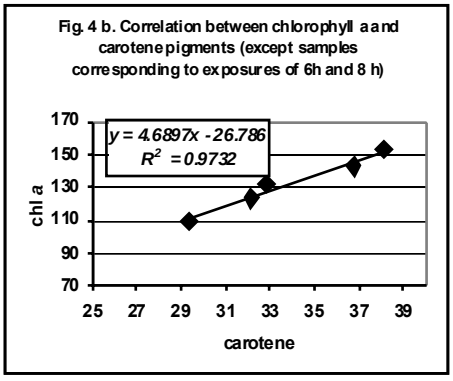


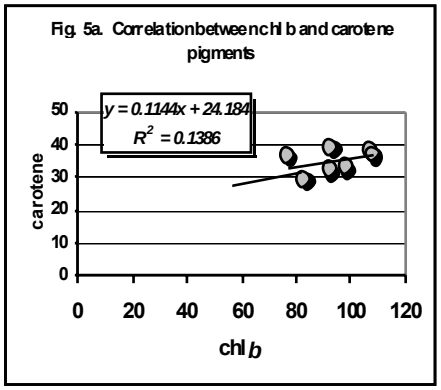


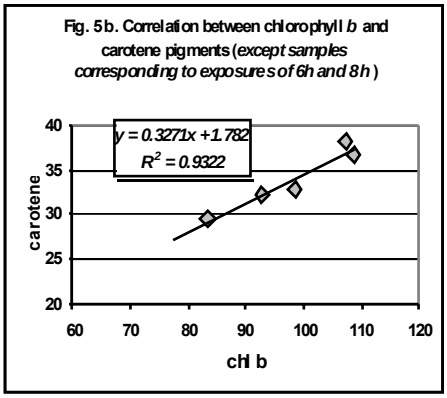
Consequently, a certain stimulation of biosynthesis happened even for low power density, as in the present experiment. But an inhibitory influence of UHF in the seedling cells may be also expected since only a putative non-thermal effect of high frequency electromagnetic waves can be related to the diminished content of assimilatory pigments in most of the exposed samples. In Fig. 6, where the sum of all assimilatory pigments is represented, the dominance of the inhibitory effect of UHF waves in all samples it is obvious - with the mention that for the exposure time of 2 hours the diminution is the lowest. In Fig. 7 the ratios between pairs of assimilatory pigment contents are given. The most significant variation is the slight increase of the chlorophylls ratio for all exposure times, ratio which represents an important criterion for any assumption regarding photosynthesis. However the correlation coefficient of the regression line is not high (only 0.3), because of some values of the pigment contents that do not represent

significant modifications (see Table 1). So, we can see that UHF waves rather damage the assimilatory pigment balance in the young seedlings of this arbor species when repeated exposures occur. Thermal effect, quite low in the case of power density of 1 mW/cm^2, is expected to cause a slight increase of temperature in the irradiated samples (due to the dielectric relaxation of water molecules), which is usually associated with a stimulation of biosynthesis, in this case the assimilatory pigment biosynthesis. But the decrease of assimilatory pigment levels may be related to a non-thermal, specific, effect of microwaves in living tissues, coexisting with the thermal one, and dominating especially for long exposure time. The inhibitory action resulted from the non-thermal UHF waves effect, may consist in the perturbation of ion channels functions at the level of membrane structures from the cell. Mainly ion transport through chloroplast membranes can be invoked in this case, though ion channels of plasma membrane and other cytoplasmatic structures are also supposed to experience microwave action (Polk and Postow, 1996). Perturbation of ion transport may lead to perturbations of various biochemical reactions which are controlled by ion messengers (especially calcium ions are known as intra-cellular messengers); so, it is possible that biochemical reactions involved in the assimilatory pigment biosynthesis are delayed for the duration of UHF wave action.

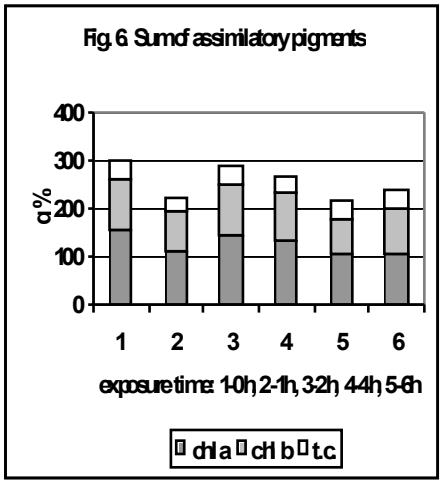


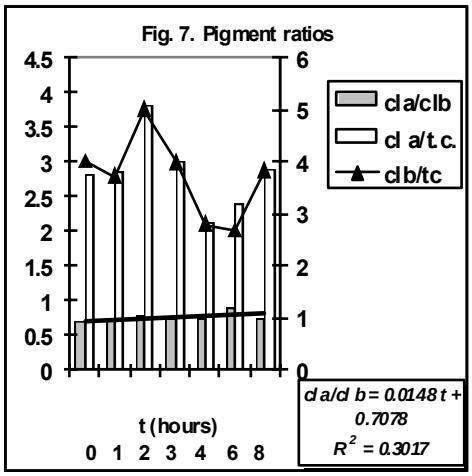






Nevertheless, molecular effect of UHF fields could occur at the level of chlorophylls, mainly since their association with water molecules is based on relatively weak forces hydrogen bonds with water molecules or even with other chlorophyll molecules.





By spectral analysis dimers, hydrated dimers and other oligomers of chlorophyll have been identified in chloroplast membranes where assimilatory pigments are located (Chapados, C., 1985, Ballschmiter, 1969). So, possibly, their molecular stability is related to the coupling with water, and consecutively, the destruction of this coupling could damage even the integrity and functions of chlorophyll molecules. Regarding chemical bonds assuring pigment molecule primary structure, it is not impossible that electromagnetic fields are able to destroy them also, since recent studies demonstrated such de

Table I. Statistical analysis of assimilatory pigment modifications induced by UHF exposure

	Exposure time (hours)							Pigment
	0 (control)	1	2	3	4	6	8	
Standard	17.17	6.73	8.32	7.89	17.32	16.29	3.42	Chlorophyll **a**
deviation	25.67	16.45	13.34	11.19	23.05	22.88	17.41	Chorophyll **b**
	6.07	4.40	10.46	5.30	21.68	3.98	3.27	Carotenes
		**	n.s.	**	**	**	n.s.	Chlorophyll **a**
T		*	n.s.	***	*	n.s.	**	Chorophyll **b**
parameter		*	n.s.	***	n.s.	n.s	*	Carotenes
(t-test)								

*p<0.01, **p<0.05, ***p=0.06, n.s. = non-significant, i.e. p>0.05

We may conclude that the balance between the two types of influence of UHF can have different results. For the oak seedlings obtained as mentioned above and for the combination power density-exposure procedure used in our experiments, the majority of the samples seems to be negatively influenced. In other species, in seedlings of other age or provenance, in other exposure conditions, the results can be different since the complexity of biophysical and biochemical processes from the living cell is tremendous. New experimental projects that we carried out on black locust saplings revealed the same trend of assimilatory pigment content decreasing except the smallest exposure time.

CONCLUSION

Pedunculate oak seedlings, after repeated exposures to UHF fields with a frequency of about 400 MHz and a power density of approximately 1 mW/cm^2, are

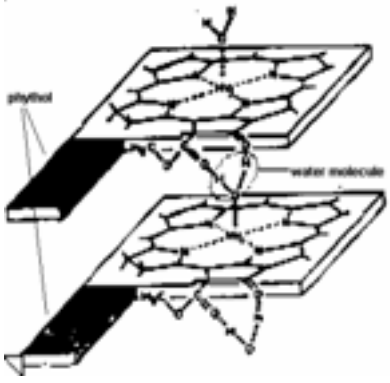


Fig. 8. Hydrated chlorophyll dimer

characterized by modified values of assimilatory pigment levels. The most remarkable change was recorded in chlorophyll **a** content where the t-test gave the smallest t-values, indicating a significant diminution in most of the samples. Carotene pigments appeared as the less sensitive to the magnetic treatment since the highest t-values have been provided by the test of statistical significance. Actually, we appreciate that this is the result of the overlapping of a thermal, stimulatory influence, and of a non-thermal, inhibitory influence of UHF absorption in the vegetal tissue, with different results in the biosynthesis of carotene and chlorophylls. The high degree of complexity of photosynthesis molecular basis is revealed in the frame of this experiment, further projects designed to get a deeper insight in the biophysical and biochemical mechanisms governing electromagnetic sensitivity of young arbor plantlets being imperiously required.

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