Final Report

Project reference: 6
Applicant's name: **Prof. J-P. Zryd, Dr. F. Rachidi**
Project title: **Influence of HF electromagnetic fields on the development and the molecular biology of the moss *Physcomitrella patens* and the nematode *Caenorabditis elegans*.**

1. State of Research.

1.1 Research activities performed, milestones and deliverables accomplished

Development of a moss biosensor system to distinguish thermal effect from other stress effect of HF electromagnetic fields.

Development of a fractal method to evaluate HF electromagnetic fields effect on the behaviour of the worm *Caenorhabditis elegans*.

1.2 Findings

1.2.1 Abstract

HF electromagnetic fields effect on the moss *Physcomitrella patens* were detected after continuous exposure to high intensity field (r.m.s of 353 V/m). This effect was identical to that of mild heat treatment. When a pulsed field with the same r.m.s was applied (20s/60s), the observed effect disappeared completely.

Caenorhabditis elegans worms respond to 100 V/m HF electromagnetic fields by increasing their motility. This is in clear contrast with heat shock treatment which results in a reduction of worms motility.

1.2.2 Main results

From what is known from our knowledge of physics, high frequency electromagnetic fields (hf EMF) are expected to act on biological material through a purely thermal effect. This view is nevertheless challenged by many experiments carried out in various laboratories; the results of those experiments are interpreted as a demonstration of a so-called non-thermal effect of hf EMF. Despite the fact that no good theory is presented to support those interpretations, the mere existence of such unexplained effect is a challenge for the scientific community.

Following the general trend to use simple organisms to study the effects of hf EMF, we decided to setup experiments using the plant (moss – Bryophytaea) *Physcomitrella patens* and the nematode worm *Caenorhabditis elegans* in an attempt to shed some light on the question. *Physcomitrella patens* is a well studied model plant of small size which could easily be placed in the restricted space of an experimental antenna. The idea was to use reporter proteins and/or genes known for their response to small temperature increase to see whether there was a correlation between temperature increase inside the organism and the response to hf EMF. This was our starting hypothesis. On the other hand, we decided to use two different ways to apply the high frequency field. One way was to expose the plants to a continuous 900MHz 500 V/m (peak value) field and the other way to a pulsed 866 V/m (peak value) field (for 20s/60s), both fields having the same r.m.s. value (353V/m, adopting the following values of conductivity and relative permittivity $\sigma=0.67\ \mathrm{S/m}$ and $\varepsilon_r=42$, the average SAR can be estimated to be 0.1 W/kg). It is expected that the thermal effect of the second treatment will be negligible due to a better and fast thermal dissipation.

We have developed a biological test (using a transgenic *P. patens*) based on the acquired protection of the reporter protein "Luciferase" toward thermal stresses. This test is based on the observations that after submitting the plant to a small increase in temperature (1-2°C), proteins are protected against "in vivo" thermal inactivation at high temperature (more than 40°C). In order to quantify more precisely the

heat shock response in further experiments, we have further created another transformed *P. patens* plant carrying a reporter gene (uidA – coding for a heat-stable protein), under the control of the soybean promoter for the heat shock protein hsp17.3.

In all following experiments we have used a 900MHz hf EMF. The results presented in figure 1 clearly indicate that exposure of *P. patens* plants during 20 hours to high energy hf EMF 500V/m peak value (r.m.s. = 353 V/m, corresponding to an estimated SAR of 0.1 W/kg) at 25°C, induces an effective protection of the protein luciferase against thermal denaturation "in vivo" at 41°C (●). This protection is similar to the protection acquired during a 20 hour treatment at 27°C that is 2 °C above the standard temperature (▪). We have also observed that the protection become negligible for low intensity field of r.m.s. = 176 V/m (estimated SAR of 0.05 W/kg).

Using the pulsed field as described above was then the critical experiment. The results are shown in figure 2. It can be seen that, if the effect of continuous field is as expected, the protective effect of a 20 hours treatment by a 353 V/m hf EMF disappears completely when a pulsed field is applied. Our results show that the pulsed field cannot elicit the same protection mechanism as for the continuous field. This observation clearly favors a simple thermal effect of high intensity, high frequency EMF on the induction of heat shock response and will challenge many claims of a non-thermal mechanism of induction.

As stated above, we have constructed a new transgenic plant containing a GUS reporter gene under the control of a small Heat Shock Protein (sHSP) promoter. This reporter system is very efficient and highly sensitive to small temperature changes as expected with a doubling of the expression for a 2°C increase in temperature (Figure 3). This new strain is a powerful tool to investigate putative weak increase of temperature in exposed plants. For the time being we cannot yet conclude about effects of an hf EMF field on this plant since preliminary results are still under processing and more experiments are needed before drawing meaningful conclusions.

Caenorhabditis elegans is a well studied animal which has already been used to investigate the biological effects of hf EMF. Animal (*C. elegans*) containing a reporter genes under the control of a small heat-shock promoter (PC72 strain) have been used. We have confirmed that, even a moderate exposure during 20 hours at 100 V/m is sufficient to stimulate the expression of the stress gene; the results of such an experiment are shown in figure 4; they confirm already made observations by de Pomerai and coworkers [1].

Many observations have shown that hf EMF are affecting the nervous system (human brain) and could influence sleep. We have therefore decided to design a test based on the wandering behavior of *C. elegans* to see whether it could be possible to use simple system to probe the effects of hf EMF on the simple nervous system of the worm. We developed a method, based on time lapse digital imaging and fractal calculations, to analyze the traces complexity of the nematodes. Not only the method works but the results are puzzling. We can conclude from figure 5 that the worm *C. elegans* responds to both heat treatment and EMF with opposite and conflicting effects. A heat shock diminishes worm mobility (fractal dimension of traces) and conversely, hf EMF stimulate the mobility. This would suggest, to the contrary of our previous observations with plants, that the thermal and hf EMF stimuli are acting in distinct ways; we should nevertheless point out that the hf EMF treatment was much longer (3 days) than the heat shock treatment (only one hour).

Our experiments constitute a proof that simple organism can give very interesting responses to questions about the nature of hf EMF effects and can allow a broad range of investigations for the future.

1. de Pomerai D, Daniells C, David H, Allan J, Duce I, Mutwakil M, Thomas D, Sewell P, Tattersall J, Jones D, Candido P: Non-thermal heat-shock response to microwaves. Nature 405: 417-418 (2000).

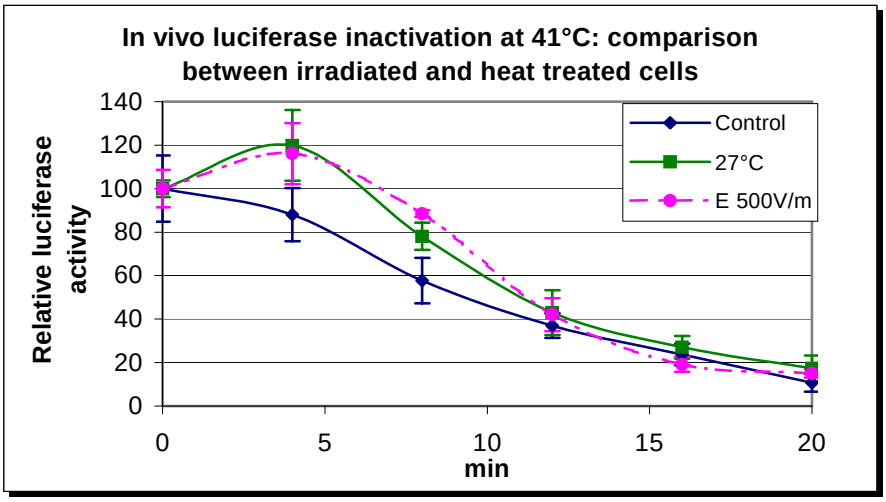

Figure 1: Comparison between luciferase inactivation curve in control cells (25°C) (◆), in cells exposed at 27°C (■) and in those exposed to 500V/m (r.m.s. 353V/m) (●).

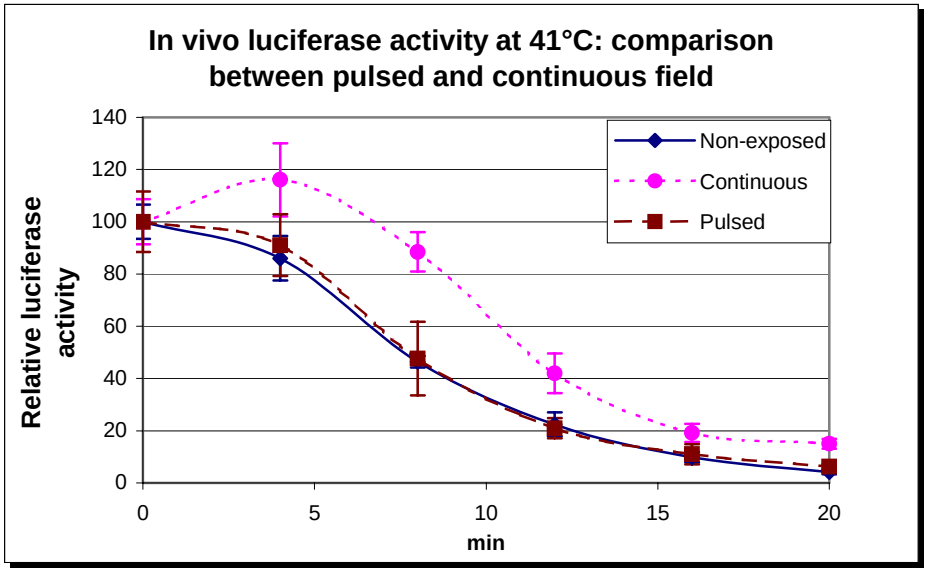

Figure 2: pulsed hf EMF (■) does not protect, but continuous hf EMF (●) induces protection (at a r.m.s of 353,55 V/m in both cases)

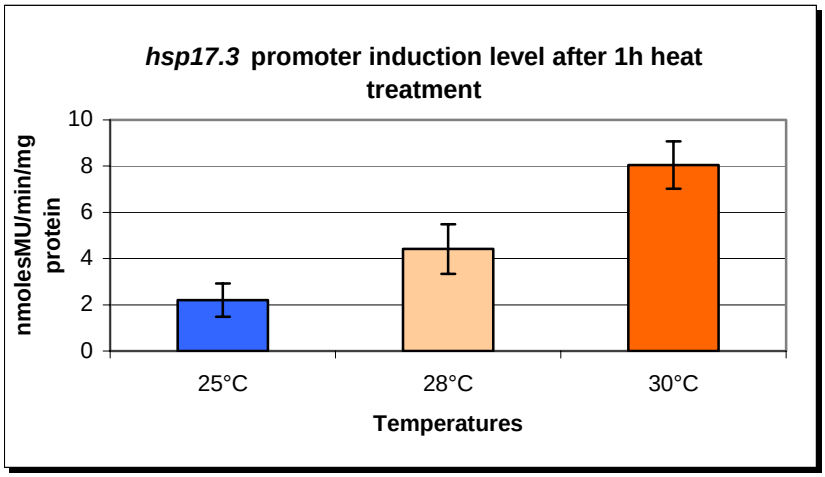

Figure 3: *hsp17.3* promoter induction level after 1h heat treatment at weak elevated temperatures.

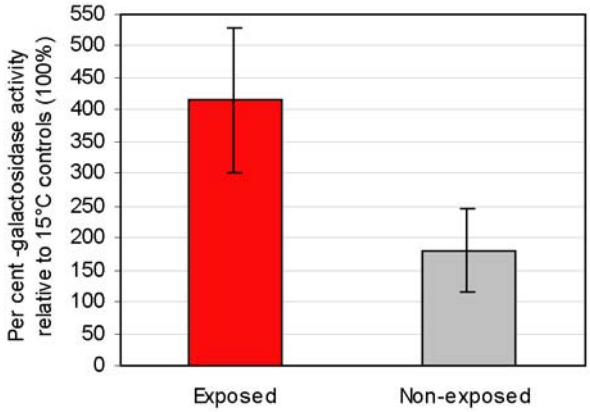

Figure 4: induction of the heat shock promoter hsp16 in the worm *C. elegans*.

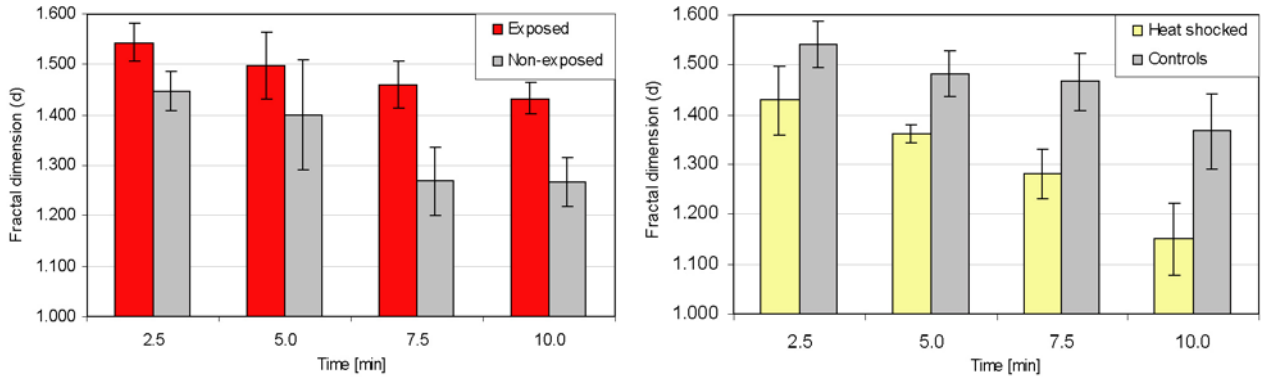

Figure 5, fractal dimension of nematodes traces. Left: exposed and non-exposed during 3 days to a 900MHz hfEMF of 100V/m at 25 °C. Right: exposed and non-exposed during one hour to a 32°C heat shock.

1.3 Problems

No major problem is to be mentioned.

2. Annex
2.1 Publications

- J.P. Zrÿd, M. Ianoz, F. Rachidi, P. Zweiacker, Influence of HF electromagnetic fields on the development and the molecular biology of the moss Physcomitrella patens and the nematode Caernorhabditis elegans, *14th International Symposium on Electromagnetic Compatibility*, **Zurich**. Proceedings Supplement pp. 179 180, February **2001**.
- Alasonati, E., Comino, E., Ianoz, M., Korovkin, N., Rachidi, F., Schaefer, D., Zrÿd, J.P. Use of fractal dimension for the analysis of biological effects of electromagnetic field on the moss P.patens and the nematode C. elegans. in *International Symposium on Electromagnetic Compatibility*. **2002**. Sorrento, **Italy**.
- Alasonati, E., Comino, E., Giudice, A., Ianoz, M., Rachidi, F., Saidi, Y., Zrÿd, J.P., Zweiacker, P. Use of the photosynthesis performance index to assess the Effects of high frequency Electromagnetic Fields on the membrane integrity of the Moss P. Patens, in: *15th International Zurich Symposium on Electromagnetic Compatibility,* **2003***,* Zurich, **Switerland**, February 18-20, 2003, 297-299.
- Alasonati, E., Comino, E., Ianoz, M., Korovkin, N., Rachidi, F., Saidi, Y., Zryd, J.P., Zweiacker, P. Fractal dimension: A method for the analysis of the biological effects of electromagnetic field. *Proceedings of the 5th International Symposium on Electromagnetic Compatibility and Electromagnetic Ecology*, St-Petersburg, **Russia**, **2003**, pp. 405-407, ISBN 5-7629-0546-2.
- Saidi, Y., Alasonati, E., Zweiacker, P., Rachidi, F., Goloubinoff, P., Zrÿd, J.P. High frequency electromagnetic radiations induce a heat shock-like response in Physcomitrella Patens, in: *The 6th Annual International Meeting for Moss Experimental Research*, **2003,** St. Louis, **USA**, September 7-10, 22.
- Zrÿd, J.P., Alasonati, E., Goloubinoff, P., Saidi, Y., Zweiacker, P., Rachidi, F. Tackling the problem of thermal versus non thermal biological effects of high frequency electromagnetic radiations, in: *Progress in Electromagnetics Research Symposium, PIERS'04*, **2004**, Pisa, **Italy**, March 28-31, 181-184.

- Comino, E., Zrÿd, J.P., Alasonati, E., Saidi, Y., Zweiacker, P., Rachidi, F. Methods for the Evaluation of Possible Biological Effects of Electromagnetic Fields, in: *Progress in Electromagnetics Research Symposium, PIERS'04*, **2004**, Pisa, **Italy**, March 28-31, 655-658.
- P. Goloubinoff, Y. Saidi, J.P Zrÿd, P.Zweiacker, F. Rachidi. Proteins and membranes as sensitive biothermometers: the role of heat-shock proteins in sensing and repairing stress damages in the Cell, in : *COST281: Influence of RF Fields on the Expression of Stress Proteins,* **2004**, Helsinki, **Finland** 28th - 29th April, 11.