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Low-amplitude, high-frequency electromagnetic field exposure causes delayed and reduced growth in *Rosa hybrida*

Alexandre Grémiaux[a], Sébastien Girard[b,c], Vincent Guérin[a], Jérémy Lothier[a], František Baluška[d], Eric Davies[e], Pierre Bonnet[b,c], Alain Vian[a,*]

[a] *Institut de Recherche en Horticulture et Semences—UMR 1345 IRHS, Université d'Angers-INRA-Agrocampus Ouest, ARCH-E, SFR 4207 QuaSaV, Campus du Végétal, CS 60057, F-49071 Beaucouzé, Cedex, France*
[b] *Université Blaise Pascal, Institut Pascal, BP 10448, F-63000 Clermont-Ferrand, France*
[c] *CNRS, UMR 6602, F-63171 Aubière, France*
[d] *Institut für Zelluläre und Molekulare Botanik Zellbiologie der Pflanzen, Universität Bonn, Kirschallee 1, 53115 Bonn, Germany*
[e] *North Carolina State University, Department of Plant and Microbial Biology, Box 7612, Raleigh, NC 27695-7612 USA*

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ABSTRACT

It is now accepted that plants perceive high-frequency electromagnetic field (HF-EMF). We wondered if the HF-EMF signal is integrated further *in planta* as a chain of reactions leading to a modification of plant growth. We exposed whole small ligneous plants (rose bush) whose growth could be studied for several weeks. We performed exposures at two different development stages (rooted cuttings bearing an axillary bud and 5-leaf stage plants), using two high frequency (900 MHz) field amplitudes (5 and 200 V m^{-1}). We achieved a tight control on the experimental conditions using a state-of-the-art stimulation device (Mode Stirred Reverberation Chamber) and specialized culture-chambers. After the exposure, we followed the shoot growth for over a one-month period. We observed no growth modification whatsoever exposure was performed on the 5-leaf stage plants. When the exposure was performed on the rooted cuttings, no growth modification was observed on Axis I (produced from the elongation of the axillary bud). Likewise, no significant modification was noted on Axis II produced at the base of Axis I, that came from pre-formed secondary axillary buds. In contrast, Axis II produced at the top of Axis I, that came from post-formed secondary buds consistently displayed a delayed and significant reduced growth (45%). The measurements of plant energy uptake from HF-EMF in this exposure condition (SAR of 7.2 10^{-4} W kg^{-1}) indicated that this biological response is likely not due to thermal effect. These results suggest that exposure to electromagnetic field only affected development of post-formed organs.

1. Introduction

Plants are continuously subjected to a wide variety of environmental stresses to which they must adapt to survive. Wind, drought, attacks of pathogens are well known to elicit rapid and more or less specific responses that dramatically affect plant metabolism and development (Maleck and Dietrich, 1999; Kozlowski and Pallardy, 2002; Obata and Fernie, 2012).

In the past couple of decades, the urban environment has been increasingly exposed to high frequency (HF) low amplitude electromagnetic background, mainly because of mobile phone and WiFi development. The possible impact of these radiations on human health was early questioned, in particular through wide scale epidemiological (Repacholi et al., 2012) or case-study investigations (Hartikka et al., 2009) and received considerable attention in the public arena. Numerous reports pointed out possible biological effects of HF electromagnetic field (HF-EMF) on living organisms (Cucurachi et al., 2013). It is important to note that biological effects are not necessarily injurious events but highlight that a living organism actually perceived the EMF and transduced it into some biological responses. In the case of tissue heating, the interaction is evaluated through the Specific Absorption Rate (SAR) that is the dissipated power of electromagnetic field per unit mass of tissues. SAR measurement requires tightly controlled experimental environment and is usually averaged over a volume of homogeneous material; therefore there are very few SAR measurements *in situ*:

Abbreviations: EMF, electromagnetic field; ELF, extremely low frequency; HF, high frequency; CW, continuous wave; SAR, specific absorption rate; MSRC, Mode Stirred Reverberation Chamber; r.m.s, root mean square.

* Corresponding author. Fax: +33 241 735 352.
E-mail address: alain.vian@univ-angers.fr (A. Vian).

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very simplified models are used instead to evaluate *in vitro* tissue heating (Bernardi et al., 2002).

Plants are specialized in the interception of EMF radiation (light). Their particular scheme of development that favor interaction with the environment involves a high surface area to volume ratio, maximizing the surface exposed to radiations. While plants are ideally suited to study interactions between EMF and living organisms (Vian et al., 2007), high frequency EMF impact on plants has received little attention over the last decade. Some reports, however, clearly demonstrated that exposing plants to continuous wave (CW) EMF evoked various kinds of biological responses including changes in enzyme activity (Sharma et al., 2009; Kouzmanova et al., 2009), gene expression (Vian et al., 2006; Jangid et al., 2010) and in growth (Tkalec et al., 2005; Jinapang et al., 2010).

In the last years, our group designed a set of experiments to establish a formal yet unequivocal link between HF-EMF exposure and modifications of plant metabolism. We performed a low amplitude, short duration EMF stimulus in order to avoid thermal effects, while a precise control of electromagnetic field characteristics was achieved using a Mode Stirred Reverberation Chamber (MSRC). Our study focused on rapid events (occurring within minutes after the end of the exposure) and address basic, early steps of biological responses (accumulation of specific stress-related transcripts). Using this methodology, we demonstrated in tomato, that low intensity ($5\,V\,m^{-1}$), short duration (10 min) and high frequency (900 MHz) EMF actually constitute an environmental signal that evoked the rapid and transient accumulation of mRNA from stress-related genes such as calmodulin, calcium–calmodulin dependent protein kinase or bZIP transcription factor (Vian et al., 2006; Roux et al., 2006, 2008). These experiments have been recently reproduced by an independent group that also showed that the molecular responses could last several days after continuous EMF stimulus (Rammal et al., 2014). Surprisingly, the plant response to EMF appears to follow an “all or nothing” law, since the measured accumulations of mRNA were similar after 5 or $41\,V\,m^{-1}$ exposure (Roux et al., 2006). The response was observed systemically when a single, aged leaf was exposed to EMF: the distant, non-exposed, terminal leaf tissue showed a similar response in amplitude, but with a slight delay (Beaubois et al., 2007). Interestingly, the distant response was not observed in *Sitiens* and *JL-5* tomato mutants that are respectively defective for ABA and jasmonic acid biosynthesis, while the local response (at the site of exposure) operated normally, suggesting that both ABA and jasmonic acid contribute to the systemic response. Furthermore, plant exposure to EMF evoked a rapid and transient (10–30 min) diminution of ATP content that was associated with a drop in the adenylate energy charge (Roux et al., 2008). Despite these strong but transient molecular responses to EMF exposure, tomato plants did not display any visible changes in their morphology and development: the growth rate, flowering time and fruit production appear identical in control and exposed plants. However, tomato was not an optimal model plant to study plant-level EMF induced development alteration, since their vigorous growth and lack of stem rigidity requires staking to prevent excessive bending. These operations involve plant manipulations that were likely to mirror eventual EMF-induced growth alterations.

In the present study, we aimed to study the morphological changes that may be observed after exposure to HF-EMF radiation. We chose to perform growth studies of EMF exposure on a different plant model, namely rose bush (*Rosa hybrida*), that is a small ligneous plant with sufficiently rigid axis to avoid the need of staking and further manipulation after exposure. Here we performed SAR measurements in planta during exposure to EMF and observed that a set of low amplitude ($5\,V\,m^{-1}$), short duration (30 min) exposures were later expressed as growth modification of some (but not all) branches. These observations are, to our knowledge, the first report of growth alteration in a ligneous plant after exposure to low amplitude HF-EMF.

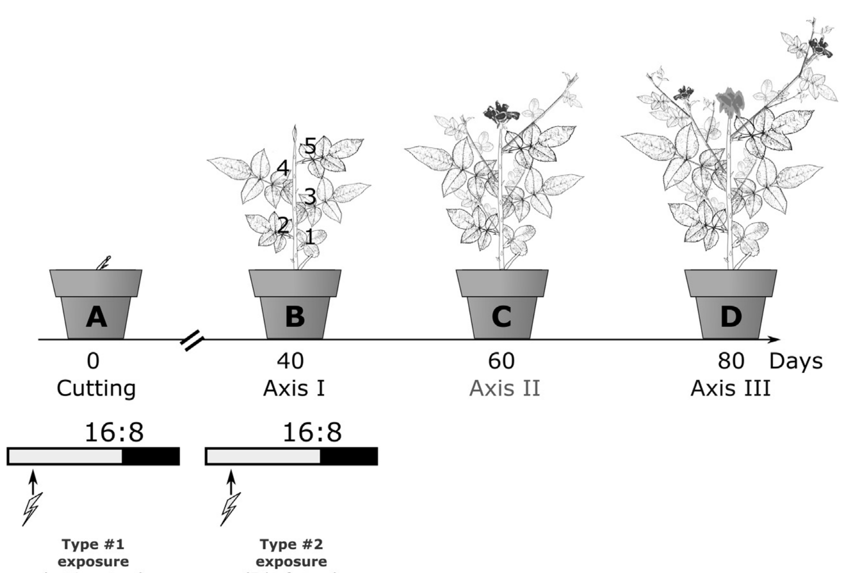

Fig. 1. Plant material and exposure.
Rosa hybrida ‘Raddrazz’ plants were obtained from cuttings and rooted in an appropriate medium. They were grown in controlled condition (L:D 16:8). Plants were exposed to EMF 3 h after light-on (see insets), either at the cut stage (type 1 exposure), a few days after rooting and before bud outgrowth (A), or after the formation of the 1st order axis bearing 5 expanded leaves (B), about 40 days after rooting and cutting insertion in the culture medium (type 2 exposure). The exposures consisted of either a single, high amplitude ($200\,V\,m^{-1}$, 30 min) 900 MHz EMF signal or a set of 3 low amplitude ($5\,V\,m^{-1}$, 30 min), each spaced of 48 h. The terminal flowering of Axis I or Axis II released apical dominance and allowed the production of Axis II (C) and Axis III (D), respectively. The buds were numbered along the axis from the oldest one (at the base of the axis) to the youngest one (at the top of the axis). We measured the subsequent bud outgrowths and axis elongation, namely Axis I and II (when using type #1 exposure) or axis II and III (when using type #2 exposure).